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ARBUSCULAR MYCORRHIZAL SYMBIOSIS OF WHEAT AND EXPOSURE TO AIR POLLUTANTS IN URBAN PAKISTAN

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ABSTRACT

The experiments were conducted in open top chamber system installed at the University of the Punjab, Quaid-e-Azam Campus, Lahore. The wheat (*Triticum aestivum* Var. Blue silver) seeds were sown in earthen pots and were kept in filtered air (FA) and unfiltered air (UFA). The pots were given three different inocula for arbuscular mycorrhizal (AM) fungi. The climatic data and concentration of ozone and nitrogen dioxide was recorded at regular intervals. In this paper the evidence is being provided that, the plants growing in FA chambers (without ozone and dust particles) responded well showing an enhancement in growth and yield parameters. There were differences among sets with variable inocula for mycorrhizal fungi. The growth performance of plants receiving inoculum with less species richness was better. We document that out of a total of 33 species, twenty-five species belonged to the genus *Glomus*, three each to *Acaulospora* and *Sclerocystis* and one each to *Gigaspora* and *Scutellospora*. The total number of species was variable during the growth phase. The number reduced to almost half in soil of UFA chambers during the growth period in all mycorrhizal treatments. Maximum number of species (33) at each harvest was recorded in the case of T1 treatment in FA chambers, where the root pieces were used as inoculum. At first harvest the number of species for T2 and control sets of FA chambers was different i.e. 25 and 28 for the two respectively, which was same (24) at the time of third harvest.

Keywords: Atmospheric pollutants, nitrogen dioxide, ozone, species richness, wheat.

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).

Wheat is a staple food in Pakistan and is cultivated The soils in wheat fields are naturally have AM fungi. There are about 40 species of these fungi which have been reported by Nasim and Bajwa (2005). In the present study we have attempted to enhance inoculum levels in the ordinary unsterilized field soil, thus trying to duplicate the natural environment rather than setting the experiments in the sterilized conditions. At this time we thought this to be a more agriculturally useful approach than non-AM vs AM and we were focused to determined that if enhancing native colonization might affect wheat resilience to ozone and NO2. The two AM enhancement treatments have been compared to control, which is field soil without inoculation.

These objectives addressed three specific hypothesis that 1: the wheat plants kept in filtered air are better health wise and in yield parameters, 2: the wheat plants getting an enhancement in AM levels in two different ways respond well to filtered air than those grown in common field soil and 3. The E1 treatment consisting of mycorrhizal root pieces of wheat are able to enhance plant

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growth and thus resulting into a better response to air pollution.

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), on a 21x15 m suburban site adjacent to agricultural fields. The experimental site was 1500m from the nearest main road, 7 km from the nearest industry and about 7.5 km 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 wheat (*Triticum aestivum* L.) cv. Pak. 81 were obtained from Ayub Agriculture Research Institute (AARI), Faisalabad. Seeds were surface-sterilized in a mixture of 70% ethanol and H_2O_2 (1:1 by v:v), washed several times with sterilized tap water, then allowed to soak in a beaker of sterilized water for 24 h. Eight seeds were then sown into each of 48 sterilized earthen pots (25 cm in diameter) at a depth of 2-3 mm on 2nd February 1995. 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 suitable moisture (18%) was maintained for germination. Seedlings were

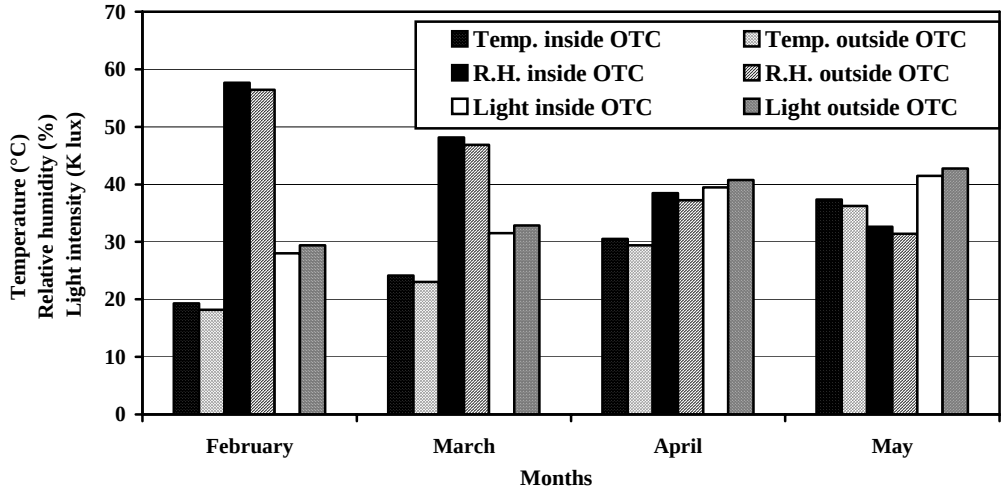

Fig. 1. Mean monthly temperature, relative humidity and light intensity inside and outside open-top chamber system in wheat season 1995.

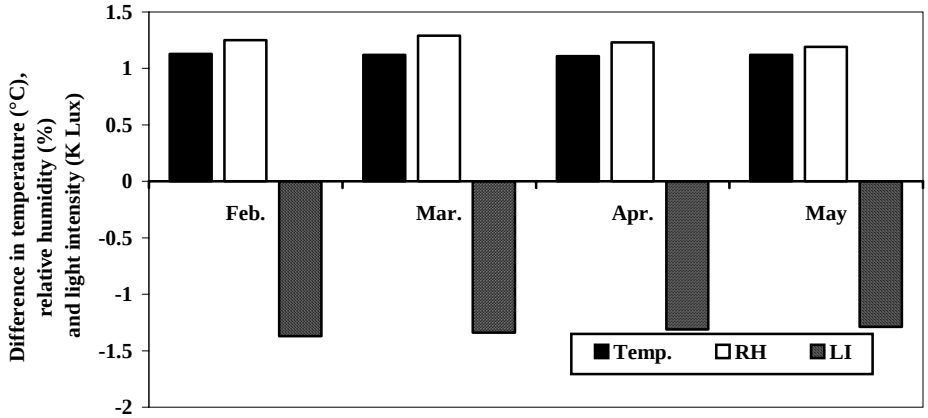

Fig. 2. Mean monthly differences in microclimatic conditions inside and outside open-top chambers.

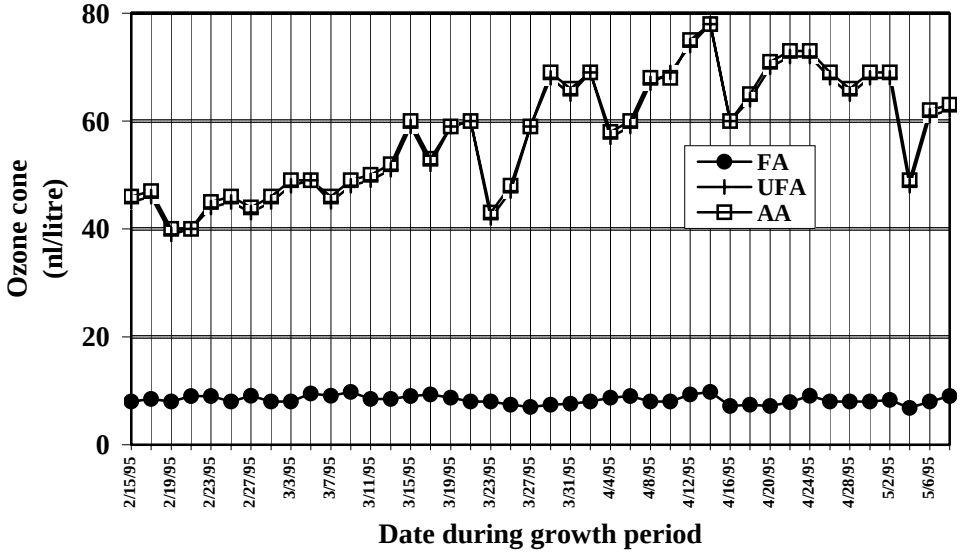

Fig. 3. Mean 6-hour (10:00-16:00 h) concentration of ozone concentration in filtered, unfiltered and ambient air in open-top chamber system during 1995 wheat season.

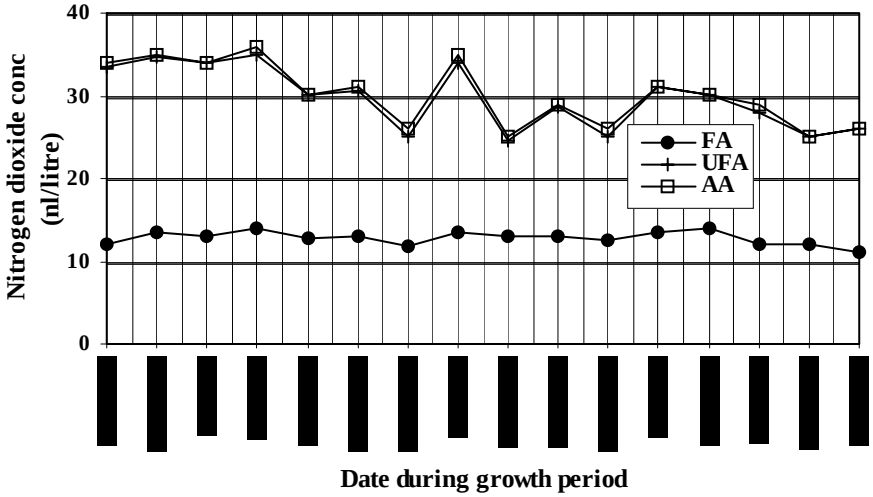

Fig. 4. Weekly mean nitrogen dioxide concentration in FA, UFA and ambient air in open-top chamber system.

grown outdoors in a wire house until they were thinned at 10 days.

Pots were labeled as enhanced AM 1 (mycorrhizal root pieces), enhanced AM 2 (scales of *Colocasia antiqourum* were supplemented with spore concentrate from the *Cycas* soil) and Control AM (without any additional innoculum) indicating various treatments. 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

The experiment included three mycorrhizal treatments: field soil (control AM), field soil + colonized wheat root pieces (enhanced AM 1), and field soil + spores (enhanced AM 2). Nonmycorrhizal wheat is an uncommon condition in our local wheat. Therefore, rather than compare nonAM and AM treatments, we used the more realistic and agriculturally useful comparison of "control" levels of mycorrhizal colonization (those occurring naturally in unsterilized field soil) with enhanced mycorrhizal colonization (plants given extra AM propagules of local genera and species). Two treatments for providing the extra propagules were studied, colonized root pieces vs. spores extracted from mycorrhizal soil, each obtained locally.

The root piece inoculum was obtained from 250 wheat plants from the wheat fields adjacent to the experimental plots. Roots were rinsed under tap water to remove

adhering soil and then cut into pieces about 1 cm in length. The spore inoculum (spore concentrate) was obtained from the rhizosphere of *Cycas circinalis*, also growing nearby in the university botanical garden due to a reduced species richness to minimize interspecific competition (Iqbal and Shahbaz, 1990). About 1 kg of soil was placed into 10 L of tap water in (1 L in each of 10 1-L beakers), the suspensions were stirred thoroughly for 5-10 min and then allowed to soak for 24 h. The clear supernatant was then suction-filtered through Whatman No. 1 filter paper to collect the glomalean spores. Spores were washed and then suspended in tap water for as inoculum. Root pieces and spores were obtained from different host species in the hope of slightly varying the fungal species composition in each of the two inocula. Inoculum was placed around each seed (about 100-120 spores per pot and 10cm^3 of root pieces per pot).

For Scale inoculum, corms of *Colocasia antiquorum* Schott were obtained from the market for the preparation of inoculum. Scales were then removed from the corms with the help of a sharp knife. These scales were washed in tap water to remove any dirt and debris. The random samples from each scale-lot were studied for the presence of arbuscular mycorrhizal structures. Only those lots having extensive (more than 80%) vesicles and mycelium were used as inoculum (Iqbal and Nasim, 1991; Nasim and Iqbal, 1991a,b; Nasim *et al*., 1992).

Air pollutant treatments

Ten days after planting, pots were thinned to 4 plants per pot, and pots were relocated for exposure to differing levels of air pollutants. Twelve plots were prepared, each 1.5 m 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 0.4 m 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. 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" 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" 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" 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" diameter holes 30cm apart along its length in two parallel rows. Two consecutive rows of holes alternated with each other. Two horizontal manifolds constructed with 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 towards the ground. The total volume of each chamber was 2.83m3, and the flow rate of the air into each chamber averaged 8m3/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 wheat 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.

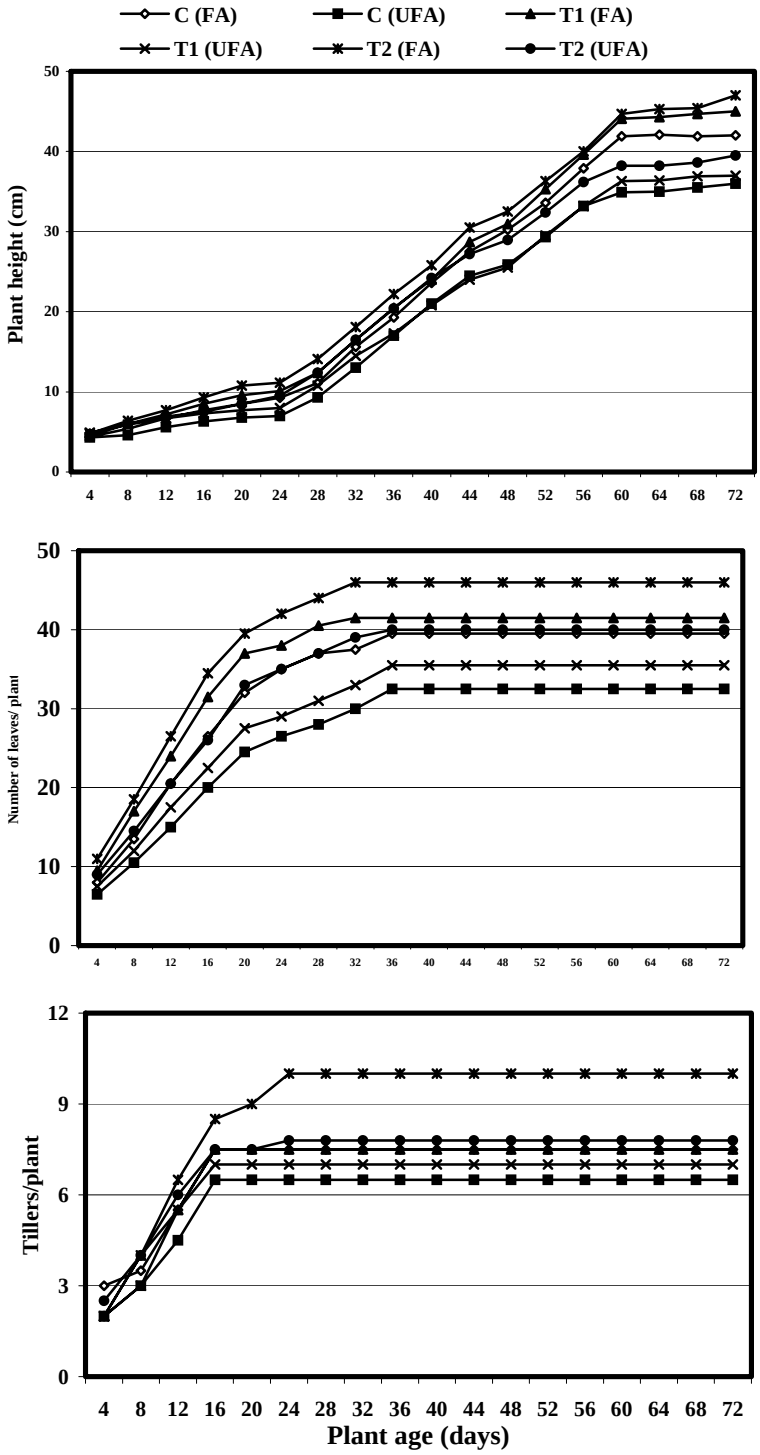

Fig. 5-7. Plant height (5), Number of leaves (6) and Number of tillers per plant (7) in control, T1 and T2 sets in FA and UFA open-top chambers.

The chambers were operated as closely as possible to the conditions prescribed under European Open-top Chamber Programme Protocol (Jager, 1993). Light intensity, air temperature and relative humidity were measured at plant height each day at 0800, 1200 and 1600 h 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 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 10.0 g of potassium iodide, 13.6 g of potassium di-hydrogen phosphate (KH_2PO_4) and 14.2 g of anhydrous di-sodium hydrogen phosphate (NaH_2PO_4) and final volume was made up to one litre with distilled water. A 0.05 N solution of iodine was then prepared by dissolving 16.0 g of potassium iodide and 3.17 g of resublimed iodide in distilled water and made the volume up to 500 ml. By diluting 5 ml of this iodine solution to 100 ml with the original neutral buffered potassium iodide and 0.0025N iodine solution was prepared.

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

- 0.8 ml of 0.0025 N iodine solution to 100 ml with KI solution = 2.54 ppm I_2.
- 1.6 ml of 0.0025 N iodine solution to 100 ml with KI solution = 5.08 ppm I_2.
- 2.4 ml of 0.0025 N iodine solution to 100 ml with KI solution = 7.61 ppm I_2.
- 3.6 ml of 0.0025 N iodine solution to 100 ml 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 10 ml 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 derived below:

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

Calculations

Let **'X'** = ppm I_2 (W/V)
Let **'Y'** = the 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 (NO2) is measured using diffusion tubes developed at the Environmetal 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 NO2 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.

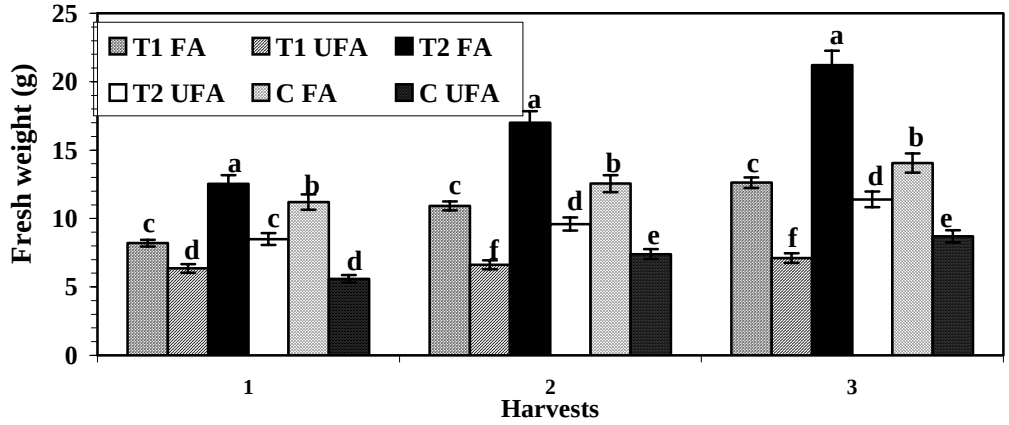

Fig. 8. Fresh weight of wheat plants after various mycorrhizal and air treatments at three different harvests. Line on data bars show S.E. while data bars with different letters are significantly different at P<0.05 according to DNMRT.

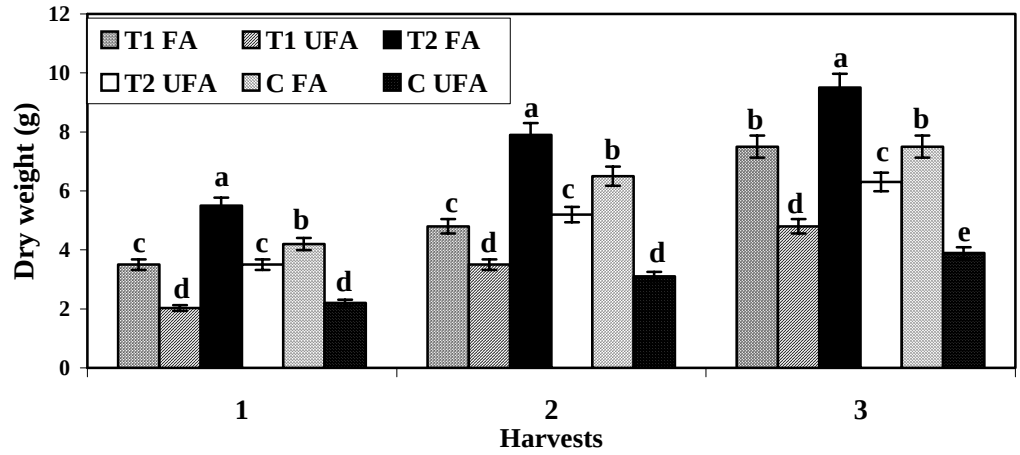

Fig. 9. Dry weight of wheat plants after various mycorrhizal and air treatments at three different harvests. Line on data bars show S.E. while data bars with different letters are significantly different at P<0.05 according to DNMRT.

The diffusion tubes comprise 7.1 cm x 1.1 cm 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 0.224 mm. 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 colored 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 (20 g sulphanilamide + 50 ml concentrated orthophosphoric acid diluted to 1000 ml with distilled water) and one tenth part N-1-naphthyl-ethylene-diamine-dihydrochloride (NEDA) reagent (0.350 g NEDA in 250 ml 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.

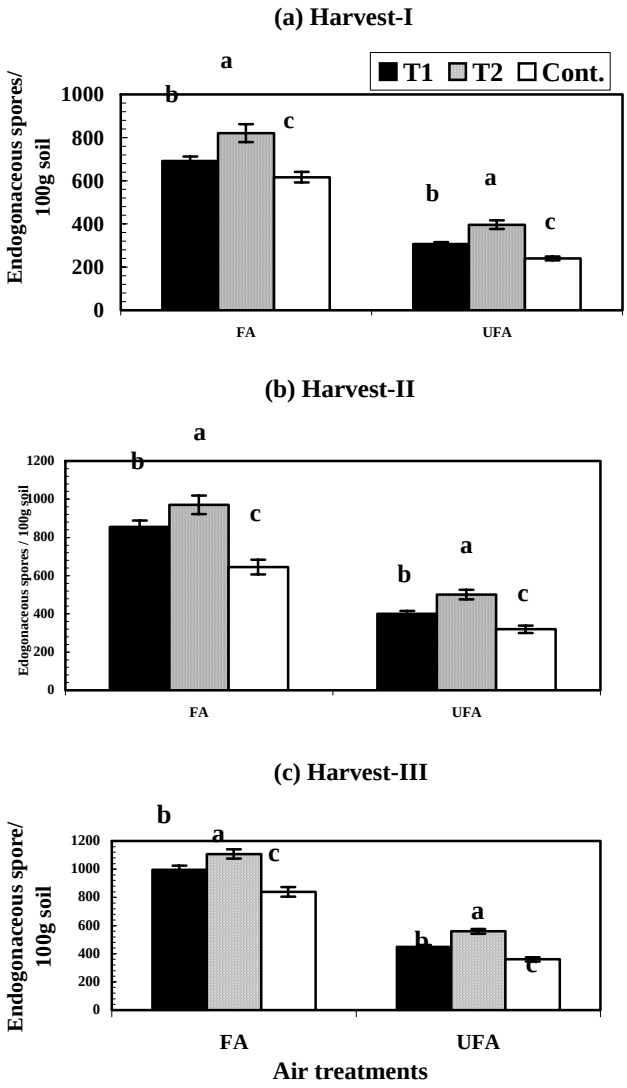

Fig. 13. Glomalean spore number in the rhizosphere soil of wheat plants kept in FA and UFA open-top chambers. Line of each of the data bar represents SE of the mean. Data bar with different letters with in each air treatment are significantly different from one another at $P<0.05$ according to DNMRT.I

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

Plants were harvested three times during the experiment, at 24, 48 and 72 days after planting seeds. These times coincided with the prime of vegetative growth (Harvest I), the onset of flowering (Harvest II) and the end of growing season when the crop had ripened (Harvest III). Three plants from each treatment were examined at each harvest.

For Harvests I and II, we recorded plant height, number of live and senescent leaves per plant, number of tillers per plant, and fresh and dry weights. Yield parameters were recorded for Harvest III: number of ears per plant, ear length, rachis length, number of spikelets per ear, number of grains per ear, number of grains per plant, grain weight per ear, 1000 grain weight 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).

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).

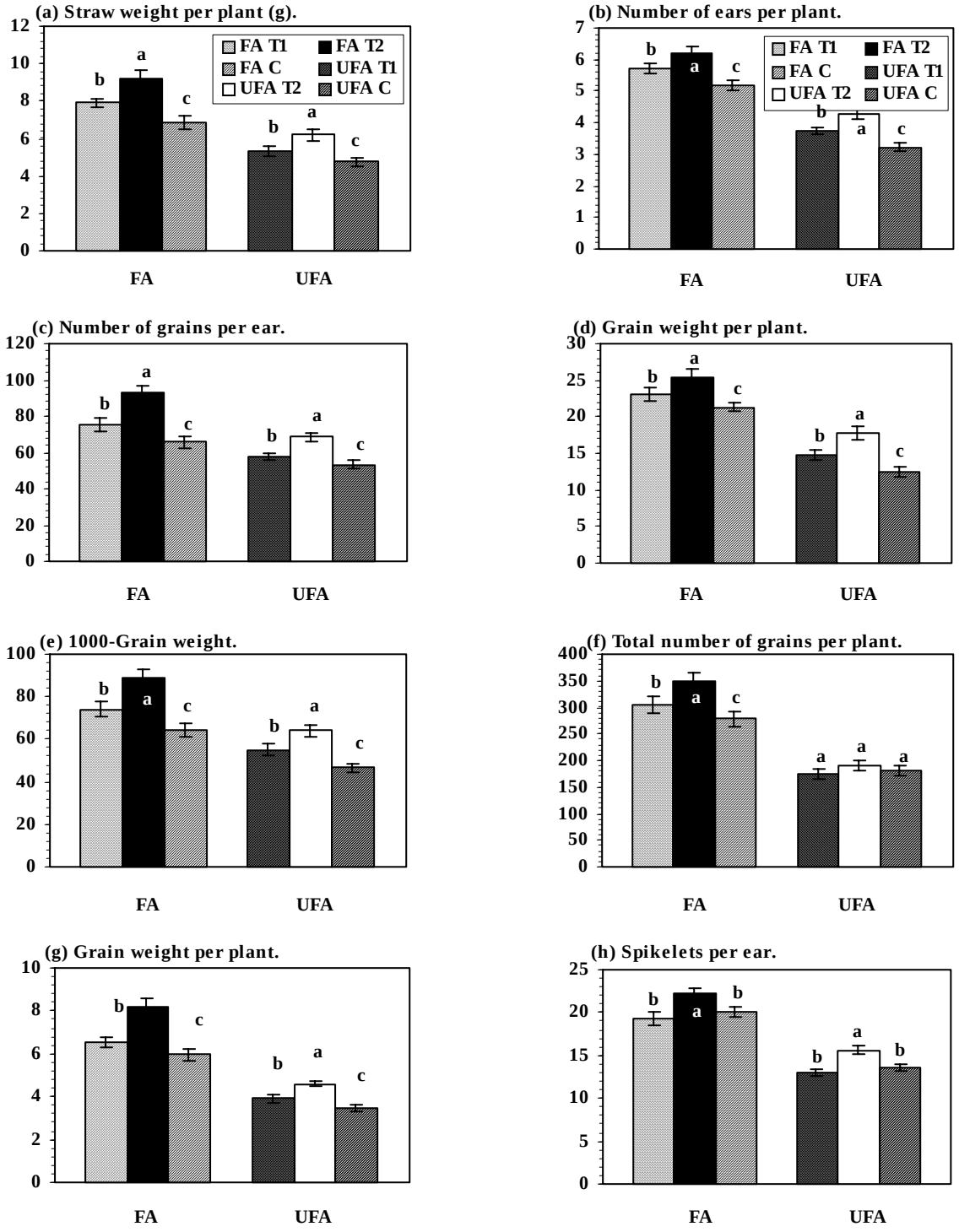

Fig. 10. (a-h): Final harvest parameter in wheat in different air and mycorrhizal treatments. Line on each of the data bar represent SE of the mean. Data bars with different letters with in air treatments are significantly different from one another at P<0.05 according to DNMRT.

RESULTS

The experiments to assess the impact of air pollution on vegetative growth, yield and associated mycoflora were conducted for wheat in the open top chamber system. The results of the aforesaid investigations are given as following:

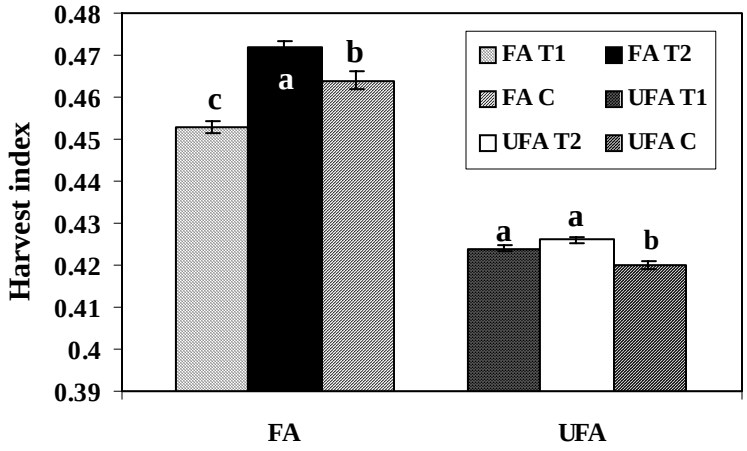

Fig. 11. Harvest index of wheat plants after various mycorrhizal and air treatments. Line on each of the data bar represent SE of the mean. Data bars with different letters with in air treatments are significantly different from one another at P<0.05 according to DNMRT.

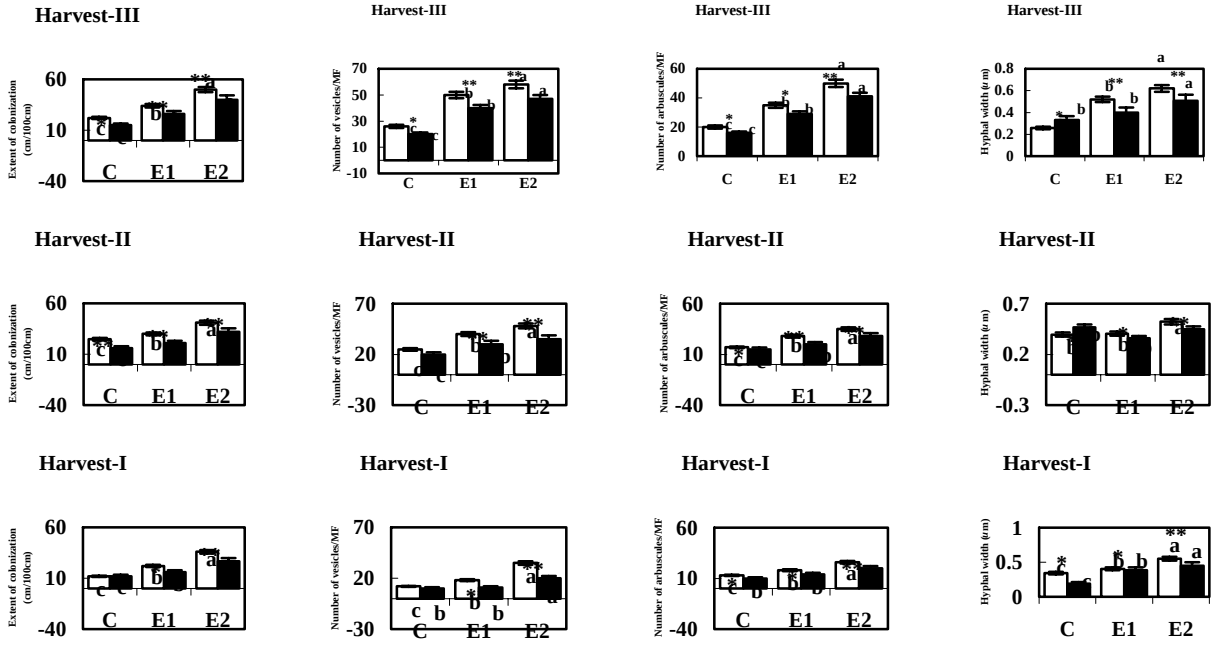

Fig. 12. Status of arbuscular mycorrhizal colonization in filtered air treatment (open bars) and unfiltered air treatment (closed bars) in open top chamber system. Line on each of the data bars represents SE of the mean. Differences between the means of two air treatments were analyzed by t-test, * and ** indicate significance at the P<0.05 and 0.01 levels respectively. (MF= Microscope field, diameter = 1430 μm & area = 4.49 cm^2).

Open-top studies with wheat

The wheat experiment in the open top chamber was conducted during wheat season of 1995. The results recorded under various heads are as follows:

Microclimatic measurements

The results of the routine measurements inside and outside the open-top chambers during the wheat growth season of 1995 showed relatively little impact upon microclimatic conditions (Fig. 1). Light level at crop height was on an average reduced by 3.64 % inside the chamber. However, the maximum reduction was in the month of February 1996. As regards the mean air temperature, it was 1.12°C higher inside the OTC. Percentage relative humidity was also relatively (1.24%) higher inside the chamber (Fig. 2). The reduction in light levels and an elevation in temperature and humidity inside OTC might be due to polyethylene vertical walls of the chamber and heating effect of the motor and fan. Air temperature increased through February to May. Light intensity also followed the same pattern being highest during the month of May. Relative humidity on the contrary decreased from February to May.

Pollutants monitoring

The data recorded for daily 6-hrs ozone (O_3) and nitrogen (NO_2) dioxide concentration showed drastic differences in filtered air chambers than in unfiltered air chambers and ambient air. The mean 6-h concentration of O_3 throughout the growing season was 54.85nl liter^{-1} with a mean NO_2 concentration of 28.82nl liter^{-1}. Ozone concentration was low in the month of February due to bad sunlight in the winter season. During the months of April and May, when there was bright summer sunshine, the values like 64.22nl liter^{-1} and 63.46nl liter^{-1} for O_3 concentration were recorded (Fig. 3). This is related to elevated light and temperature levels during these months (Fig. 1).

Filtration efficiencies were almost 90% and 65% overall for O_3 and NO_2 respectively. Ozone concentration in FA chambers did not exceed 8.38nl liter^{-1}, while NO_2 concentration in FA chambers did not exceed 13.48nl liter^{-1} (Figs. 3 and 4).

Assessment of vegetative growth parameters

An appreciable difference was recorded in plant growth parameters and the development of mycorrhizal associations in filtered air (FA) and unfiltered air (UFA). The parameters were recorded regularly at the time of three harvests. These three harvests were taken during a period of 72 days. For the plant height parameter, the values were significant for those kept in FA chambers. This was true for control plants and those given AM treatments i.e. T1 and T2 sets (Fig 5-7). At first, second and third harvest maximum values for plant height were recorded in the case of FA plant of T2 set. These values were 11.5, 32.52 and 47.0cm respectively. A reduction in height for UFA set for T2 treatment at all the three harvests was 14.79, 10.94 and 15.95 % respectively at three harvests. At the time of second harvest, the values of plant height for T1 and control set of FA chambers were non-significantly different than those of T2 plants in the same air treatment.

At third harvest too, the figures were highest (47.0cm) for T2 plants of FA chambers followed by close values (45.0) of T1 of the same air treatment. At this stage the values for control set differed significantly for the same air treatment (Fig. 5-7).

As regards the **number of leaves** per plant, at first harvest highest values (42 leaves per plant) were recorded for T2 plants kept on filtered air while lowest values (26.5 leaves) were noted for control plants in unfiltered air (Fig. 6). At second harvest same trend in the values for number of leaves was recorded. Highest values (46.5 leaved per plant) were again noticed for T2-FA plants and minimum values (32.5 leaves per plant) were for control-UFA plants. The number of leaves did not increase beyond this time and the same figures were recorded at the time of third harvest (Fig. 6).

The **number of tillers** went maximum up to 10 tillers per plant. This highest value was recorded for plants of T2-FA set. While control-UFA set had the lowest value (6.5 tillers per plant) for number of tillers per plant. The number of tillers was the same at the time of second and the third harvests (Fig. 7).

The maximum values for **plant fresh weight** were noticed for T2-UFA set at all the harvests. The minimum values were recorded for control-UFA at the time of first harvest. These lowest figures however varied insignificantly from T1-UFA set. At second harvest, the fresh weight values for the T1-UFA set had minimum values but these values were overlapping with the values for control-UFA set (Fig. 3.1.8). At the time of third harvest, the trend in fresh weight values was the same as it was at the time of second harvest. The lowest values (7.11g) of T1-UFA set vary insignificantly from those (8.7g) of control-UFA plants.

The values for dry weight of the plant also showed the same trend at least as far as the highest figures were concerned. At all the harvests plants of T2-FA sets turned up with maximum oven dry mass. The minima however varied from time to time. At first harvest smallest dry weight values (2.03g) were observed in the case of T1-UFA plants while at second and third harvest the minimum values (3.1 and 3.9g respectively) were reported for control-UFA set (Fig. 8).

Yield analysis

The parameters recorded at the time of final harvest were the number of ears, ear length & rachis length per plant, number of spikelets, number of grains per ear, number of grains per plant, grain weight per ear, 1000 grain weight and harvest index. It has been shown in figure 10 and 11 that filtered air has significantly improved the yield of the wheat plants. A reduction in yield from 40.28 to 44.77 % was recorded in UFA plants of various AM treatments. In all the yield parameters, T2-FA showed significantly high values amongst FA sets (Fig. 10). The difference was worth noticing in the case of straw weight, number of grains per ear, 1000-grain weight and grain weight per plant. However as regards the UFA treatments, the values for AM treatment were sometimes overlapping with T2, keeping an upper edge in all the parameters.

The harvest indices were also high for FA sets (Fig. 11). A reduction of 6.4, 9.75 and 9.48% was recorded in the harvest index of T1, T2 and control plants of unfiltered air chambers.

Mycorrhizal assessments

Extent of general infections, number of vesicles per microscope field, number of arbuscules per microscope field, width of hyphae and density and diversity of Glomalean spore flora were the parameters noticed

regularly and or at the time of final harvest. The set of plants kept in filtered air shown as open bars in figure 12 had appreciable arbuscular infections in control, T1, T2 and control sets. On the other hand, plants of unfiltered air ended up with a regular depression in values (shown as closed bars in Fig. 12).

As is obvious in figure 12, T2-FA set has maximum values (35.5, 42 and 50cm/100cm) for extent of infection of arbuscular infection at all the three harvests. While minimum values (12, 18 and 20cm/100cm) were recorded for control set of UFA chambers. Same was true for the number of vesicles and arbuscules / microscope field. The comparison at all the harvests have shown that the highest number of vesicles recorded per field of microscope was 58 which was for T2 set kept in filtered air. The control-set kept in unfiltered air had smallest number of vesicles (10 vesicles /microscope field) in the root cortex. For the number of arbuscules all the same trend was observed. It means that the maximum number (50 arbuscules /microscope field) of arbuscules was recorded for T2-FA set while the control-UFA set was the lowest achiever in the series. It had 10 arbuscules /microscope field. For hyphal width, however an irregular trend was observed and this parameter did not seem to correlate with the air or mycorrhizal treatments.

With reference to diversity and density of Glomalean spore flora, the results of the present study indicate that the total number of spores remains highest in FA-T2 set at all three harvests (Fig. 13). The T1 mycorrhizal treatment had root piece inoculum while T2 treatment included scales of corms of *Colocasia antiquorum* and spores concentrate from the *Cycas* soil. The number was greatly reduced in UFA set. Thus, minimum number of spores at each of the harvest was recorded for UFA-control set. In control set, no amendments as regards mycorrhizae were made. The number of spores per 100 g rhizosphere soil was markedly high in plants of FA chambers as compared to those kept in UFA chambers. Within mycorrhizal treatments, the number of spores per 100 g soil was maximum and was significantly high as determined at $P<0.05$ level (Fig. 13).

An overall output of the identification process of the Glomalean spores recovered indicated that a total of 33 spore types were present in rhizosphere soil of potted plants including FA and UFA plants receiving different mycorrhizal inoculations (Table 1 & 2). Out of this total of 33 species, twenty-five species belonged to the genus *Glomus*, three each to *Acaulospora* and *Sclerocystis* and one each to *Gigaspora* and *Scutellospora*. The total number of species was variable during the growth phase. The total number of species reduced in soil of UFA chambers with the passage of time in all mycorrhizal treatments. The number of species reduced to almost half in UFA plants as compared to FA plants (Table 1).

The impact of mycorrhizal treatments was also clearly visible on the total number of species of AM fungi. Maximum number of species (33) at each harvest was recorded in the case of T1 treatment in FA chambers, where the root pieces were used as inoculum. At first harvest the number of species for T2 and control sets of FA chambers was different i.e. 25 and 28 for the two respectively, which was same (24) at the time of third harvest (Table 1).

Species of the Genus *Glomus* were highly abundant species at various harvests in all mycorrhizal and air treatments. Amongst most abundantly recovered *Glomus* species were *G. fasciculatum, G. mosseae, G. aggregatum, G. caledonicum, G. deserticola, G. geosporum, G. monosporum, G. multicaul,* and *G. reticulatum* (Tables 2-4). The pattern of abundance kept on varying at various harvests for different air and mycorrhizal treatments. In the case of plants of UFA treatment, only two species of *Glomus* were abundant namely *G. fasciculatum* and *G. geosporum*. Species of *Acaulospora* and *Gigaspora* in particular and *Scutellospora* and *Sclerocystis* in general were sensitive to polluted air (Table 2-4).

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 and Meharg, 1999; McCardy and Anderson, 2000; Miller *et al*., 1997; Yoshida *et al*., 2001; Shafer and Schoenberger, 1991). 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 and 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 wheat 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 and 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.,* 1995 a,b; Nasim *et al.,* 1995; Bajwa *et al.,* 1997) in Pakistan. Very few studies have

Table 1. Variations in the total number of AM spore types at different stages of growth of wheat and in various air and mycorrhizal treatments.

Harvests ↓ / Air → treatments	Mycorrhizal treatments					
	Root inoculum		Spore/scales Inoculum		Un inoculated Control	
	FA	UFA	FA	UFA	FA	UFA
Harvest-I	33	20	25	20	28	18
Harvest-II	33	18	24	15	27	15
Harvest-III	33	15	24	15	24	14

Table 2. Frequency of occurrence of Glomalean spore per 100 g of soil of pots of wheat at first harvest kept in Filtered air (FA) and unfiltered air (UFA) open-top chambers.

Sr. No.	Species of AMF	Root inoculum		Spore/scales Inoculum		Un inoculated Control	
		FA	UFA	FA	UFA	FA	UFA
1.	*Acaulospora bireticulata*	++	++	+++	++	++	++
2.	*A. foveata*	+	-	+++	-	+	-
3.	*A. rehmii*	++	-	-	-	++	-
4.	*Gigaspora decipiens*	+	-	-	-	+	-
5.	*Glomus aggregatum*	++++*	++	++	++	++++*	-
6.	*G. albidum*	+	+	-	+	+	-
7.	*G. caledonicum*	+++	++	-	++	++	++
8.	*G. cerebriformis*	++	-	+++	-	+++	+
9.	*G. clarum*	+++	+	++	+	++	++
10.	*G. constrictum*	+++	++	++	++	+++	-
11.	*G. dilhiense*	++	++	++	++	++	+
12.	*G. deserticola*	++	+	++	+	++	++
13.	*G. dimorphicum*	+++	-	+++	-	+++	++
14.	*G. fasciculatum*	++++*	+++	++++*	++++*	++++*	+
15.	*G. ficundisporum*	++	-	++	-	+	-
16.	*G. geosporum*	++	+	++	+	++	+++
17.	*G. halonatum*	+	-	-	-	+	-
18.	*G. intraradices*	+	-	-	-	+	+
19.	*G. leptotichum*	++	+	+++	++	+++	-
20.	*G. microaggregatum*	+++	++	++++*	++	+++	-
21.	*G. microcarpum*	++	+	+++	+	+++	-
22.	*G. monosporum*	++	++	++++*	++	+++	++
23.	*G. mosseae*	++++*	++	++++*	++	++++*	+
24.	*G. multicaul*	++	++	+++	++	+++	++
25.	*G. reticulatum*	++	-	+++	-	++	++
26.	*G. tenuis*	+++	-	++	-	+++	++
27.	*Glomus sp. I*	+++	-	++++*	-	++	-
28.	*Glomus sp. II*	+	-	-	-	-	-
29.	*Glomus sp. III*	+	-	-	-	-	-
30.	*Sclerocystis micrcarpus*	++	+	+++	+	+	+
31.	*S. pakistanica*	++	+++	+++	+++	+++	-
32.	*S. sinuosa*	+	+	+++	+	++	+
33.	*Scutellospora pellucida*	+	++	+++	++	++	+++

Key: + = rare(25%); ++ =frequen(50%)t, +++ =abundant(75%) & ++++ = * = highly abundant species(100%).

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

Table 3. Frequency of occurrence of Glomalean spore per 100 g of soil of pots of wheat at second harvest kept in Filtered air (FA) and unfiltered air (UFA) open-top chambers.

Sr. No.	*Species of AMF*	*Root inoculum*		*Spore/scales Inoculum*		*Un inoculated Control*	
		FA	**UFA**	**FA**	**UFA**	**FA**	**UFA**
1.	*Acaulospora bireticulata*	++	++	+++	++	++	++
2.	*A. foveata*	+	-	+++	-	++	-
3.	*A. rehmii*	++	-	-	-	++	-
4.	*Gigaspora decipiens*	++	-	-	-	++	-
5.	*Glomus aggregatum*	+++	+	-	+	+++	-
6.	*G. albidum*	++	-	+++	+	-	-
7.	*G. caledonicum*	++++*	++	++++*	++	++++*	-
8.	*G. cerebriformis*	++	-	-	-	-	-
9.	*G. clarum*	++++	++	-	++	++++	++
10.	*G. constrictum*	+++	-	++++	-	+++	+
11.	*G. dilhiense*	+++	-	-	-	+++	++
12.	*G. deserticola*	++++*	++	++++*	++	++++*	-
13.	*G. dimorphicum*	+++	++	+++	++	+++	+
14.	*G. fasciculatum*	++++*	+	+++	-	+++	++
15.	*G. ficundisporum*	+++	-	+++	-	+++	++
16.	*G. geosporum*	+++	+++	++++*	++++*	++++*	+
17.	*G. halonatum*	+++	-	+++	-	+++	-
18.	*G. intraradices*	++	+	++	-	++	+++
19.	*G. leptotichum*	+	-	-	-	-	-
20.	*G. microaggregatum*	+++	-	-	-	-	+
21.	*G. microcarpum*	+++	-	+++	-	+++	-
22.	*G. monosporum*	++++*	++	++++*	++	++++*	-
23.	*G. mosseae*	++++*	+	+++	+	+++	-
24.	*G. multicaul*	+++	++	++++*	++	++++*	++
25.	*G. reticulatum*	+++	++	++++*	++	++++*	+
26.	*G. tenuis*	+++	++	+++	++	+++	++
27.	*Glomus sp. I*	+++	-	+++	-	+++	++
28.	*Glomus sp. II*	+	-	+++	-	+++	++
29.	*Glomus sp. III*	+	-	++++	-	++++	-
30.	*Sclerocystis micrcarpus*	++	+	-	-	-	-
31.	*S. pakistanica*	++	+	+	-	-	-
32.	*S. sinuosa*	+	++	++	++	++	-
33.	*Scutellospora pellucida*	+	+	++	+	++	-

Key: + = rare(25%); ++ =frequen(50%)t, +++ =abundant(75%) & ++++ = * = highly abundant species(100%).

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 and 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

Table 4. Frequency of occurrence of Glomalean spore per 100 g of soil of pots of wheat at third harvest kept in Filtered air (FA) and unfiltered air (UFA) open-top chambers.

Sr. No.	Species of AMF	*Root inoculum*		*Spore/scales Inoculum*		Un inoculated Control	
		FA	UFA	FA	UFA	FA	UFA
1.	*Acaulospora bireticulata*	+++	++	++++	++	+++	++
2.	*A. foveata*	++	-	+++	-	++	-
3.	*A. rehmii*	++	-	-	-	-	-
4.	*Gigaspora decipiens*	++	-	-	-	-	-
5.	*Glomus aggregatum*	++++	-	+++	-	+++	-
6.	*G. albidum*	++	-	+++	-	++	-
7.	*G. caledonicum*	++++*	++	++++*	++	++++*	-
8.	*G. cerebriformis*	++	-	-	-	-	-
9.	*G. clarum*	++++	++	-	++	-	++
10.	*G. constrictum*	+++	-	++++	-	+++	-
11.	*G. dilhiense*	+++	-	-	+	-	++
12.	*G. deserticola*	++++*	+ +	++++*	++	++++*	-
13.	*G. dimorphicum*	+++	++	+++	++	+++	+
14.	*G. fasciculatum*	++++*	+++	++++*	+++	++++*	+++
15.	*G. ficundisporum*	+++	-	+++	-	+++	++
16.	*G. geosporum*	++++*	+++	++++*	++++*	++++*	+
17.	*G. halonatum*	+++	-	+++	-	+++	-
18.	*G. intraradices*	++	+	++	+	++	+++
19.	*G. leptotichum*	+	-	-	-	-	-
20.	*G. microaggregatum*	+++	-	-	-	-	+
21.	*G. microcarpum*	+++	-	+++	-	+++	-
22.	*G. monosporum*	++++*	+	++++*	++	++++*	-
23.	*G. mosseae*	+++	+	+++	+	+++	+
24.	*G. multicaul*	++++*	++	++++*	++	++++*	-
25.	*G. reticulatum*	++++*	++	++++*	++	++++*	+
26.	*G. tenuis*	+++	++	+++	++	+++	++
27.	*Glomus sp. I*	+++	-	+++	-	+++	++
28.	*Glomus sp. II*	+	-	+++	-	+++	++
29.	*Glomus sp. III*	+	-	++++	-	++++	-
30.	*Sclerocystis micrcarpus*	++	-	-	-	-	-
31.	*S. pakistanica*	++	-	-	-	-	-
32.	*S. sinuosa*	+	++	++	++	++	-
33.	*Scutellospora pellucida*	+	+	++	-	++	-

Key: + = rare(25%); ++ =frequen(50%)t, +++ =abundant(75%) & ++++ = * = highly abundant species(100%).

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,b).

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,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 and Zeiger, 2006).

In the present study, the results presented for wheat are quite in line with those obtained by Wahid *et al*. (1995a,b). Particularly in terms of yield the total grain weight per plant is reduced which may be attributed to the number of ears per plant, number of grains per ear and number of grains per plant. 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,b). However, the results cannot be compared with those of the fumigation studies (Wahid *et al.*, 1995a,b). 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,b). Similarly, a very positive trend was noticed in nodulation and N-fixation ability of mungbean and 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 and Gupta, 1988; Kohut *et al.*, 1986; Heggestad and Lee, 1990; Mulchi *et al.*, 1988). However, there is only one research paper as yet regarding the interaction between *Glomus geosporum* (a AM forming fungus) and exposure of soybean to ozone, (Brewer and Heagle, 1983). 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 and 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.

Previous studies have shown that mycorrhizal plants have significantly high values for growth and yield parameters than non-mycorrhizal plants in all air treatments. In wheat, the variously inoculated sets had intertreatment variations but in all respects, FA plants had significantly high values for different parameters than UFA plants. The plants of T2 treatment showed promising results. This set was given the inoculum of *Colocasia* scales and supplemented with spore concentrate from *Cycas* rhizosphere. These two inocula have 3-5 species of arbuscular mycorrhizal fungi hence offering a minimum of interfungal interaction. On the contrary, the T1 set received root inoculum, which had mixed inoculum of 15-20 species. Thus, T1 plants exposed to these interactions were lagging a little bit behind the other treatment. For mungbean plants the values for FA set remained highest followed by UFA and AA. In another study with soybean, 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 resulted in reduced growth of host plant (Nasim and 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 and 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 wheat 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 wheat are typically mycotrophic and also responds strikingly to the air treatments. The results have also shown that the pollutants reduced plant growth of mycorrhizal plants (plants with enhancement treatments) but growth reduction was even greater in plants with no enhancement 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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