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RESPONSE OF ARBUSCULAR MYCORRHIZAL FUNGI AND *BRADYRHIZOBIUM JAPONICUM* TO AIR POLLUTION STRESS IN SOYBEAN

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ABSTRACT

Filtered air (FA) resulted in greater plant height, more number of leaves and shoots per plant, greater number of flowers and pods per plant as compared to unfiltered air (UFA) and ambient air (AA). As regard to soil treatment, the treatment with sterilized soil with AM and Rhizobium inoculation showed an overall significant performance as compared to other cases. The efficacy of N-fixation enhanced in filtered air as compared to other treatments. The number of root nodules also increased. As far as arbuscular mycorrhizal (AM) colonization is concerned, all the fungal organs showed maximum intensity. Spores formation was also significantly high in plants of filtered air grown in sterilized inoculated soil than other air or soil treatments. In the unfiltered air (UFA and AA), mycorrhizal plants showed better results than non-mycorrhizal plants in all respects. The mycofloral composition of soybean at the time of final harvest constituted a total of (29) species of AM fungi, majority of which belonged to the genus *Glomus*. While some sensitive species failed to sporulate indicating the prevalence of unfavorable conditions. A few AM species like *G. tenue* were sensitive to dust pollution and could not form spores in ambient air.

Keywords: soybean, fungi, air, pollution, nitrogen, ozone, species.

INTRODUCTION

Ozone and nitrogen dioxide are major elements of urban smog that can affect plant growth and health. Negative impacts include reduced growth and seed production, and increased susceptibility to insects and disease (Lefohn *et al.*, 1997; Samuelson and Kelly, 2001; Castagna *et al.*, 2001). Long-term ozone stress may lead to changes in species composition and biodiversity (Burroughs, 2001; Ranieri *et al.*, 1996 and 2000; Crutzen, 2002; Allen and Rincon, 2003).

Most plants form mycorrhizae, and the role of AM symbiosis in mediating plant responses to atmospheric change may be an important consideration in predicting effects of atmospheric pollutants on plants in agricultural lands and managed ecosystems (Shafer and Schoeneberger 1991). Although the effects of atmospheric pollutants on mycorrhizal symbiosis have received some study in recent decades (Fitter *et al*. 2000, Egerton-Warburton *et al.*, 2001, 2002), most research on ozone and mycorrhizae has involved coniferous plants and ectomycorrhizae. Less information is available regarding how ozone or nitrous pollutants affect arbuscular mycorrhizal (AM) colonization of roots and growth of AM plants (Duckmanton and Widden, 1994, McCool 1984; McCool *et al.*, 1982, 1983). McCool (1979) reported that higher than normal concentrations of ozone inhibited the growth and spread of *Glomus fasciculatum* in a *Citrus* sp. Air containing higher than ambient concentrations of ozone reduced photosynthetic capacity and inhibited mycorrhizal formation (Heath 1980, Heath *et al.* 1982). With exposure to 0.1 ppm ozone for three months, root weight and intensity of mycorrhizal formation declined in the forage grass *Festuca arundinacea* (Ho and Trappe 1984). Soybean inoculated with *Glomus geosporum* and *Rhizobium* was less sensitive to adverse growth and yield effects of ozone (Brewer and Heagle 1983).

Soybean is grown over vast areas in Pakistan for a rich protein supplementing vegetable and for extracting cooking oil. The soils in soybean fields naturally have AM fungi. There are about 40 species of these fungi reported by Nasim and Bajwa (2005). In the present study we have attempted to compare the response of soybean plants in sterilized and unsterilized conditions. One set of plants in sterilized conditions was given the inoculum of AM and *Bradyrhizobium japonicum*. At this time we thought this to be a more agriculturally useful approach to compare non-AM vs AM and Rhizobium non rhizobium scenarios. We were focused to determine that inducing colonization with known microbes might affect soybean resilience to ozone and NO_2. The two sterilized soil treatments have been compared to control, which is field soil without inoculation.

These objectives addressed three specific hypothesis that 1: the soybean plants kept in filtered air are better health wise and in yield responses, 2: the plants getting an inoculation of known AMF and *Rhizobium* respond well to filtered air than those grown in sterilized soil and common field soil and 3. The mycorrhizal plants are better able to withstand air pollution stress as compared to non-mycorrhizal ones.

MATERIALS AND METHODS

Site

The study was conducted in the Botanical Garden of Punjab University, Quaid-e-Azam Campus, Lahore (31° 35_00_N, 74° 21_00_E), Pakistan, on a 21m x 15m suburban site adjacent to agricultural fields. The

experimental site was 1500m from the nearest main road, 7km from the nearest industry and about 7.5km from the city center. Ambient ozone levels here during the study generally ranged between 40 and 80 ppb and NO_2 between 25 and 35 ppb. In preparation for planting, the site was cleared of vegetation, ploughed and leveled.

Plant material and culture

Certified seed of soybean cv. NARC-I was obtained from National Agricultural Research Centre (NARC), Islamabad. Healthy looking seeds were sorted out by rejecting empty and shriveled seeds. These seeds were surface sterilized in a mixture of 70% alcohol and H_2O_2 in 1:1 ratio. After giving a couple of washings with sterilized tap water the seeds were used for sowing.

Application of *Rhizobium* inoculum

The inoculum of *Bradyrhizobium japonicum* used in soybean experiment was obtained from NARC, Islamabad. The inoculum was in the form of black powder, which included the culture of *Rhizobium* mixed with *Sphagnum* peat (an inert material). Seeds to be inoculated were moistened in sugar solution made in water (10%). The seeds were then rolled in the inoculum and sown. The inoculum was applied @2ml per kg of seeds, (pamphlet, NARC).

Rhizobium coated and plain seeds (5-6) were sown in empty yogurt glasses in sterilized soil mix on 5th August 1998. Three holes were made in the bottom of these containers and a layer (1.5" thick) of course gravel was spread before filling these with sterilized soil. The seedlings were shifted to pots after a week of germination. At the time of transplantation, arbuscular mycorrhizal inoculum was provided to the seedlings of AM inoculated set.

The potting medium was an unsterilized field soil (fine-loamy) mixed with sand (medium-to-coarse, mined, sieved) in 3:1 ratio. The soil-sand mix was air-dried and sieved (passed through 2mm screen). This was mixed with thoroughly sieved good quality composted farmyard manure in 6:1 ratio by volume. The soil mix was sterilized by fumigation. For this purpose soil was spread in the form of 5-7" layer on a clean polythene sheet. Holes were made 2-3" deep and 1-inch square with the help of a clean wooden stick. The inter-hole distance was maintained at 10". The fumigant (formaline) was released into a glass beaker (capacity 1L). This fumigant (2-3 ml) was then poured into the holes under a heavy and clean polythene sheet buried under the soil around the edges (Bowalda, 1983, 1985). Three days after application of the fumigant, the sheet was removed and soil was mixed thoroughly. About 5 kg of this soil was then filled in clean earthen pots (12" in diameter). In to these pots, 5 days later, seedlings were transferred along with the inoculum. Great care was taken to avoid contamination of the fumigated soil.

The suitable moisture (18%) was maintained for germination. Seedlings were grown outdoors in a wire house until they were thinned at 10 days.

Pots were labeled as **SS** (sterilized control soil) **SS+Inoc** (Sterilized soil + inoculation) and UFS (unsterilized field soil). Half of the pots in each treatment were placed in filtered air chambers while the other half was placed in unfiltered air chambers.

AM inoculation

Maize plants inoculated with *Glomus mosseae* and *G. fasiculatum* in aseptic conditions. These spores were picked from the common field soil following wet sieving and decanting technique of Gerdemann and Nicolson (1963). The roots of these plants were examined for the presence of AM endophytic mycelium. Root systems heavily infected with mycorrhiza (more than 80%) were used as inoculum. Roots were cut up into 1cm pieces. 5ml of these root pieces infected with AM endophytes were mixed in the upper 1cm zone of each pot at the point of placement of seedling except in the sets which served as AM inoculated sterilized soil set.

Air pollutant treatments

10 days after transplanting, pots were thinned to one plant per pot, and pots were relocated for exposure to differing levels of air pollutants. 12 plots were prepared, each 1.5m in diameter, with 3 replicate plants of each treatment placed in each plot. Prior to placing experimental plants, each plot was excavated to a depth of 0.4m and had a concrete barrier applied to its perimeter to prevent the entry of rodents and soil erosion during monsoons. Open-top chambers were constructed on eight of the plots (Figure-1). Circular angle iron frames were attached to the concrete at the tops of each of these eight plot and open-top chambers fitted into these frames.

The cylindrical shape of the chamber was produced by connecting two angle iron frames (each 1.5m in diameter), by five 1.5m long vertical angle iron bars. The frames were fabricated locally. The curved walls were covered with plastic sheet (PVC 1000 gauge sheeting; transatlantic plastics), apart from an aperture between two adjacent support bars (approximately 0.5m across), which was covered separately to create a doorway. The plastic was used only for one growth season and replaced at the beginning of each season, as with age it becomes more opaque and brittle with exposure to ultra-violet light and pollutant gases. Single inlet centrifugal blowers (fan) and motors of appropriate specifications were obtained from local manufacturers and used to blow air through lengths of plastic drain-piping of 6 inch diameter that were connected to the individual chambers. Emcel air filters (dust or pre-filters and charcoal filters) were imported from U.K. Pre-filters remove dust or particulate matter form the air while charcoal filters remove various pollutant gases from the air as they are absorbed by the activated charcoal. An activated charcoal filter along with a pre-filter connected to a 3HP fan-motor was linked to a pair of chambers through a 6 inch diameter drain-piping bifurcated at the distant end, each leading to individual chambers of the pair and connected to a vertical manifold. Similarly a pre-filter connected to a 2HP fan-motor was

also linked to a pair of chambers. A vertical manifold which was simply a perspex pipe of 6 inch diameter blocked at its top end and with a butter fly valve at its base (allowing for the adjustment and equalizing of the air flow into each chamber) was fixed inside the wall of the chamber and connected to in-coming drain-piping. Vertical manifold was studded with 2 inch diameter holes 30cm apart along its length in two parallel rows. Two consecutive rows of holes alternated with each other. Two horizontal manifolds constructed from plastic lay flat tubing (transatlantic plastics) were connected to two parallel pairs of holes in the vertical manifolds (at heights of 50 and 80cm) so that they would be expanded by the air pressure. Holes (2.0cm in diameter) were punched at 20cm intervals in the horizontal manifolds to allow the introduction of air blown through the piping system into the chambers. The horizontal manifolds were fixed so that these holes were oriented at 45 degrees to the horizontal and directed to wards the ground. The total volume of each chamber was 2.83m^3, and the flow rate of the air into each chamber averaged 8m^3/min. The resulting airflow therefore gave approximately 3 changes per minute. Shading of motors, fans and filters with metal housing minimized any rise in temperature of air entering the chambers. Dust was washed from the outside of the chamber walls regularly.

Four of the open-top chambers were fan-ventilated with ambient unfiltered air by passing air through a dust filter (to remove particulate matter). The remaining four open-top chambers were supplied with the same air but after passage through an activated charcoal filter in addition to the pre-filter to remove dust. The remaining 4 un-chambered control plots were not ventilated or filtered but were simply exposed to ambient atmospheric conditions. The purpose of the filters was to remove ozone and NO_2 from the atmosphere around the soybean foliage. This system was constructed following the methods of Bell and Ashmore (1986) and Wahid *et al.* (1995a, b). The open-top chambers were arranged in four pairs, with the remaining four un-chambered plots located a row adjacent to the chambered plots. The four plots not supplied with fan-blown air or chambers were control plots.

The chambers were operated as closely as possible to the conditions prescribed under European Open-top Chamber Programme Protocol (Jager *et al.*, 1993). Light intensity, air temperature and relative humidity were measured at plant height each day at 0800, 1200 and 1600h using a portable light meter (horticultural Lux meter, model Dm-28, OGAWA Seiki Co., Japan) and temperature-humidity probe (thermo-hygrometer, model HI-8564, Hanna Instruments, USA) throughout the experiment. Micro-climatic measurements began when plants were transferred to the experimental plots. Transparent walls of the open top chambers were kept clean by washing regularly early in the morning in order to minimize any differences in the light levels between inside and outside of the open top chambers.

Ozone and NO_2 were continuously monitored in each air treatment during the course of experiment by wet chemical methods.

Measurement of ozone

Ozone concentrations in air were measured using the KI buffered method (Saltzman and Gilbert 1959) on alternate days (three times per week). Air was sampled in the center of the experimental plots between 1000 to 1600 h. at crop height. This sampling height varied as the crop matured.

Initially for the calibration a neutral buffered solution of potassium iodide (KI) was prepared containing 10.0g of potassium iodide, 13.6g of potassium di-hydrogen phosphate (KH_2PO_4) and 14.2g of anhydrous di-sodium hydrogen phosphate (NaH_2PO_4) and final volume was made up to one liter with distilled water. A 0.05 N solution of iodine was then prepared by dissolving 16.0g of potassium iodide and 3.17g of resublimed iodide in distilled water and made the volume up to 500ml. By diluting 5ml of this iodine solution to 100ml with the original neutral buffered potassium iodide and 0.0025 N iodine solution was prepared.

The solutions of iodine concentration for the calibration were then prepared as follows:

- 0.8ml of 0.0025 N iodine solution to 100ml with KI solution = 2.54 ppm I_2.
- 1.6ml of 0.0025 N iodine solution to 100ml with KI solution = 5.08 ppm I_2.
- 2.4ml of 0.0025 N iodine solution to 100ml with KI solution = 7.61 ppm I_2.
- 3.6ml of 0.0025 N iodine solution to 100ml with KI solution = 11.42 ppm I_2.

The optical density of these solutions was then measured against the neutral buffered potassium iodide as reference, at a wavelength of 352 nm on a UV Spectrometer (Hitachi Model U-1100). A calibration graph was drawn for ozone estimation.

The air in the chambers was sampled by drawing the air through 10ml of buffered potassium iodide solution at a known flow rate and for a known period of time. The ozone oxidized the potassium iodide to release iodine, the amount of iodine formed being measured on the spectrophotometer at 352 nm. The optical density value was converted to ppm iodine using the calibration graph.

Determination of Ozone concentration in ppm by substituting I_2 values with O_3 values in the equation are derived as follows:

$$2KI + O_3 + H_2O \longrightarrow I_2 + 2KOH + O_2$$
$$1 \text{ mol. Wt. } O_3 \longrightarrow 1 \text{ mol. Wt. } I_2$$
$$22.4 \text{ litres } O_3 \longrightarrow 254 \text{ g } I_2$$
$$22400 \text{ cc } O_3 \longrightarrow 254 \times 10^3 \text{ mg } I_2$$

Calculations

Let **'X'** = ppm I_2 (W/V)
Let **'Y'** = volume of air sampled /time

$$\text{ppm } O_3 \text{ (V/V)} = \frac{224 \times 10^3 \times \textbf{'X'}}{254 \times 10^3 \times \textbf{'Y'}}$$

$$O_3 \text{ ppm} = \frac{224 \times \textbf{'X'}}{254 \times \textbf{'Y'}}$$

Measurement of Nitrogen dioxide

Nitrogen dioxide (NO_2) monitoring in open top was done over weekly periods in Chambers following Atkins *et al.*, (1978) using triethanol amine coated discs and their subsequent analysis calorimetrically. Diffusion tubes were exposed in triplicate at weekly intervals in the each of the 12 plots. Tubes were fixed on wooden rods anchored in the middle of the chambers at points which varied according to crop height during the course of the experiment at crop height, like for ozone. Concentration of O_3 and NO_2 inside the chambers was also monitored 3x per month at different heights (0.3, 0.6, 0.9 and 1.2 m), to gauge vertical pollution levels inside the chamber environment.

Nitrogen dioxide (NO_2) is measured using diffusion tubes developed at the Environmental and Medical Sciences Division, AERE Harwell (Atkins *et al.*, 1978). Gas was samples passively by molecular diffusion along a tube to an absorbing medium. The absorbent used is triethanolamine (TEA) impregnated onto stainless steel mesh. NO_2 is determined as nitrite ion colorimetrically.
The principles of diffusion tubes are clearly explained by this method. For a tube of known length and internal diameter with an efficient absorber at one end, the NO_2 sampling rate may be calculated using Fick's Law of the unidirectional flow of gas through a mixture of gases under conditions of constant temperature. The temperature dependence of the diffusion coefficient is 0.2% per °C and the collection of gases in diffusion tubes is independent of pressure. The sampling rate of the tubes used here is calculated to be 62.3ml of air per hour with a diffusion tube constant of 10437.8.

The diffusion tubes comprise 7.1cm x 1.1cm internal diameter acrylic tubes fitted with one coloured (blue or red) and one natural airtight polythene end-cap. Before used tubes are acid washed (5% HCl) thoroughly rinsed with distilled water and dried in an oven at 30°C. One cm diameter discs which fit tightly inside the blue end-caps, are cut from 34 gauge stainless steel wire of mesh 0.224mm. They are also acid washed and dried before dipping in 50% V/V triethanolamine/acetone solution. Discs are then placed on filter paper to allow the acetone to evaporate and thus leaving a fine coating of triethanolamine (TEA). Next two or three prepared discs are placed inside the blue polythene end-caps and these are refitted to the diffusion tubes, other end is also closed with natural polythene end-caps during transport and storage. It is important that prepared discs are placed inside coloured end-caps rather than other cap to prevent possible light induced reactions which may affect pollutant adsorption. During sampling the natural coloured end caps are removed and the tubes are mounted vertically, with absorbent uppermost and open end pointing down wards to prevent the entry of rain water and dust particles. Weekly sampling periods are employed and the starting and finishing times are recorded accurately. Duplicate tubes should be a minimum requirement to avoid any hazard and for accurate analytical analysis.

Nitrogen dioxide (NO_2) is determined colorimetrically as NO_2. One part distilled water; one part sulphanilamide reagent (20g sulphanilamide + 50ml concentrated orthophosphoric acid diluted to 1000ml with distilled water) and one tenth part N-1-naphthyl-ethylene-diamine-dihydrochloride (NEDA) reagent (0.35g NEDA in 250ml distilled water) are added to each sample tube at the opposite end to the mesh collectors. After addition of chemical reagent, the open-end cap is closed. After 10 minutes, shake the tubes gently and leave them for more than 20 minutes. The nitrate ion is released from the mesh collectors in solution and deoxidizes the suphanilamide. The salt formed, become coupled with NEDA and a purple red azo dye is produced whose optical absorption is measured on a UV range spectrophotometer at 520 nm with a reagent blank in the reference cell. Standards were made up by dilution from 100 ppm solution of 'analar' sodium nitrite and a range of 0.05 to 1.0 ppm was sufficient to cover all samples.

For a tube of known length and internal diameter with an efficient absorber at one end, the NO_2 sampling rate may be calculated by using Fick's first law of the unidirectional flow of gas through a mixture of gases under conditions of constant temperature. The temperature dependence of the diffusion coefficient is 0.2 % per °C and collection of gases in diffusion tubes is independent of pressure. Total microgram of nitrite was found from this analysis and parts per billion (ppb) NO_2 was derived from this figure and the collection rate of the tubes thus:

$$\text{ppb } NO_2 = \text{Sample } NO^-_2\ (\mu g) - \text{blank } NO^-_2\ (\mu g) \times \frac{10437.8^*}{t(\text{hrs})}$$

*Diffusion tube constant

Plant and fungal characters

During the course of study the regular plant growth parameters recorded were plant height (cm), number of leaves per plant, number of shoots per plant, number of flowers per plant and number of pods per plant
Two harvests were taken during the entire duration of experiment. First harvest (mid-season harvest or Harvest-I) was carried out at the prime of vegetative growth of plants while the second harvest (final harvest or harvest - II) was taken at the end of growing season.

Plants in triplicate were harvested from each treatment and root systems were gently washed. The parameters recorded at the time of first mid season harvest were length of shoot and root (cm), number of nodules per plant, Acetylene reduction test for N-fixation, Fresh weight of root and shoot (g), Total fresh weight of plant (g), and Oven dry weight of root and shoot (g).

At the time of final destructive harvest following parameters were recorded:

- Number of pods per plant
- Average number of seeds per pod
- Average weight of seeds per pod (g)
- Total weight of seeds per plant (g)
- Eandogonaceous spore flora of rhizospheric soil and Harvest index (Harvest index was defined as the ratio of grain yield to total plant mass (Sinclaire, 1998).

Several mycorrhizal characters were assessed at each harvest: total, vesicular and arbuscular mycorrhizal colonization; hyphal width; and density and diversity of Glomalean spores. Roots were washed thoroughly and fixed in separate test tubes overnight or stored in mixture of formaline, acetic acid and ethyl alcohol (FAA) in 5:5:90 ratios. These were processed and stained following the methods of Phillips and Hayman (1970) with some modifications (Iqbal and Nasim 1986). Stained root pieces (20 per slide) were examined under the microscope by randomly selecting the field and averaging the data for five such observations. AM spore extraction was done using the wet sieving and decanting technique of Gerdemann and Nicolson (1963) and Nasim and Iqbal (1991). Spores were mounted in water or stain (trypan blue in lactophenol) study under the microscope. Identification followed the synoptic keys of Morton (1988) and Schenck and Perez (1990).

Acetylene reduction test

The test was conducted at NIAB, Faisalabad. Root systems along with rhizosphere soil were carried to NIAB for further processing, for acetylene reduction test (Hardy *et al.*, 1973).

Statistical analyses

Standard error (SE), standard deviation (SD), Student's T test, analysis of variance (ANOVA) and Duncan's New Multiple Range Test (DNMRT) were performed to analyze the data following Steel and Torrie (1980) and Rosner (2000) using SPSS 10.0 (Carver and Nash 2000).

RESULTS

These open chamber studies for soybean were conducted in soybean growth season for summer crop in 1998.

Microclimate measurements

The results of the routine measurements inside and outside the chamber showed relatively little impact upon microclimate (Figures 2 to 5).

Light levels at crop height were, on average reduced by 3.18% inside the chamber, while the mean air temperature inside the chamber was 1.03°C higher than outside. Relative humidity was also 1.13% higher in the chamber (Figures 2 to 5). The reduction in light levels and increase in temperature and relative humidity may be due to polyethylene vertical walls of the chambers and due to the heating effects of the fan and motor. Air temperatures were higher in August to September, and decreased in October and November. While light levels were highest in August and decreased in later months. Relative humidity of the air was maximum in September. In August, the values were also in the higher range. The relative humidity decreased in October and November (Figure-2).

Pollutants monitoring

The daily 6-hour ozone (O_3) and nitrogen (NO_2) dioxide concentration showed drastic differences in filtered air chambers than in unfiltered air chambers and ambient air (Figures 4 and 5). The mean 6-h concentration of O_3 throughout the growing season was 61.24nl liter^{-1} with a mean NO_2 concentration of 31.68nl liter^{-1}. Ozone concentration was low in the month of early August due to monsoon season. During the month of September, when the monsoon season ended the highest values (84.74nl liter^{-1}) for O_3 concentration were recorded. Ozone concentration dropped in the later part of the experiment with a mean concentration of 64.09nl liter^{-1} in October and 52.58nl liter^{-1} in November (Figure-4). This is related to a fall in light and temperature levels during these months (Figure-2).

Filtration efficiencies were almost 90% and 65% overall for O_3 and NO_2 respectively. Ozone concentration in FA chambers did not exceed 8nl liter^{-1}, while NO_2 concentration in FA chambers did not exceed 13nl liter^{-1}. In UFA chambers O_3 and NO_2 concentration were 1-1.5 nl liter^{-1} below the open plot values for the same, (Figures 4 and 5).

Regular assessment of plants' vegetative growth parameters

The plant growth and development along with arbuscular mycorrhizal status showed worthwhile differences in filtered air (FA), unfiltered air (UFA) and ambient air (AA). The data presented in Figure-6 shows weekly increase in **plant height** measured for a period of 13 weeks. The results clearly indicate highly significant enhancement effect of air treatments on plant height in all the soil treatments in FA compared to UFA or AA, (Figure-6). The increase in height was prominent in FA plants right from the seedling phase of growth and continued till the maturation of crop. On the other hand, an early growth depression and slower biomass increment were clearly evident in plants under unfiltered or ambient air. As regards the soil treatments, the plants grown in sterilized soil but inoculated with AM endophytes and *Rhizobium* (SS+Inoc.) showed significant improvement in results for plant height for all three air treatments i.e. FA, UFA and AA. While those grown in sterilized control soil (SS) showed minimum values for plant height parameter.

The increase in height was maximum during the early period of growth. The growth almost stopped in 8th to 9th week of the growth period, (Figure-6). Maximum growth in plant height was recorded in the month of August and it continued till early September (Figure-6). At the end of

the experiment there was an estimated reduction in plant height of up to 47% of UFA and AA sets as compared to FA set.

The results recorded for the **number of leaves** per plant for soybean showed that total number of leaves was significantly high in plants grown in filtered air in all soil treatments (Figure-7). These differences in number of leaves per plant were however maximum when the plants were 9/10 weeks old. For soil treatment results, the trend followed the same lines as for the plant height. Inoculated plants in sterilized soil showed maximum number of leaves in all the three air treatments. Plants grown in sterilized control soil ended up with minimum values for the number of leaves (Figure-7). At the time of final harvest, the plants of all soil treatment sets kept in FA chambers had 48 to 61% greater number of leaves as compared to their counterparts kept in UFA or AA condition.

Number of shoots per plant showed a remarkable increase in FA plants in 3rd week of growth i.e. in August, (Figure-8). In later phases, this increase became slow and steady. The pattern for this growth parameter was almost the same as in the previous cases. Differences in FA plants and UFA/AA plants were highly significant. Maximum shoots were recorded in FA plants than other two situations, which varied insignificantly, (Figure-8). As far as the soil treatments are concerned, the plants grown in sterilized soil but inoculated with arbuscular mycorrhizal and N-fixing organisms (SS+Inoc.) showed appreciably high values than rest of the treatments i.e. unsterilized field soil (UFS) and sterilized control soil (SS). There was a reduction in number of shoots up to 46 to 48% in case of plants kept in UFA and AA chambers as compared to plants of FA treatments at the end of the experiments.

Regular assessment of plants' reproductive growth parameters

Like vegetative growth, reproductive growth of the experimental plants was monitored regularly throughout the growth period, (Figures 3d and 4). Number of flowers per plant and number of pods per plant was also affected in UFA and AA as compared to FA treatment.

It is evident from the Figure-9 that **flowering** started in 3rd week after shifting the pots of experimental soybean plants into open-top chamber system. It started in 2nd week of August. Flowering started at the same time in FA plants in all the soil treatments but it was delayed in UFA and AA plants. In UFA plants flowering was delayed by one week in sterilized soil (SS) control set. In the case of AA plants it was delayed by one week in unsterilized field soil (UFS) and sterilized soil (SS). Number of flowers per plant remained consistently higher in FA plants followed by significant reduction in UFA and AA plants in specific soil treatments. A decline in the number of flowers per plant occurred in 8th week after transferring pots into the chamber system. At this stage FA plants had 30 to 33% more flowers than UFA or AA plants. Flowering stopped earlier in UFA and AA plants as compared to FA plants.

As regards soil treatment, FA plants showed a drop in number of flowers in 4th-5th week. While in UFA and AA plants the trend remained the same as it was for the vegetative parameters i.e. the plants grown in SS+Inoc. soil showed maximum values and SS control plants showed minimum values for the number of flowers per plant (Figure-9).

Pods appeared in experimental plants in 4th week after shifting plants to open-top chamber system in FA plants while in the case of UFA and AA plants ripening started in 5th week (Figure-10). It is clear from the figure that the number of pods per plant was maximum in FA plants through out the maturation phase of the life cycle of plants followed by a remarkable reduction in UFA and AA plants in all soil treatments. Plants from FA chambers grown in sterilized soil inoculated with selected AM endophytes and *Rhizobium* showed highly significant results for the pod number per plant. These highest values were followed by those recorded for FA plants grown in common unsterilized field soil. The FA plants grown in fumigated soil had minimum number of pods amongst filtered air treated plants, (Figure-10). The plants grown in UFA and AA showed overlapping values but in both the cases, values were maximum for soil treatment # 1 i.e. sterilized inoculated soil. At the end of the study a reduction in pod number of up to 44% was recorded in UFA and AA plants.

Mid-term harvest

First harvest was taken on 2nd of September. This was the prime period of vegetative growth. The data for plant growth parameters, nodulation, rate of N-fixation, arbuscular mycorrhizal status and density and diversity of endogonaceous spore populations in the rhizosphere soil was recorded at the time of first harvest.

Shoot and root length

Mid season harvest was taken when the plants had almost completed their vegetative growth i.e. 6 weeks old plants. The results are summarized in Table-1 and Figure-11 clearly shows the effects of air filtration on shoot length of soybean in various soil treatments. Plants grown in FA treatment were healthier with lush green leaves as compared to their UFA and AA counterparts. Table-1 shows that FA treated plants had significantly greater root (52-60%) and shoot (48-52%) length, (Figure-11). While values for UFA and AA plants varied insignificantly. As far as soil treatments are related, the fumigated sterilized soil given the inoculation of microbes ended up with promising results. While the sterilized control had minimum values. Root length figures were significantly high for FA plants grown in sterilized inoculated soil than in any other air or soil treatment, (Figure-11).

Plant biomass studies

Figure-11 shows the effect of air and soil treatments on **fresh and dry weights** of experimental plants. Total fresh weight of plants was highest in FA

plants followed by a significant reduction of up to 47% in UFA and AA treated plants. There was a significant difference as regards various soil treatments in FA and UFA plants for fresh weight parameter, while for AA plants soil treatments varied insignificantly, (Fig. 5). Sterilized soil with inoculation showed maximum values in this respect than its other two counter parts. Shoot fresh weights were higher than those of root. Roots and shoot fresh weights when recorded separately also followed the same lines as did total plant weight. The values for fresh weight of root and shoots were significantly high for plants kept in filtered air (FA) followed by UFA and AA counterparts. Plant's dry weight to fresh weight ratio was almost 1:5. The values for plant dry weight were significantly high for FA plants in three soil treatments, (Figure-11). For UFA plants, the dry weight values reduced to almost more then half (62%) in contrast to FA treated plants. However, these show overlapping with AA results. In the case of UFA and AA plants, the soil treatment # 2 and 3 showed insignificantly different results. The sterilized soil supplied with microbial inoculation showed worth mentioning results following the basic trend of the experiment (Figure-11).

Arbuscular mycorrhizal studies

The growth and spread of arbuscular mycorrhizal hyphae, formation of arbuscules and vesicles, their density in root system and occurrence of other pathogenic fungi was recorded at the time of mid-harvest stage. The number and types of endogonaceous spores was recorded at the time of mid-term harvest and final harvest (Figures 12 and 13).

Arbuscular mycorrhizal hyphal spread was almost up to 100% in FA Plants than their UFA and AA counterparts, (Figure-12). No infections could be observed in plants grown in sterilized soil control in all air treatments. The soil, which was sterilized and inoculated with AM & *Rhizobium*, showed significantly high values. For AA plants however, the results for the soil treatment overlapped and the values were significantly greater in the case of unsterilized soil than sterilized inoculated soil. Percentage of vesicles was significantly high in FA plants in all soil treatments (Figure-12). There were significant variations as regards soil treatments. Maximum infections were recorded in plants growing in sterilized but inoculated soil. For plants growing in common field soil in unsterilized conditions, the values were not very much different but were significantly high than their counterparts growing in sterilized inoculated soil (Figure-12). Percentage of arbuscular infections showed significant reductions in UFA and AA treated plants than FA plants (Figure-12). Soil treatments ended up with a remarkable difference in the establishment of arbuscular infections. Maximum percentage of arbuscules was recorded in sterilized inoculated soil followed by the values for unsterilized field soil. The values were very slightly different but were significantly high as regards air treatments, maximum being for FA treatments (Figure-12).

As regards the percentage of occurrence of extramatrical mycelial infections, the FA treatment provided a conducive environment for the proper spread of fungal hyphae. The figures for FA plants had highly significant values (47.67%) for the parameter as compares to UFA and AA plants, (Figure-12, Table-3). However, plants grown in soil, which was fumigated and inoculated with AM endophytes and *Rhizobium*, had worthwhile differences than the field soil, which was used without sterilization and with the same indigenous micro-flora and micro-fauna. The soil, which served as sterilized control did not show up any infections as regards AM. The results for the number of arbuscules and number of vesicles per unit length of root system of experimental plants followed the same lines as were seen in the previous cases. Values were significantly high (79.33 and 89%, respectively) for both the parameters in FA treated plants than the other two treatments i.e. UFA and AA. The values were maximum for plants grown in soil treatment # 1, followed by figures for plants grown in soil treatment #2 and 3. The figures for extent of AM mycelium actually indicated the real spread of infections. There was a significant difference in between all air treatments in various soil treatments. Results were promising for FA plants (815.92μm/cm of root) than UFA (617.75μm/cm and AA (309.32μm/cm) plants (Table-3). The air treatments having polluting gases (UFA) inhibited the ramification of the hyphae of these symbiotic fungi in the root system of their host plant. While the air having polluting gases along with dust particles (AA) caused further inhibition (Table-3). The mycelia of some fungi other than AM were observed in the case of unsterilized soil. The frequency of occurrence of the non-mycorrhizal fungi was significantly greater (65%) in roots of plants kept in open plots while filtered air reduced the incidence of occurrence (29.67%) of these fungi in the root system of soybean plants (Table-3).

The results revealed that the **total number of spores** per 100g of rhizosphere soil was maximum (715 spores/100g soil) in filtered air (FA) at the time of first harvest (Figure-13). The number was greatly reduced in plants of the other two air treatments. Minimum (250 spores/100g soil) in this respect was noticed for ambient air (AA). As far as the number of spores in various soil treatments is concerned, it was maximum in the case of plants which were grown in fumigated soil which was later on inoculated with AM and N-fixing bacteria. These values were followed by those for common unsterilized field soil. The aseptic conditions reduced the number of spores considerably in all three air treatments. However, the difference was slightly more pronounced in FA plants than UFA and AA plants (Figure-13). The soil which was left as such after fumigation (SS soil treatment), served as total control. Spores were totally lacking in this soil treatment.

When the spores isolated were identified, the data revealed that only two types of spores were present in the rhizosphere soil of the experimental soybean plants which were grown in fumigated soil supplemented with the inocula of arbuscular mycorrhizal roots and *Rhizobium*

(Tables 1 and 2). The spores belonged to two species of *Glomus* i.e. *G. mosseae* and *G. fasciculatum*. These were the species with which the maize roots (used as inoculum) were infected. While on the whole, a total of twenty nine (29) species of endogonaceous spores were recovered from the rhizosphere soil of experimental plants including first and second harvest (Table-2). This total included three (3) species of *Acaulospora*, one (1) species of *Gigaspora*, twenty one (21) species of *Glomus*, three (3) species of *Sclerocystis* and one (1) species of *Scutellospora*. Out of these twenty nine species, a total of twenty one species of AM spores were present in the rhizosphere soil of FA plants (Table-2). This species composition *Acaulospora bireticulata, Glomus aggregatum, G. cerebriforme, G. clarum, G. constrictum, G. delhiense, G. deserticola, G. dimorphicum, G. fasciculatum, G. facundisporum, G. geosporum, G. halonatum, G. intraradices, G. microaggregatum, G. microcarpum, G. monosporum, G. mosseae, G. multicaule, G. tenue, Sclerocystis microcarpus,* and *S. pakistanica)* was dominated by species of *Glomus* while a couple of other species were also present This number decreased in soil of unfiltered plants but the species composition (*Acaulospora bireticulata, Glomus aggregatum, G. clarum, G. fasciculatum, G. microaggregatum, G. microcarpum, G. monosporum, G. mosseae,* and *Sclerocystis microcarpus)* in this air treatment was also dominated by *Glomus* spp.It further reduced in AA plants, *(Glomus aggregatum, G. clarum, G. fasciculatum, G. microaggregatum, G. microcarpum, G. mosseae,* and *Sclerocystis microcarpus).* The difference in number of species of AM fungi between UFA and AA plants was insignificant. The sterilized soil did not have any spores (Table-2).

Nodulation and N-fixation studies

As nodulation and N-fixation studies are concerned various parameter recorded in this respect were, number of nodules per plant, fresh weight of nodules per plant and oven dry weight of nodules per plant. For the assessment of N-fixation acetylene reduction test was also performed for all treatments (Figure-14). The nodule formation was maximum in filtered air plants of soybean (Figure-14). These significantly high values were recorded in plants grown in soil treatment # 1. The fumigated soil inoculated with AM and *Rhizobium* created a remarkable difference. The values for nodule number in UFA and AA plant showed a considerable reduction than FA plants. The values however were close and differed insignificantly in UFA and AA plants. Nodules of FA treated plants were bigger in size as is evident from the data on fresh and dry weight of nodules (Figure-14). Fresh and oven dry weight of nodules per soybean plant showed highly promising results for plants kept in filtered air and the soil used was the fumigated and inoculated one. The inoculum of *Rhizobium* was well suited to the soil and host thus showing significant figures in air treatment, which was free of pollutant gases and dust particles. These highest values were followed by the readings for UFA and AA Plants. Plants treated with UFA had slightly better results than AA plants. The soil which served as sterilized control completely inhibited nodule formation in experimental soybean plants. While nodule formation was scanty in the case of common unsterilized field soil (Figure-14). As far as nitrogenous activity in nodules of experimental plants is concerned, an extremely high difference was observed for FA plants as compared to their UFA/AA counterparts (Figure-14). The amount of acetylene reduced per gram weight of nodules per hour was highly significant. The sterilized inoculated soil showed best results for this parameter (Figure-14).

Arbuscular mycorrhizal structures were also studied in the nodules of soybean plants. Only mycelial hyphae and vesicles were observed in the nodule tissue after staining with trypan blue and microscopic observation (Table-3). The results followed the same lines as in the previous cases. Values being highly significant for plants kept in filtered air than other two air treatments. Values were high for sterilized inoculated soil than for common field soil. The soil, (SS), free of microbial populations served as total control. Number of vesicles per $100\mu m^2$ was also maximum for FA plants than UFA and AA plants.

Final harvest

Final harvest was taken at the end of growth period when the plants were mature. It was taken in first week of November. At this time, yield analysis was performed along with the isolation of AM spores in the rhizosphere soil.

Arbuscular mycorrhizal spore counts and identification

Number of AM spores per 100g of the rhizosphere soil was recorded in all the soil and air treatments. The data revealed that a total of twenty nine (29) species (*Acaulospora bireticulata, A. foveata, A. rehmii, Gigaspora decipiens, Glomus aggregatum, G. albidum, G. caledonium, G. cerebriforme, G. clarum, G. constrictum, G. delhiense, G. deserticola, G. dimorphicum, G. fasciculatum, G. facundisporum, G. geosporum, G. halonatum, G. intraradices, G. leptotichum, G. microaggregatum, G. microcarpum, G. monosporum, G. mosseae, G. multicaule, G. tenue, Sclerocystis microcarpus, S. pakistanica, S. sinusa* and *Scutellospora pellucida)* were present in FA plants grown in fumigates and inoculated soil (Table-2). While UFA plants in the same soil treatment had fifteen species (*Acaulospora bireticulata, Glomus aggregatum, G. cerebriforme G. clarum, G. deserticola, G. fasciculatum, G. geosporum, G. halonatum, G. microaggregatum, G. microcarpum, G. monosporum, G. mosseae, G. multicaule, G. tenue* and *Sclerocystis microcarpus)* of AM spores. The plants of AA treatment twelve species (*Acaulospora bireticulata, Glomus aggregatum, G. cerebriforme, G. clarum, G. deserticola, G. fasciculatum, G. geosporum, G. microaggregatum, G. microcarpum, G. monosporum, G. mosseae,* and *Sclerocystis microcarpus)* of AM spores in

the rhizosphere soil (Table-2). The FA plants grown in fumigated & inoculated soil had only two types of spores i.e. *Glomus fasciculatum* and *G. mosseae*.

The number of spores per 100g soil increased at the time of final harvest. The trend however remained the same (Figure-13, Table-2). Highest number was recorded for FA plants in sterilized inoculated soil followed by UFA and AA sets in the same soil. In unsterilized field soil, the number per 100g soil was less although there was significant variety of spores in this soil treatment.

Yield analysis

Yield meters were recorded to analyze the effect of air treatments on plant productivity. The parameters recorded were number of pods per plant, pod length, number of seeds per pod, number of seeds per plant, weight of seeds per plant and 1000 seed weight.

The set of soybean plants which was kept in filtered air chambers, showed maximum productivity, (Table-4). The soil treatment # 1, which is the soil sterilized by fumigation and inoculated with AM and nodulating bacteria, gave promising results. The number of pods per plant reduced to almost half in UFA plants. Further reduction in yield was observed in AA treated plants. The FA plants grown in sterilized inoculated soil yielded worthwhile results as compared to those of FA sets, which were raised in common unsterilized field soil, (UFS) or sterilized control soil, (SS). The same situation prevailed for UFA and AA plants as regards soil treatments.

The pods were healthier and greater number of seeds per pod was recorded in plants of FA set in same soil treatment as for the previous parameter. There was a significant reduction in pod length and number of seeds per pod in other two air treatments. In the case of soil treatment the results followed the same trend i.e. values being highest for plants grown in fumigated and inoculated soil. The above mentioned three parameters lead to greater number of seeds per plant, greater weight of seeds per plant and greater weight of 1000 seeds in plants of filtered air treatment. These were followed by values for UFA/AA plants. In some cases, the figures for UFA and AA plants differed insignificantly. For soil treatments, the minimum values were recorded for sterilized control soil and maximum for sterilized inoculated soil (Table-4).

The harvest index calculated indicated a substantial difference between FA plants and the other two sets (Figure-15).

Table-1. List of Endogonaceous fungi isolated from potted plants of soybean grown in open top chambers.

S. #	Species of AM Endophytes	S. #	Species of AM Endophytes
1.	*Acaulospora bireticulata* Rothwell &	15.	*G. facundisporum* Schenck & Smith &
2.	Trappe	16.	Sanders
3.	*A. foveata* Trappe & Janos	17.	*G. geosporum* (Nicol. & Gerd.) Walker
4.	*A. rehmii* Sieverding & Toro	18.	*G. halonatum* Ross & Trappe
5.	*Gigaspora decipiens* Hall & Abbott	19.	*G. intraradices* Schenck & Smith
	Glomus aggregatum Schenck &	20.	*G. leptotichum* Schenck & Smith
6.	Smith emend. Koske	21.	*G. microaggregatum* Koske, Gemma &
7.	*G. albidum* Walker & Rhodes	22.	Olexia
	G. caledonium (Nicol. & Gerd.)	23.	*G. microcarpum* Tul. & Tul.
8.	Trappe & Gerd.	24.	*G. monosporum* Gerd. & Trappe
9.	*G. cerebriforme* McGee	25.	*G. mosseae* (Nicol. & Gerd.) Gerd. Trappe
10.	*G. clarum* Nicol. & Schenck	26.	*G. multicaule* Gerd. & Bakshi
11.	*G. constrictum* Trappe	27.	*G. tenue* (Greenall) Hall
	G. delhiense Mukerjee,	28.	*Sclerocystis microcarpus* Iqbal & Bushra
12.	Bhatacharjee & Tewari	29.	*S. pakistanica* Iqbal & Bushra
13.	*G. deserticola* Trappe, Bloss and Tewari		*S. sinusa* Gerd. & Bakshi
14.	*G. dimorphicum* Boyetchko & Tewari		*Scutellospora pellucida* (Nicol. & Schenck)
	G. fasciculatum (Thaxter) Gerd. &		Walker & Sanders
	Trappe emend. Walker & Koske		

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Table-2. Effect of air filtration on species of chlamydospores of arbuscular mycorrhizal fungi (number per 100g rhizosphere soil) of soybean plants at the time of mid-season and final harvest stage in various air and soil treatments.

Harvest	Air treatment	Soil treatment		
		SS+Inoc.	UFS	SS
Harvest-I	**FA**	*Glomus fasciculatum* and *G. mosseae*	**21 Spore types of AM recovered** *Acaulospora bireticulata, Glomus aggregatum, G. cerebriforme, G. clarum, G. constrictum, G. delhiense, G. deserticola, G. dimorphicum, G. fasciculatum, G. facundisporum, G. geosporum, G. halonatum, G. intraradices, G. microaggregatum, G. microcarpum, G. monosporum, G. mosseae, G. multicaule, G. tenue, Sclerocystis microcarpus,* and *S. pakistanica*	
	UFA	*Glomus fasciculatum* and *G. mosseae*	**9 Spore types of AM recovered** *Acaulospora bireticulata, Glomus aggregatum, G. clarum, G. fasciculatum, G. microaggregatum, G. microcarpum, G. monosporum, G. mosseae,* and *Sclerocystis microcarpus*	
	AA	*Glomus fasciculatum* and *G. mosseae*	**7 Spore types of AM recovered** *Glomus aggregatum, G. clarum, G. fasciculatum, G. microaggregatum, G. microcarpum, G. mosseae,* and *Sclerocystis microcarpus*	
Harvest-II	**FA**	*Glomus fasciculatum* and *G. mosseae*	**29 Spore types of AM recovered** *Acaulospora bireticulata, A. foveata ,A. rehmii, Gigaspora decipiens, Glomus aggregatum, G. albidum, G. caledonium, G. cerebriforme, G. clarum, G. constrictum, G. delhiense, G. deserticola, G. dimorphicum, G. fasciculatum, G. facundisporum, G. geosporum, G. halonatum, G. intraradices, G. leptotichum, G. microaggregatum, G. microcarpum, G. monosporum, G. mosseae, G. multicaule, G. tenue, Sclerocystis microcarpus, S. pakistanica, S. sinusa* and *Scutellospora pellucida*	
	UFA	*Glomus fasciculatum* and *G. mosseae*	**15 Spore types of AM recovered** *Acaulospora bireticulata, Glomus aggregatum, G. cerebriforme G. clarum, G. deserticola, G. fasciculatum, G. geosporum, G. halonatum, G. microaggregatum, G. microcarpum, G. monosporum, G. mosseae, G. multicaule, G. tenue* and *Sclerocystis microcarpus*	
	AA	*Glomus fasciculatum* and *G. mosseae*	**12 Spore types of AM recovered** *Acaulospora bireticulata, Glomus aggregatum, G. cerebriforme, G. clarum, G. deserticola, G. fasciculatum, G. geosporum, G. microaggregatum, G. microcarpum, G. monosporum, G. mosseae,* and *Sclerocystis microcarpus.*	

* AM spores not found.

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Table-3. Effect of air filtration on AM status of nodules on root systems of soybean plants at the time of mid-harvest stage in various air and soil treatments.

S. #	Parameter studied	Air treatment	Soil treatment		
			SS+Inoc.	UFS	SS
1.	**General mycelium** (%)	FA	73.33*a*±1.67	58.00*a*±1.55	00.00
		UFA	44.67*b*±0.88	33.33*b*±0.88	00.00
		AA	30.33*c*±0.88	25.65*c*±1.20	00.00
2.	**Vesicles** (%)	FA	67.67*a*±2.19	42.67*a*±1.20	00.00
		UFA	28.33*b*±0.88	18.00*b*±1.15	00.00
		AA	18.33*c*±1.45	9.67*c*±0.45	00.00
3.	**Number of vesicles per 100µm²**	FA	19.90*a*±0.21	11.31*a*±0.16	00.00
		UFA	3.55*b*±0.03	2.16*b*±0.03	00.00
		AA	2.09*b*±0.01	2.04*b*±0.02	00.00

Treatment means ± S.E. followed by different letters in the same column differ significantly from one another at P < 0.05 according to DNMRT.

Table-4. Effect of air filtration on yield of soybean plants at the time of final harvest stage in various air and soil treatments.

S. #	Parameter studied	Air treatment	Soil treatment		
			SS+Inoc.	UFS	SS
1.	Number of pods per plant	FA	39.33*a*±0.88	31.67*a*±0.88	29.00*a*±0.48
		UFA	22.00*b*±0.58	19.33*b*±0.33	17.33*b*±.23
		AA	19.67*c*±0.33	17.00*c*±0.58	16.67*c*±0.33
2.	Pod length (cm)	FA	3.96*a*±0.03	3.85*a*±0.02	3.75*a*±0.02
		UFA	3.33*b*±0.01	3.19*b*±.01	3.09*b*±0.01
		AA	3.20*b*±0.01	3.10*b*±0.01	3.02*b*±0.01
3.	Number of seeds per pod	FA	3.33*a*±0.33	2.90*a*±0.06	2.70*a*±0.10
		UFA	2.77*b*±0.23	2.70*b*±0.11	2.63*b*±0.20
		AA	2.43*c*±0.25	2.1*c*±0.06	1.90*c*±0.12
4.	Number of seeds per plant	FA	81.67*a*±1.66	72.67*a*±1.45	58.33*a*±0.88
		UFA	43.33*b*±2.01	41.27*b*±0.41	38.20*b*±0.55
		AA	36.67*c*±1.20	32.97*c*±1.02	29.33*c*±0.38
5.	Weight of seeds per plant	FA	23.67*a*±0.88	19.80*a*±0.12	16.67*a*±0.67
		UFA	12.70*b*±0.67	10.30*b*±0.36	8.76*b*±0.23
		AA	10.36*c*±0.33	9.10*c*±0.12	7.90*b*±0.25
6.	1000 seed weight (g)	FA	215.33*a*±2.91	192.33*a*±2.85	180.33*a*±1.45
		UFA	176.00*b*±3.06	156.67*b*±2.33	144.33*b*±2.96
		AA	144.67*c*±2.60	128.00*c*±1.53	115.67*c*±2.33

Treatment means ± S.E. followed by different letters in the same column differ significantly from one another at P < 0.05 according to DNMRT.

DISCUSSION

In the present investigation, absolutely new dimensions in the research of AM fungi have been discussed. The impact of air pollution on plants has been proved by a series of researches in the past (Cairney & Meharg, 1999). The positive role of arbuscular mycorrhizae in alleviating the effect of soil and sediment pollution has been shown variously in the literature (Oliveira *et al.*, 2001; Berreck & Haselwandter, 2001; Khan *et al.*, 2000; Chaudhry *et al.*, 1998; Leyval *et al.*, 1997). However, very scanty information is available in the literature related to the effect of polluted air on microbes especially AM fungi associated with roots of the plants. The influence of anthropogenically produced ozone, nitrogen dioxide and particulate matter on soybean plants and their associated microflora has been a primary focus of this study. Comprehensive study was conducted focusing the issue and the results obtained were highly promising. The gaseous pollutants have been shown to reduce plant growth, yield and mycorrhizal development. This is inline with the studies of Reich *et al.*, (1988) and Ho & Trappe (1984).

The present study was carried out in an open-top chamber system. This system has been found to be an excellent technique to assess the impact of ambient air pollution on growth and yield performance of wheat, rice and mungbean, (Wahid *et al.*, 1995a, 1995b; Nasim *et al.*, 1995; Bajwa *et al.*, 1997) in Pakistan. Very few studies have however been done on the impact of air pollution on vesicular arbuscular mycorrhizal status of test plants along

with the study of growth parameters. Only a couple of investigations have been done on arbuscular mycorrhiza of wheat and mungbean in the same set up, (Nasim *et al.*, 1995; Bajwa *et al.*, 1997). According to Tiaz and Zeiger (2006), experiments aimed at determining the impact of chronic exposures to low concentration of gases should allow plants to grow under near-natural conditions. One method is to grow plants in open-top chambers into which the gases are carefully metered, or where plants receiving ambient polluted air are compared with controls receiving air that have been scrubbed of pollutants,

In the present study, results have shown that the climate inside the chambers hardly differed from that prevailing outside the chamber through out the season. This may be due to complete open-top design of the chamber. The chambers (FA & UFA) were connected with motor blower system to filter dust particles. While FA chamber has an additional filter set up to filter ozone (O_3) and nitrogen dioxide (NO_2). The filters for gases showed high filtration efficiency through out the season. Another reason for the maintenance of pollution free environment inside FA chamber may be that, the conditions were not very windy at the experimental site resulting into a reduced ingress of outside air.

In the present study as it was conducted in open top chamber system rather than being a fumigation experiment, the levels of gaseous pollutants like O_3 and NO_2 were not regulated. However daily monitoring was done which showed a concentration of around 5-8 ppb of O_3 in FA and up to 80 ppb in UFA chambers and ambient air. Similarly, for NO_2 10-12 ppb in FA chambers and up to 40-45 ppb in UFA chambers and ambient air. Thus there were two drastically different sets of environmental conditions which ultimately affected plant growth, yield and mycorrhizal development. Besides O_3, NO_2 and other which were not measured on site like SO_2, NH_3, hydrogen peroxide (H_2O_2) and peroxyacetyle nitrate cannot be excluded (Wahid *et al.*, 1995a and 1995b).

The results of the present study show that various growth parameters related to vegetative growth such as plant height, number of leaves per plant, number of shoots per plant significantly increased in FA sets as compared to UFA and AA sets of plants. The differences became more pronounced in later phases of growth. The concentration of polluting gases, or their solutions, to which the plants were exposed were highly variable, depending on wind direction, rainfall, and sunlight. Polluting gases, such as SO_2 and NO_2 enter leaves through stomata, following the same diffusion pathway as CO_2. NO_x dissolves in cells and gives rise to nitrite ions (NO_2-, which are toxic at high concentrations) and nitrate ions (NO_3^-) that enter into nitrogen metabolism as if they have been absorbed through the roots. In some cases, exposure to pollutant gases, particularly SO_2, causes stomatal closure, which protects the leaf against further entry of the pollutant but also curtails photosynthesis. In the cells, SO_2 dissolves to give bisulfite and sulfite ions; sulfite is toxic, but at low concentration it is metabolized by chloroplast to sulfate, which is not toxic. At sufficiently low concentration, bisulfite and sulfite are effectively detoxified by plants, and SO_2 air pollution then provides a sulfur source for the plant. Dissolution of NO_x and SO_2 in water droplets in the atmosphere causes the pH of the rain to decrease to 3 to 4 or some times up to 1.7 as compared to unpolluted rain, which has pH in the range of 5.6. This H^+ addition into the soil in the form of wet and dry deposition can result in the release of ammonium ions from soil minerals, causing aluminum toxicity. Air pollution is considered to be a major factor in the decline of forests in heavily polluted areas of Europe and North America (Tiaz and Zeiger, 2006). In urban areas, these polluting gases may be present in such a high concentration that they cannot be detoxified rapidly enough to avoid injury. Ozone is presently considered to be the most damaging air pollutant in North America (Heagle 1989; Krupa *et al.*, 1995). It has been estimated that where ever the mean daily O_3 concentration reaches 40, 50, or 60 ppb (parts per billion, or per 10^9), the combined yields of soybean, maize, winter wheat, and cotton would be decreased by 5, 10, and 16% respectively.

Ozone is highly reactive and binds to plasma membrane and it alters metabolism. As a result, stomatal apertures are poorly regulated, chloroplast thylakoid membranes are damaged, rubisco is degraded, and photosynthesis is inhibited. Ozone reacts with O_2 and produces reactive O_2 species, including hydrogen peroxide (H_2O_2), superoxide (O_2^-), singlet oxygen ($^1O_2$*), and the hydroxyl radicle (.OH). The denatured proteins, damage nucleic acids and thereby give rise to mutations, and cause lipid peroxidation, which breaks down lipids in membranes. Many deleterious changes in metabolism caused by air pollution precede external symptoms of injury, which appear only at much higher concentrations. For example, when plants are exposed to air containing NO_x, lesions on leaves appear at an NO_x concentration of 5ml L^{-1}, but photosynthesis starts to be inhibited at a concentration of only 0.1ml L^{-1}. These low, threshold concentration refer to the effects of single pollutant. However, two or more pollutants acting together can have a synergistic effect, produce damage at lower concentrations than if they were acting separately. In addition, vegetation weakened by air pollution can become more susceptible to invasion by pathogens and pests (Moldau, 1999).

The results of the present study also show that both fresh and dry weights of FA plants were higher than their UFA and AA counterparts. This may be considered as a reflection of more vigorous growth of FA plants through out the experimental period. These results are also inline with the previous studies carried out in North America (Kats *et al.*, 1985; Amundson *et al.*, 1987), Europe (Adaros *et al.*, 1991 ; Bender *et al.*, 1991; Pleijel, *et al.*, 1991), Japan (Nouchi *et al.*, 1995) and Pakistan, (Wahid *et al.*, 1995a, 1995 b). The difference in growth between UFA and AA plants may be considered due to dust particles. Although dust pollution is of localized importance near roads, quarries, cement works, and other industrial areas. Apart from screening out sunlight, dust on leaves blocks stomata and lowers their conductance to

CO_2 simultaneously interfering with photosystem II, (Taiz & Zeiger, 2006).

In the present study, the results presented for soybean are quite in line with those obtained for wheat by Wahid *et al*., (1995a and 1995b) Nasim *et al*.,(1995) and Nasim and Bajwa (2007) and for Mungbean by Bajwa *et al*, 1997 and Nasim and Bajwa, (2007) particularly in terms of yield parameters. This is consistent with the observations by Heagle *et al.,* (1979), Mulchi *et al.,* (1988), Pleijel *et al*., (1991), Fuhrer *et al.,* (1997) and Wahid *et al.,* (1995a & 1995b). However, the results cannot be compared with those of the fumigation studies (Wahid *et al.,* 1995a & 1995b). Further more the pants in the present study were grown in pots which also makes the comparison with other work (conducted in the field) difficult. It has been indicate by Skarby *et al.,* (1998) that pot-grown plants are less sensitive to O_3 than those in the field, possibly due to reduced nutrients and water status. Thus, it can be concluded that pollutants has reduced plant growth of mycorrhizal plants but as Ho and Trappe (1984) have said that the growth reduction is even greater in non-mycorrhizal plants.

Air filtration also resulted in better reproductive growth in plants of FA treatment. Significantly high flower production by FA plants ended up with appreciably high yields than plants of other air treatments, (Mathy, 1993; Wahid et al., 1995a, 1995b). Similarly, a very positive trend was noticed in nodulation and N-fixation ability of soybean plants in filtered air. In the case of soybean, the number of nodules per plant, fresh weight of nodule per plant, oven dry weight of nodules per plant and rate of N-fixation was significantly high in FA plants following a reduction in UFA and AA plants.

Earlier studies have shown reduced plant growth and biomass in soybean cultivars due to air pollution, Sabaratnum & Gupta, 1988; Kohut *et al.,* 1986; Heggestad and Lee, 1990; Mulchi *et al.,* 1988). However, there is only a few research papers as yet regarding the interaction between *Glomus geosporum* (a AM forming fungus) and exposure of soybean to ozone, (Brewer and Heagle 1983, Nasim and Bajwa 2007). There are indeed very few studies of interactions/ or involvement of fungi in pollution studies, (Nasim *et al.,* 1995; Bajwa *et al.,* 1997; Krupa *et al.,* 1995). The present study was therefore undertaken to review the effects of the gaseous pollutants in causing reduction not only in plant growth and yield but also affecting the development of vesicular arbuscular mycorrhizal association in roots of soybean. This study is in line with those of Brewer and Heagle (1983), Reich *et al.,* (1988), Ho and Trappe (1984) and Kraigher *et al.,* (1996). In the case of ectomycorrhizae (EM) there are evidences of a decline in the frequency of EM forming fungi in Netherlands associated with pollutants like sulfurdioxide (SO_2) and ammonia, (Termorshuzen and Schaffers, 1987). There is also one report by Shaw *et al.,* (1992), of reduced EM formation in conifers in increased SO_2 and O_3.

Human activities are changing the environment in which mycorrhizal roots function on a global as well as on local scale. Mycorrhizal associations being largely dependent on the photosynthates of the host may indirectly be affected by reduced rates of photosynthesis, (Graham *et al.,* 1982). According to the study of Graham, *et al.,* (1982), reduced photon irradiances adversely affected the frequency of arbuscules and vesicles formed by arbuscular mycorrhizal fungi. In the case of ambient air pollution, O_3 inhibits the rate of photosynthesis ending up with chlorosis and necrosis of leaves (Heath *et al.,* 1982; Heath & Tylor, 1997; Heath, 1988). These indirect effects of pollutant in reducing photosynthesis and hence carbon allocation to the root system may also inhibit mycorrhizal development. This finally results in reduced growth and yield of plants.

The study however showed that mycorrhizal plants had significantly high values for growth and yield parameters than non-mycorrhizal plants in all air treatments. The difference was clear between sterilized soil (SS) and sterilized inoculated soil (SS+Inoc.). The over lapping values of SS soil and common field soil (UFS) used without sterilization may be due to interaction of a variety of AM fungi in the soil which usually results in reduced growth of host plant, (Nasim & Zahoor, 1996).

There is an apparent lack of host specificity among AM fungi but some of these may be associated with a certain host plant, (Fox and Spasoff, 1971). Most taxa of AM fungi forming spores are in *Glomus* (Morton, 1988). The existence of *Glomus* as the dominant genus in the root zone indicates either the influence of the soil or the plant type, (Schenck and Kinloch, 1980, Nasim & Iqbal, 1991). The present study is also in line with the previous investigations indicating the presence of *Glomus* as a dominant and most resistant genus. The species, which were sensitive to air pollution, disappeared while the resistant species remained there and sporulated. Among sensitive species were *Acaulospora foveata*, *A. rehmii*, *Gigaspora decipiens*, *Glomus albidum*, *G. caledonium*, *G. constrictum*, *G. delhiense*, *G. dimorphicum*, *G. facundisporum*, *G. intraradices*, *G. leptotichum*, *S. pakistanica*, *S. sinusa* and *Scutellospora pellucida*. The absence of spores of these AM species from normal mycofloral composition in the rhizosphere of soybean may be considered as an alarming sign indicating the pollution environment. This attempt is in line with the study of Kraigher *et al.,* (1996) which they carried out for ectomycorrhiza forming fungi.

The study can be concluded by stating that the test plants like soybean is typically mycotrophic and also respond strikingly to the air treatments. The results have also shown that the pollutants reduced plant growth of mycorrhizal plants but growth reduction was even greater in non-mycorrhizal plants confirming the inferences made by Ho and Trappe, (1984) and Brewer and Heagle (1983). Relatively long-term experiments at appropriate concentration of pollutants are necessary to establish the real impact of air pollution on vegetation. The reactions of test plants to high concentration of pollutants in short-term experiments may overwhelm the plant's defense mechanism and may provoke abnormal symptoms.

As air pollutant levels are likely to continue to rise over the coming decades in many developing countries including Pakistan, therefore further investigations are immediately needed to screen pollution tolerant/resistant mycorrhizal species for all the important crop plants. It is also needed to evaluate the possible role of mycorrhiza in inducing resistance in plants to air pollution in developing countries like Pakistan.

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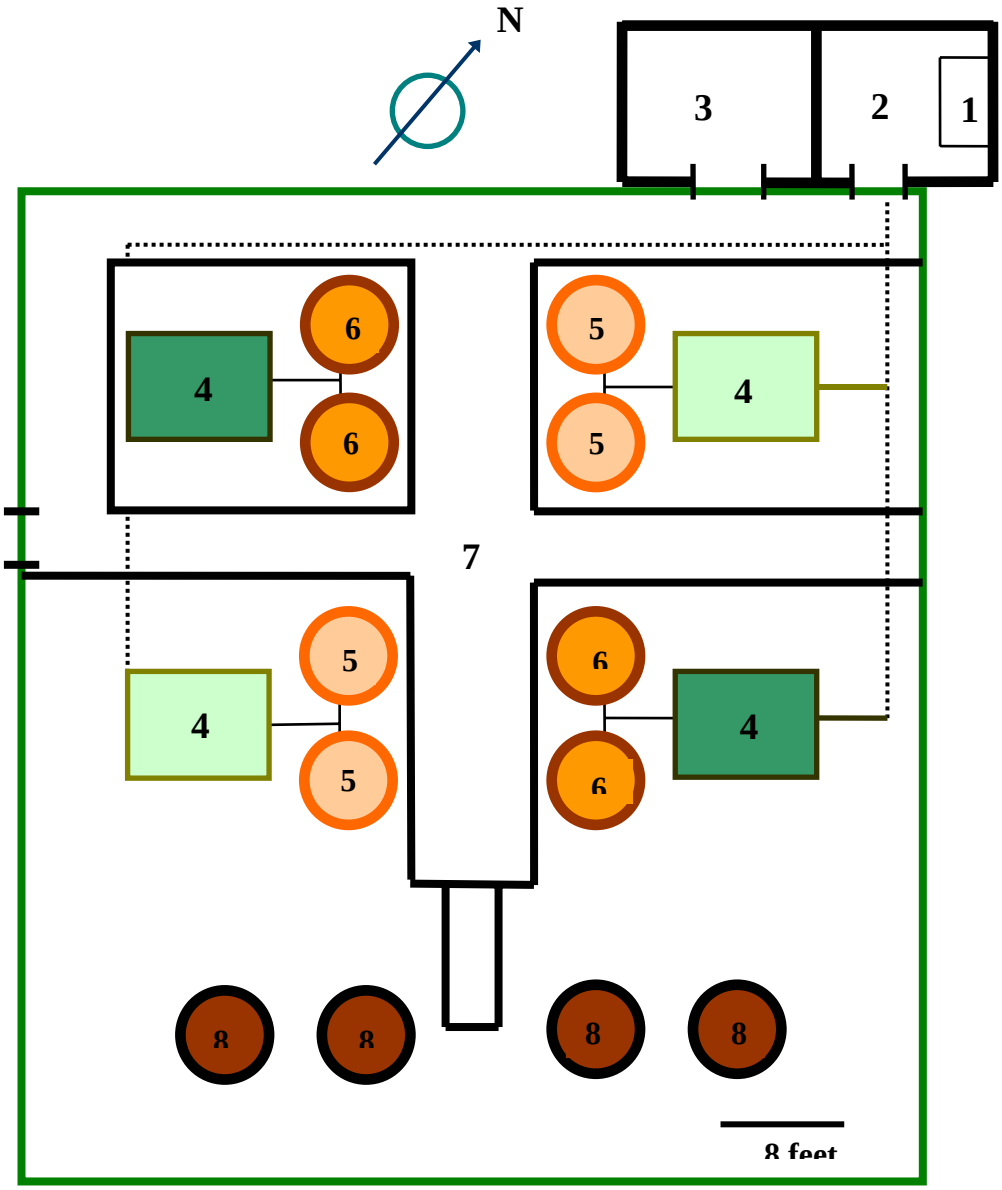

Key:
1. Electricity mains
2. Control room
3. Storage shed
4. Platform
5. Filtered air chambers
6. Unfiltered air chambers
7. Brick pavement
8. Un-chambered field plots

-- Electricity cable

▬ Security fence

Figure-1. General layout of site for open top chamber system at Botanical Garden, University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan.

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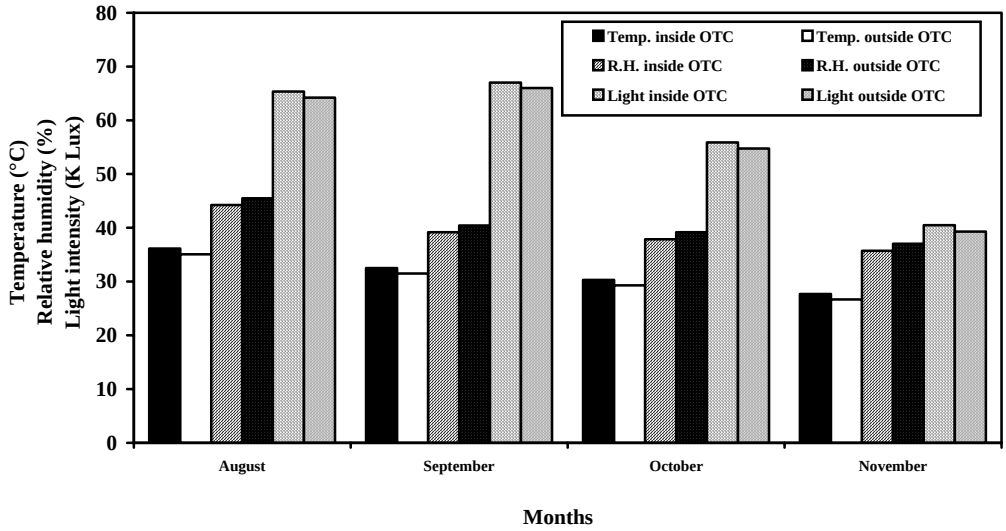

Figure-2. Mean monthly temperature, relative humidity and light intensity inside and outside open top-chambers during 1998 soybean season.

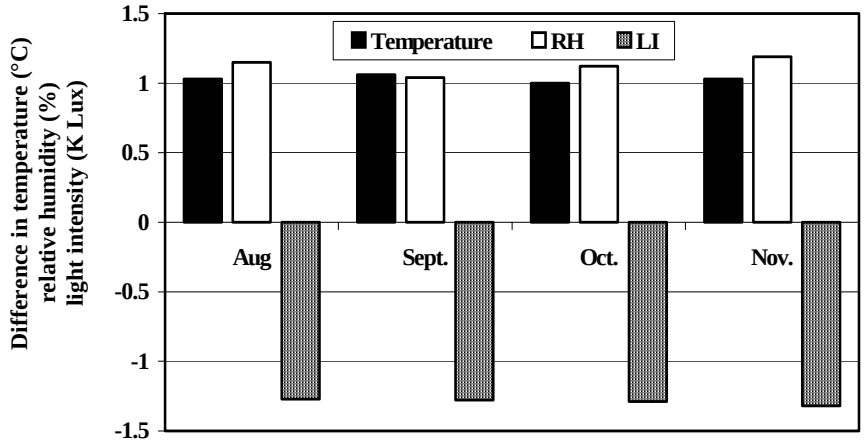

Figure-3. Mean monthly microclimatic differences inside and outside open top chambers.

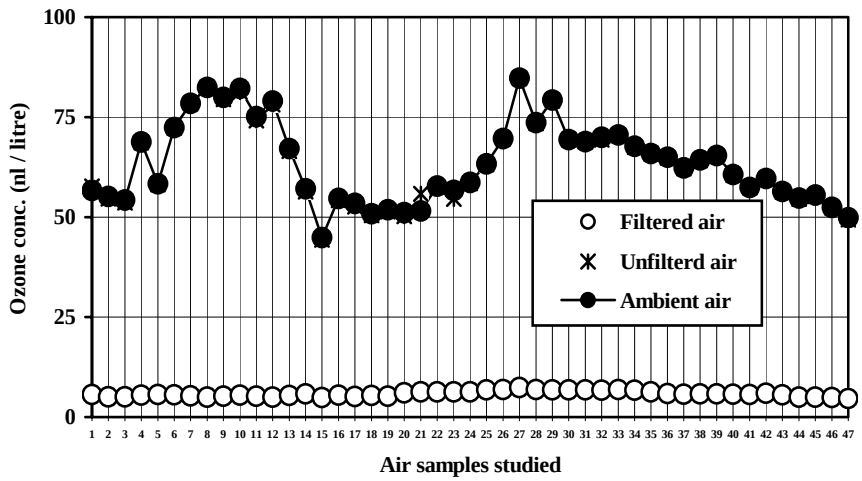

Figure-4. Mean 6-hours (10:00 -16:00 h) ozone concentration in FA, UFA open top chambers and ambient air during 1998 soybean season.

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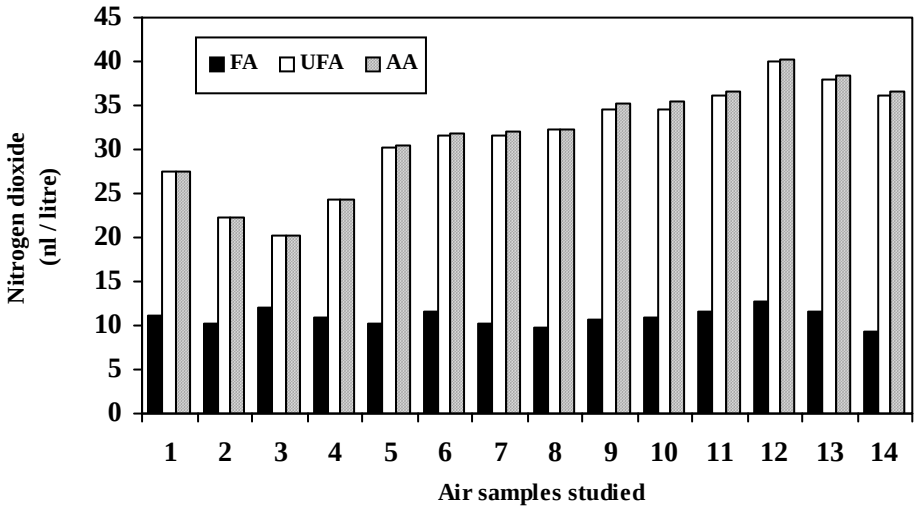

Figure-5. Weekly mean nitrogen dioxide concentration in FA and UFA open-top chambers and ambient air during 1998 soybean season.

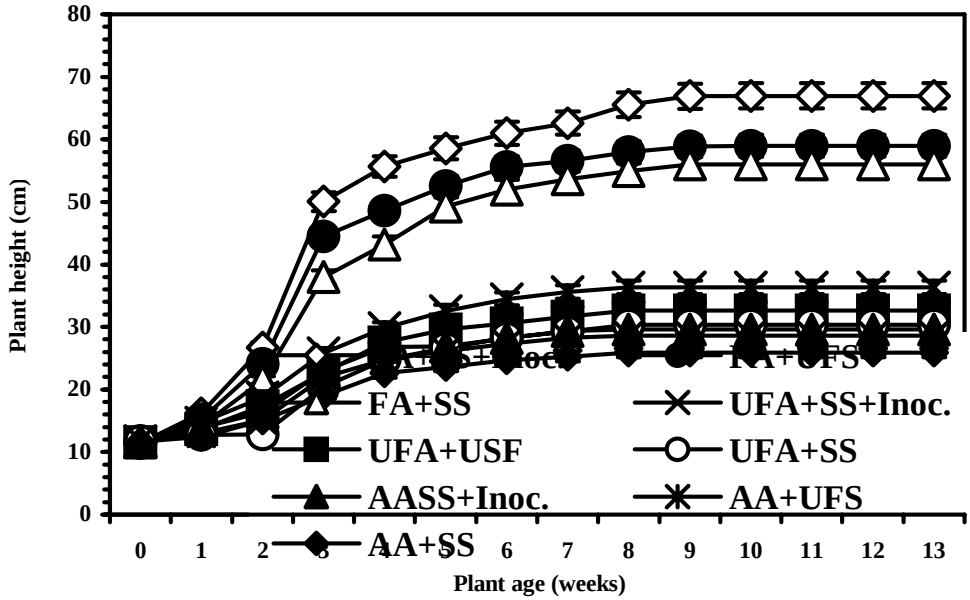

Figure-6. Effect of air treatments on plant height in soybean during the growth period in different soils in open-top chamber system. Line on each data point represents SE of the mean.

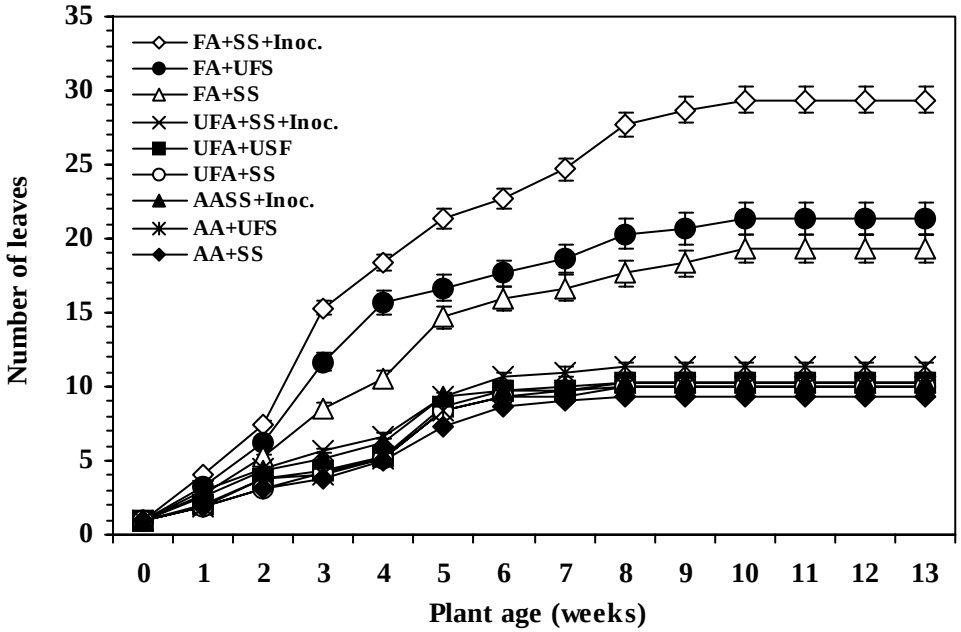

Figure-7. Effect of air treatments on number of leaves in soybean during the growth period in different soils in open top chamber system. Line on each data point represents SE of the mean.

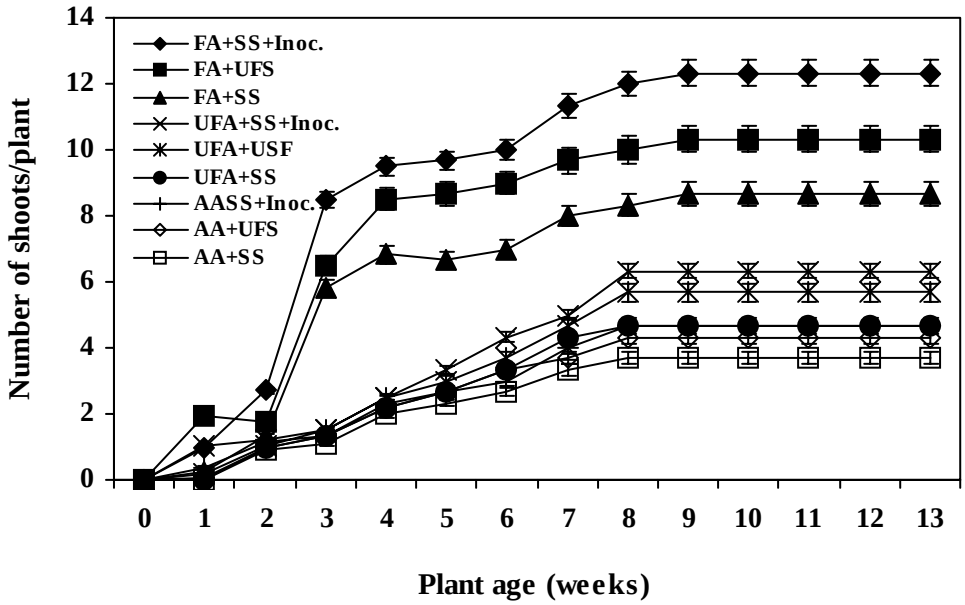

Figure-8. Effect of air treatment on number of shoots per plant in soybean during the growth period in different soils in open top chamber system. Line on each data point represents SE of the mean.

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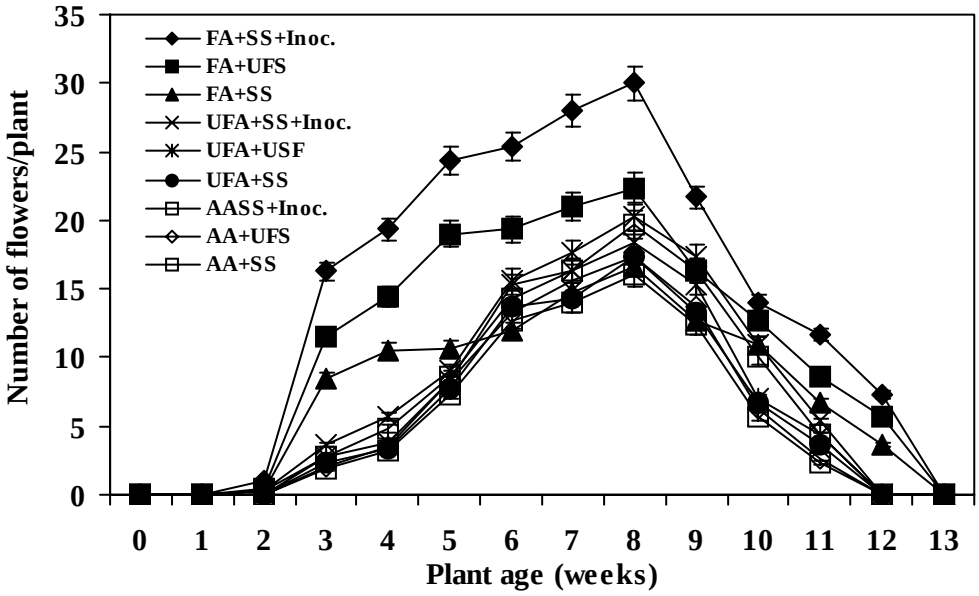

Figure-9. Effect of air treatments on number of flowers per plant in soybean during the growth period in different soils in open top chamber system. Line on each data point represents SE of the mean.

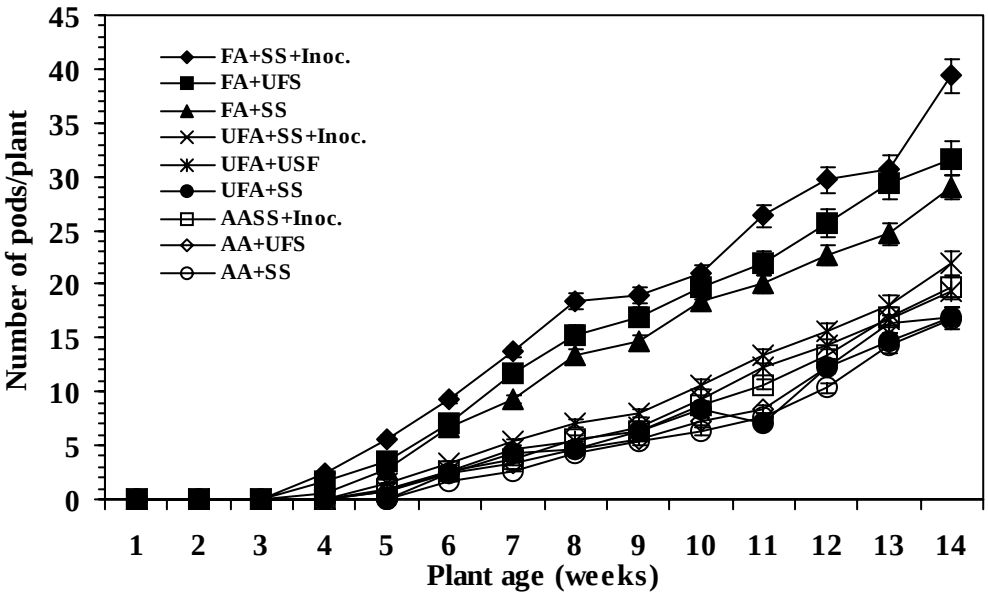

Figure-10. Effect of air treatments on number of pods per plant in soybean during the growth period in different soils in open-top chamber system. Line on each data point represents SE of the mean.

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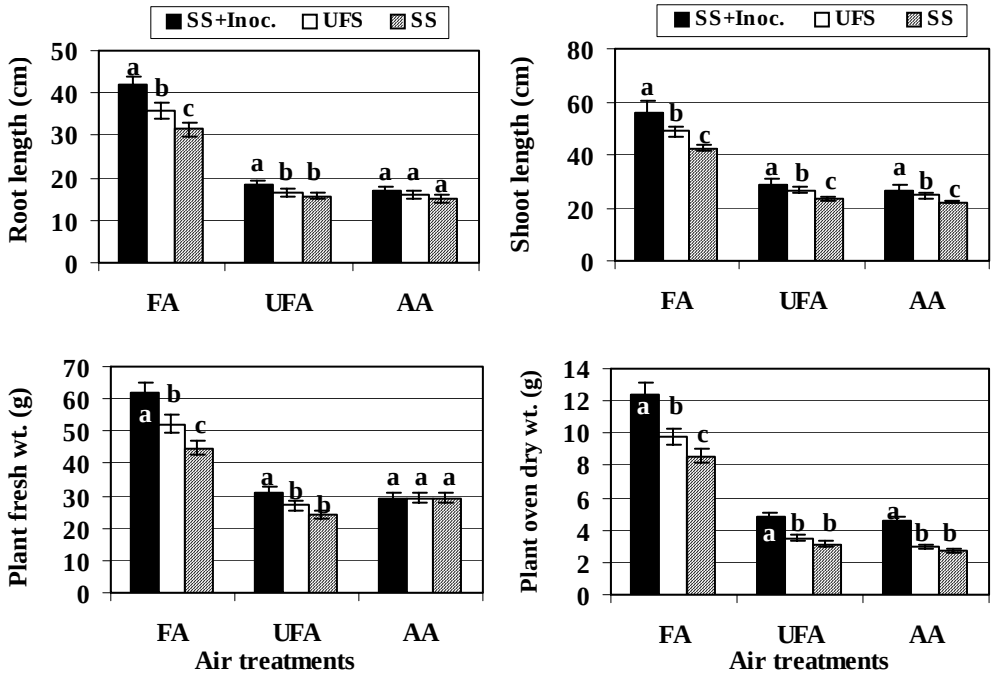

Figue-11. Plant growth and biomass assessment of soybean at the time of mid-term harvest in filtered air (FA), unfiltered air (UFA) and ambient air (AA) treatments in open top chambers in variously treated soils. Line on each data point represents SE of the mean. Data bars with different letters with in each air treatment are significantly different from one another at P<0.05 according to DNMRT.

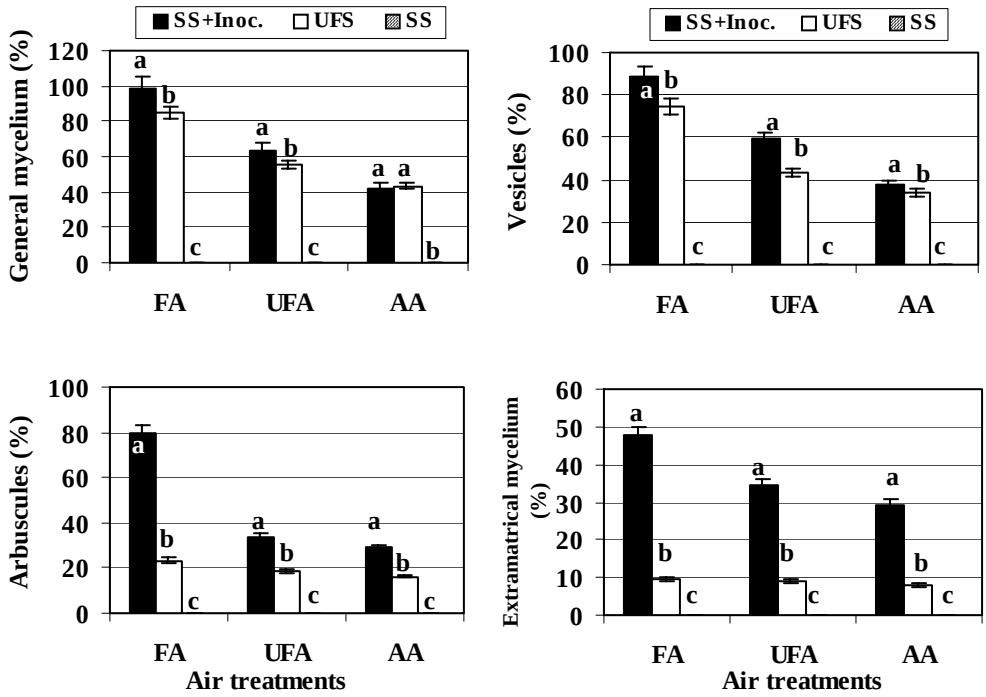

Figure-12. Status of arbuscular mycorrhizae in root corticle cells of soybean at the time of mid term harvest in filtered, unfiltered and ambient air treatments in open top chambers in variously treated soils. Line on each data point represents SE of the mean. Data bars with different letters with in each air treatment are significantly different from one another at P<0.05 according to DNMRT.

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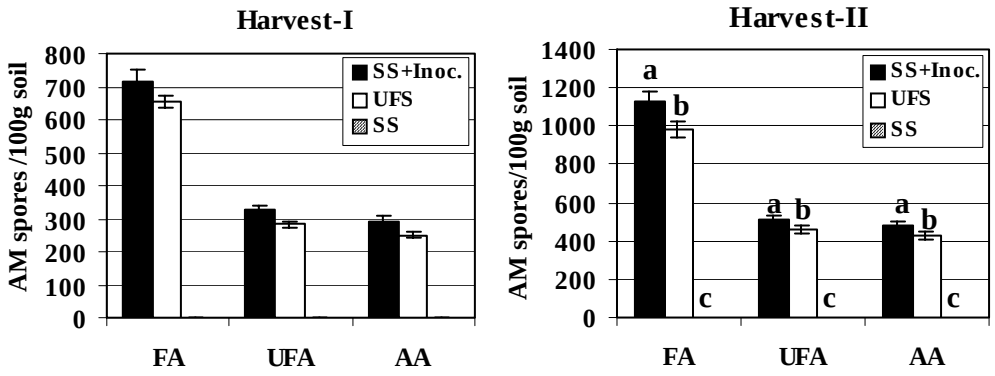


Figure-13. Number of Endogonaceous spores in rhizosphere soil of soybean plants at the time of mid-term and final harvests in variable air and soil treatments. Line on each data point represents SE of the mean. Data bars with different letters with in each air treatment are significantly different from one another at P<0.05 according to DNMRT.

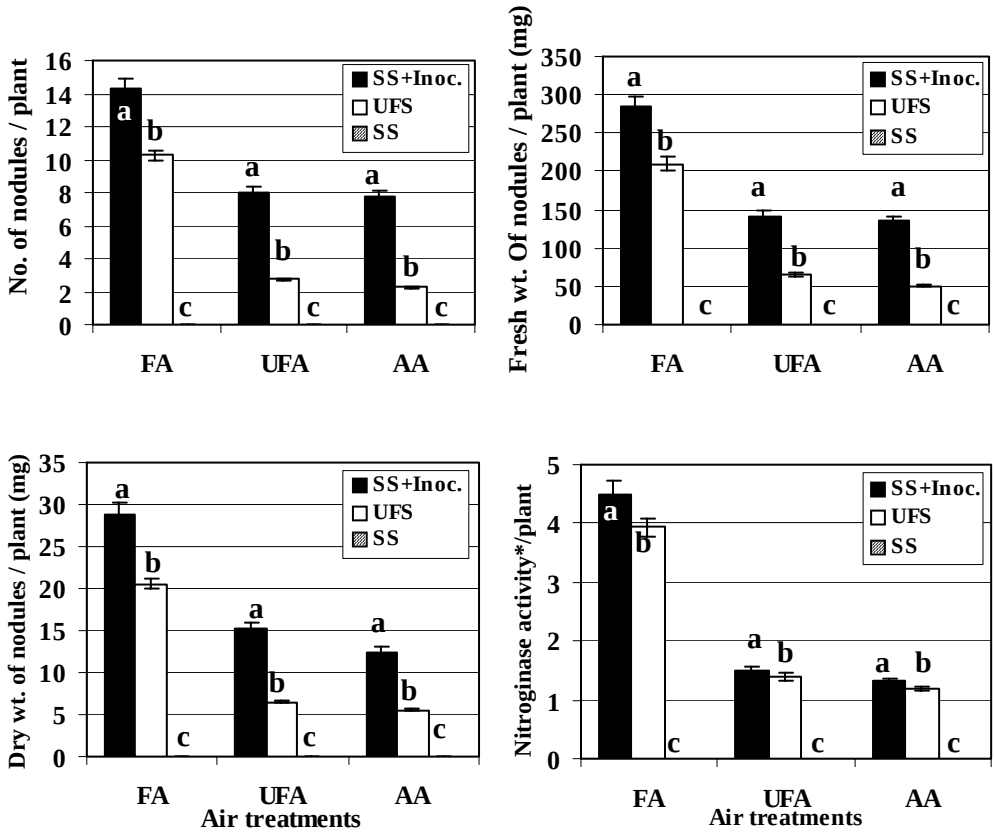
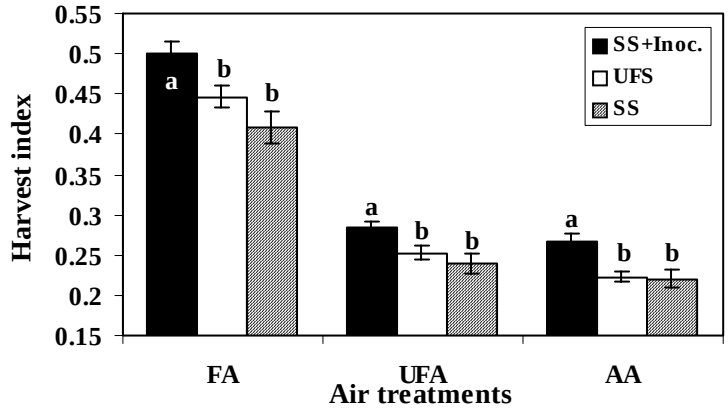


Figure-14. Number and biomass of nodules and nitroginase activity of soybean plants at the time of mid-term harvests in variable air and soil treatments. Line on each data point represents SE of the mean. Data bars with different letters with in each air treatment are significantly different from one another at P<0.05 according to DNMRT (* *u* mole C_2H_2/g weight of nodules /hr).



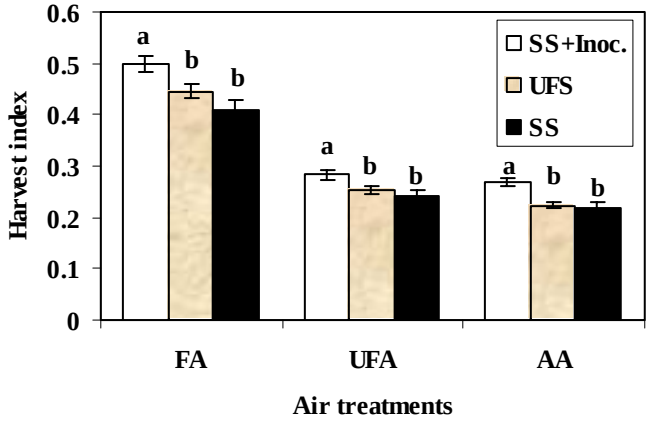


Figure-15. Harvest index of soybean plants after various soil and air treatments. Line on each of the data bar represent SE of the mean. Data bars with different letters with in air treatments are significantly different from one another at $P<0.05$ according to DNMRT.