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Impacts of vehicle exhaust black soot on germination of gram seed (*Cicer arietinum* L.)

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ABSTRACT

An investigation was initiated to examine the effects of carbon soot collected from exhaust tube of 15 years old petrol and diesel operated vehicles on gram seed germination and biochemical changes of seedling. In view of the widespread cultivation of gram seed in India and long-term impact of black carbon is the warming of the atmosphere as per the recommendation of IPCC (2007). Black soot were separately treated with different doses and the effects of these treatment had on seed germination, seedling vigor, chlorophyll and carotenoid content, root and shoot growth, protein, sugar, phenol and proline estimation were studied. The treatment T_6 significantly affected on seed germination (84%) as well as seedling vigor and chlorophyll content. But other treatment promoted both seed germination and seedling vigor along with enhancement of other biochemical constituents. On the other hand micrograph study revealed that treatments T_1 and T_4 both showed negative effects on stomata rather than the ultra-structure of xylem and phloem.

Keywords: Carbon black soot, Germination, Vigor index, Biochemical constituints, Micrograph study

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INTRODUCTION

The characteristics of urban air pollution have changed significantly over recent decades. Concentration of traditionally important pollutants such as sulphur dioxide (SO_2) and black smoke have declined substantially, whilst road traffic emission have emerged as the major cause of poor air quality (Brophy et al. 2007). Diesel and petrol fuelled vehicles are responsible for the generation of wide range of pollutants, with concentrations and relative proportions of pollutants, depending on vehicle technology and operating conditions (Colvile et al. 2001).

Although complete combustion of diesel fuel produces water and carbon dioxide, but use of diesel in motor vehicles normally results in incomplete combustion and the formation of various gases, liquids and solid particles. Atmospheric black carbon (BC) is released from incomplete combustion of biomass and fossil fuels (Anda and Illes 2012) and it is commonly referred as soot (Andreae and Crutzen 1997). Soot is a particle-phase product of incomplete combustion of carbon containing fuels. Its main components are black carbon (BC) and organic carbon (OC). BC emission from road transport is smaller from residential than industrial sector due to rapid growth of industrial sector (Song et al. 2012). Streets et al. (2001) reported that the transport sector contributed only 7.2 % in 1995, but would grow to 11.3 % in 2020. However, Klimont et al. (2009) reported that BC emissions from vehicles in India in 2020 and 2030 would be lower than China in the baseline scenario.

BC is important from an environmental standpoint because these various compounds possess different physical and chemical properties, and therefore, can affect climate and be affected by climate in different ways (Goldberg 1985, Schmidt and Noack 2000). Recent report (Zhan et al. 2012) show that BC can make up a significant proportion of the organic carbon in soils, but the amount has been found to differ considerably among soil types. Diesel exhaust BC is especially dangerous, containing nearly forty (40) hazardous pollutants. The mixture contains carbon particles that are exceptially small in size, less than one micron (1<µm). These fine particles may be deeply inhaled into the lung and penetrates into pulmonary alveoli and causes respiratory disease (Song et al. 2012, Wargo et al. 2006). It also contributes to premature death due to cardio-vascular impacts (Song et al. 2009, Scwartz et al. 2008, Pope et al. 1995, Dochery et al. 1993, Wargo et al. 2006).

Most studies of the human health effects of air pollution had not been composition specific, but there is evidence that tiny soot particles, usually include toxic organic carbon and metals, are carcinogenic and among the most harmful pollutants (Künzli et al. 2000). These toxic materials such as carbon particles, unburned and partially burned hydrocarbons, fuels, tar materials, lead compounds and other elements, which are the constituents of petrol and lubricating oils, are deposited on the surface of plants. These pollutants have individual as well as synergistic effects on plants (Qadir and Iqbal 1991). Soot reduced atmospheric transparency and visibility, by enough in India and China to reduced agricultural productivity an estimated 10-20% (Chameides et al. 1999) with additional productivity loss from soot deposited on plant leaves (Bergin et al. 2001). Moreover, soot also esthetically displeasing as it is responsible for the brown appearance of urban hazes and soiling of buildings (Novakov and Hansen 2004). Moreover, long-term impact of black carbon is the warming of the atmosphere as published by the last IPCC report (2007) and short-term influences are the local impacts on humans and vegetation.

Road transport, mainly vehicle exhaust is responsible in deterioration of town's air quality and composition. Grantz et al. (2003) summed direct and indirect influences of atmospheric PM on vegetation. Direct impact means deposition in foliar surface, while indirect one by changing soil nutrient cycle-plant nutrient uptake or incoming solar radiation. In urban areas the relative proportion of fine particles, playing major role in hampering the overall growth of plants exceeded the rate of ultrafine coarse particles (Rai et al. 2010). Moreover, vehicle origin these pollutants adversely affect the germinability of seeds (Guderian 1977, Wong et al. 1984, Türkan 1988, Qadir and Iqbal 1991). Mehmood and Iqbal (1989) collected the seeds of *Dalbergia sissoo* Roxb. from different polluted areas of the city and showed significant reduction in germination as compared to the seeds collected from the less polluted areas. Similarly, Siddiqui and Iqbal (1994) collected the seeds of *Cassia surattensis* Burm. f., *Leucaena leucocephala* (Lam.) de Wit, *Parkinsonia aculeata* L., and *Sesbania sesban* (L.) Merr. from the polluted areas and showed significant reductions in germination as compared to the control. In spite of above mentioned research, there is still a serious lack of knowledge of the impact of air quality on vegetation in the both rural and urban areas.

Keeping in mind the above facts the present work is dedicated to check the effects of black soot collected from vehicle exhaust tube on germination and biochemical effects of gram seed (*Cicer arietinum*).

MATERIAL AND METHODS

Black Carbon (BC) collection. BC were collected in sterilized plastic packet from exhaust tube of 15 years

old vehicle exhaust of diesel and petrol engine by using a nickel spatula and stored in desiccators for further use.

Seed germination and seedling growth. Healthy uniform gram seed was chosen as test plant and pre-soaked in distilled water for overnight. Before germination the gram seeds were surface sterilized with 0.1 % $HgCl_2$ solution for 30 seconds and washing in double distilled water thoroughly for several times to remove excess of chemical and dried on absorbent to eliminate fungal attack. Twenty selected seed were placed on filter paper inside a sterilized Petri plate (9 cm diam. and 1.5 cm depth) for seed germination and seedling growth. The treatment concentration was considered as T_1 (0.04 g); T_2 (0.08 g) and T_3 (0.1g) black soot taken from vehicle exhaust tube of petrol engine and T_4 (0.04 g), T_5(0.08 g) and T_6(0.1 g) taken from the vehicle exhaust tube of diesel engine soot sprayed on the germinating seeds. The control set up was made without black soot. Petri dishes were placed in a cage box which is equilibrium with room temperature 30 ± 1 ^{0}C for twelve days. Watering was given to all petri plates regularly.

The primary root length, shoot length and germination percentage was measured on every day after the start of the experiment up to twelve day. The length of shoot and root were recorded by using a centimeter scale and germination was calculated based on the number of seeds germinated in a petri plate and expressed as germination percentage. Seedling Vigor Index (SVI) was calculated by the formula described by Abdul-Baki and Anderson (1993):

Seed Vigor Index = Germination % (root length + shoot length).

Biochemical Parameters of seedling. Biochemical parameters of plant materials viz. soluble sugar (Montgomery 1970), Proline content (Bates et al. 1973), plant pigment of chlorophyll (Arnon 1949) and protein (Lowry et al. 1951) were estimated by spectrometer on twelve day after start of the experiment.

Chlorophyll and Carotenoid assay. Fresh young leaves (0.1g) were selected from plants under each treatment at the last day of the experiment, and washed with de-ionized water. The leaves were cut into small pieces. Chlorophyll fractions 'a', 'b' and total chlorophyll were determined in the acetone extract (80% v/v) (Bates 1973) measured in a spectrophotometer at 645, 652nm and 663 nm and the concentration were expressed as mg chlorophyll g^{-1} fresh weight by using the following equations (4-7):

$$Chl"a"(\mathrm{mgg^{-1}f.w}) = [12.7x\mathrm{D}_{663} - 2.69x\mathrm{D}_{645}]x\frac{vw}{1000}$$

$$Chl"b"(\mathrm{mgg^{-1}f.w}) = [22.9x\mathrm{D}_{645} - 4.68x\mathrm{D}_{663}]x\frac{vw}{1000}$$

$$TotalChl(\mathrm{mgg^{-1}f.w}) = \mathrm{D}_{652}x1000x\frac{vw}{1000}$$

Where D = optical density; v = final volume of 80% acetone; w = weight of sample; f.w. =fresh weight of the sample

Estimation of Proline. Proline was extracted from the leaves and estimated by the methods of Bates et al. (Bates et al. 1973). Homogenates of the leaf samples were prepared in 3% sulphosalicylic acid. Pink colour was developed by a reaction with glacial acid and ninhydrin. The colour was separated in toluene layer and intensity of the colour was measured at 529 nm., spectrophotometrically.

Estimation of Protein. Protein content of the plants (untreated and treated) with BS was estimated by the method of Lowry et al. (Lowry 1951). To avoid interference by pigments, the trichloroacetic acid (TCA) precipitate was washed twice with 90% acetone. BSA was used as the standard. Absorbance was recorded spectrophotometrically at 660 nm.

Estimation of Soluble Sugars. Soluble sugars were estimated by the method of Montgomery (Montgomery, 1970). Plant tissue (0.2 g) was homogenized in 2.0 mL of 80% ethanol (10% homogenate) using a Potter Elvehjem glass homogenizer and centrifuged at 3023 g for 20 min. To 0.1 mL supernatant was added 0.9 mL water, 0.1 mL of 80% phenol, and 5.0 mL conc. H_2SO_4, and the mixture was allowed to stand at room temperature for 30 min. The absorbance was measured spectrophotometrically at 490 nm.

Micrograph study. Scanning Electron Microscopic (SEM) Study. The changes in external morphology of root; shoot and leaf of *C. arietinum* seedling were studied using a scanning electron microscope (SEM). Root, shoot and leaf specimens were prepared for SEM using the protocol adapted from standard procedures (O'Brien et al. 1981). The fresh root, shoot and leaf samples (of 5 mm square from similar middle portion) of nine root, shoot and leaves each from the control and cadmium treatments were dissected and immediately fixed in a solution of 2% gluteraldehyde prepared in a 0.1M sodium phosphate buffer (pH 7.0) for 12 h at room temperature. The specimens were washed three times in 25 mM sodium phosphate buffer (pH 6.8) overnight at 4 ^{0}C and then dehydrated to

absolute ethanol using 10 minutes series samples of 25%, 50%, 75%, 95% and 100% ethanol and then stored at -20 ^{0}C until required time of examination, the specimens were rinsed, post fixed in 2% osmium tetraoxide, critical point dried and sputter coated with gold palladium before being mounted aluminum stubs. The specimens were viewed and photographed using a 15 KV scanning electron microscope (HITACHI, S-530, SEM and ELKO Engineering).

Statistical analysis. The data were analyzed statistically by Duncan Multiple Range Test (DMRT) and correlation study. Significance between control and treatment was compared at 0.05 probability levels.

RESULTS AND DISCUSSION

The present experimental results depicted in the Figure 1(a) and it is clearly indicate that during five days of incubation period almost all treated seeds showed 100% germination except treatment T_6. All the treatments from T_1 to T_4 showed complete germination (100%) after 3rd days of incubation. But after 24 hours of incubation highest germination was recorded for treatment T_4 (92%), followed by T_1 (88%), T_2 and T_5 (84%); T_3 (80%) and lowest in T_6 (60%) (Table 1). But after 48 hours highest germination was found at T_1 and T_4 (96%) followed by T_2, T_3 and T_4 and little improvement in T_6 treatment. Again after 72 hours, T_5 and T_6 showed 88% and 76% of germination respectively. But the maximum germination of T_6 was recorded about 84% after five days of incubation (Figure 1a). During germination it has been found that seed coat changes to brown with respect to control. This is probably due to oxidation of phenol in the seed coat (Rashid et al. 2005).

Phenols are commonly present in many parts of the seeds including the coat and embryo, and they are primarily related to the regulation of seed germination as well as defense against herbivores and pathogen infestation (Muscolo et al. 2004, Rashid et al. 2005). From the growth (root and shoot length) study it has been found that all treatment showed steady increase of root and shoot growth like control (Table 2). But after eight days of incubation root growth was completely stunned and shoots growth stopped after ninth days of incubation with respect to the control treatment. This pattern of root and shoot growth was little difference in different treatments.

It is interesting to note that during eighth days of incubation in control no further growth of root was found, but during this incubation period treatment T_3, T_4 and T_5 and T_6 showed higher root length than control but T_1 and T_6 showed little lesser root length than control. Almost similar finding was reported by Ashenden et al. (2003). Again all treatment showed reduced shoot length than control during eighth days of incubation period. The growth study showed a most interesting observation which indicate root and shoot growth almost constant during eleventh days of incubation in treatment T_1 to T_3. But such constant growth was started from ninth days of incubation for treatment T_5 and tenths days of incubation for the treatment T_4 and T_6 (Table 2).

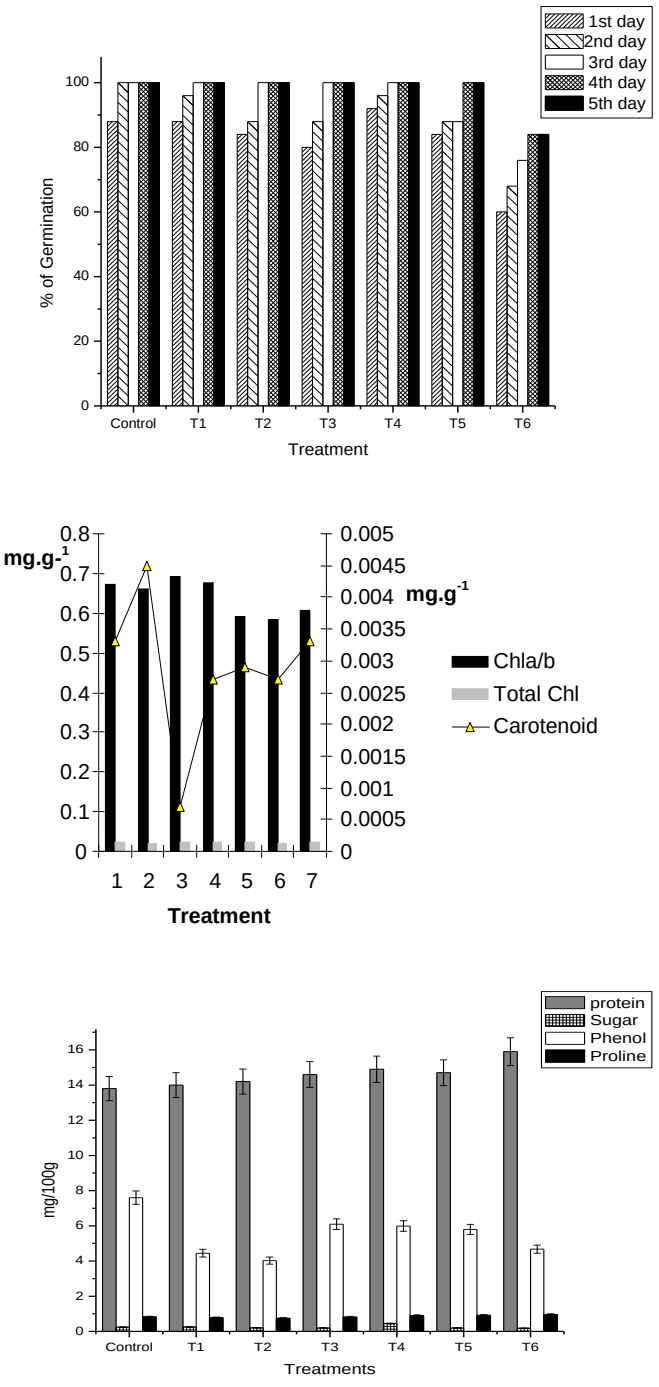

Figure 1. % germination of plant material (up), plant pigments (middle), and biochemical parameters (down) under different treatment after twelve day of sowing.

Table 1. Variation of root and shoot length (cm) of gram seedling during experimental period in different treatments.

Observation Time	Control	T_1	T_2	T_3	T_4	T_5	T_6
1st day	**0.63**	**1.17**	**1.10**	**1.50**	**0.80**	**1.20**	**0.67**
	0.53	0.77	*1.30*	*1.23*	*0.17*	*1.10*	*1.20*
2nd day	**1.23**	**1.33**	**1.33**	**1.70**	**1.05**	**1.45**	**1.05**
	1.03	1.10	*1.33*	*0.80*	*0.40*	*1.15*	*1.40*
3rd day	**1.55**	**1.5**	**1.65**	**1.80**	**1.25**	**1.70**	**1.30**
	1.40	1.45	*1.60*	*1.45*	*0.60*	*1.40*	*1.60*
4th day	**1.85**	**1.90**	**2.00**	**2.05**	**1.70**	**2.05**	**1.40**
	1.65	1.70	*2.00*	*1.65*	*0.50*	*1.70*	*1.80*
5th day	**2.05**	**2.10**	**2.25**	**2.40**	**2.30**	**2.40**	**1.60**
	1.95	2.00	*2.25*	*1.90*	*1.30*	*1.90*	*2.00*
6th day	**2.20**	**2.20**	**2.55**	**2.45**	**2.45**	**2.30**	**2.45**
	2.15	2.05	*2.30*	*2.05*	*2.05*	*1.45*	*2.00*
7th day	**2.35**	**2.25**	**2.55**	**2.45**	**2.45**	**3.05**	**2.05**
	2.20	2.10	*2.30*	*2.30*	*1.70*	*2.25*	*2.30*
8th day	**2.45**	**2.30**	**2.55**	**2.55**	**2.70**	**3.10**	**2.20**
	2.55	2.20	*2.45*	*2.45*	*1.90*	*2.35*	*2.45*
9th day	**2.45**	**2.30**	**2.55**	**2.55**	**2.70**	**3.15**	**2.25**
	2.65	2.50	*2.60*	*2.60*	*2.05*	*2.50*	*2.55*
10th day	**2.45**	**2.40**	**2.60**	**2.55**	**2.85**	**3.15**	**2.40**
	2.70	2.85	*2.70*	*3.00*	*2.15*	*2.60*	*2.70*
11th day	**2.50**	**2.45**	**2.70**	**2.75**	**2.85**	**3.15**	**2.40**
	2.70	2.85	*2.70*	*3.05*	*2.30*	*2.60*	*2.75*
12th day	**2.50**	**2.45**	**2.90**	**2.80**	**2.90**	**3.15**	**2.40**
	2.70	2.85	*2.75*	*3.05*	*2.35*	*2.70*	*2.85*

OBS.: Bold and Italic number indicates Root length and Shoot length respectively.

The results also showed that the content of chlorophyll reduced significantly in all treatments over control (Figure 1(b). Moreover, the reduction of chlorophyll-a is higher than chlorophyll-b under all treatments except T_2. The status of total chlorophyll was recorded highest in treatment T_2 followed by T_6 and lowest in treatment T_5. The reduction of chlorophyll content is due to the deposition of dust particles and iron dusts on the leaves (Czaja 1961, Parthasarthy et al. 1975, Lerman 1992, Das et al. 2012). Moreover soot particles reduced the growth of plants and subsequently inhibition of photosynthesis which affects the chlorophyll metabolism due to presence of cadmium in soot (Somashekaraiah et al. 1992). Similar observation reported by Ara et al. (1996) and Khalid et al. (1996). However, previous research has shown that at high concentrations, many of the pollutants present in exhaust gases can be damaging to plants (Honour et al. 2009, Ackerly and Bazzaz 1995, Grantz et al. 2003, Wellburn 1990).

On the other hand correlation study indicate that total chlorophyll level significantly related with total phenol ($p<0.05$) and proline ($p<0.05$) but protein also depends on chlorophyll level but not significantly (Table 4). Almost similar results reported by Gratani et al. (2000) and they showed positive relationships between traffic density and photosynthetic activity, stomatal conductance, total chlorophyll content and leaf senescence of *Quercus ilex* L. in Rome. The shoot length showed negative different relationship with carotenoid, but significantly ($p<0.05$) negatively related with sugar. Proline showed significant ($p<0.05$) positive relationship with total chlorophyll ($r = 0.838$, $p<0.05$) and protein ($r = 0.858$, $p < 0.05$).

The vigor index of seedling in the all treatments increased with increasing concentration of carbon soot except treatment T_4 and T_6 (Table 3). The total protein, sugar, phenol and proline range from 13.8-15.9 g/100g; 18.2-24.9g/100g; 4.02-6.1g/100g and 7.51-9.5g/100g respectively (Figure 1(c). The results indicate that the high dose of diesel exhaust soot has more negative impact than petrol exhaust soot on both protein and sugar level. This is probably due to the reduction of leaf surface area, destroying individual cells and finally reducing the plants ability to produce food (Shafiq and Iqbal 2009, Shafiq and Iqbal 2012). Moreover, Shafiq and Iqbal (2009) expressed in their paper that automobile emission significantly reduced the productivity, leaf area and leaf dry weight of *Guaiacum officinale* L., *F. bengalensis* and *Eucalyptus* sp. At the polluted site of Karachi as compared to control. However, from Figure 1(c) it is also clear that sugar and phenol content greatly influenced by soot than protein and proline level.

From the micrograph study (Figures 2.1-2.9.) it has been found that in control, distinct hexagonal structure of xylem and phloem in root and shoot (Figures 2.1 and 2.2) and normal structure of stomata (Figure 2.3). It is interesting to note that treated plant (T_1) did not show distinct structure of xylem and phloem (Figures 2.4 and 2.5) and also abnormal structure of stomata with enlarged guard cell (Figure 2.6). Similar observation was reported by Kazmi et al. (2002) with higher frequencies of elongated stomata (with particulate matter) in the leaves of roadside plants (*Albizzia Jublibrissin* and *Ficus bengalensis*) compare to those from unpolluted locality probably due to vehicle exhaust emission.

CONCLUSION

From the present study it may be concluded that diesel exhaust soot particles has much negative impact on germination and biochemical changes on *C. arietinum* over petrol exhaust soot particles. So far as mutual

understanding between plant and the environment is concern, it is essential to maintain a sustainable clean environment. Present study also confirms that the black soot which is originated from vehicle exhaust not only pollutes our air but plant community and their physiological pathways affected too.

Table 2. Effect of vehicle black soot on gram seed germination.

Sample collection from	Treatments	Concentration (g)	% of germination	Root length (cm)	Shoot length (cm)	SVI
15 years old vehicle (petrol)	T_1	0.04	100	2.029 ± 0.05^a	2.035 ± 0.08^a	530 ± 4.04^a
	T_2	0.08	100	2.440 ± 0.05^a	2.190 ± 0.04^a	545 ± 5.20^a
	T_3	0.10	100	2.295 ± 0.10^b	2.128 ± 0.03^b	575 ± 4.62^b
15 years old vehicle (diesel)	T_4	0.04	100	2.167 ± 0.01^a	1.456 ± 0.02^a	520 ± 7.51^a
	T_5	0.08	100	2.488 ± 0.02^c	1.975 ± 0.03^a	585 ± 5.20^c
	T_6	0.10	100	1.848 ± 0.03^d	2.133 ± 0.03^a	441 ± 5.77^a
	Control	0	100	2.018 ± 0.04^a	2.017 ± 0.06^a	520 ± 3.61^a
	CD at 5%		0.0	0.192	0.155	34.779

*Significant at p (level of probability) less than 0.05. Each value is the mean ± SE of three replicates.

Table 3. Chemical components that have been found in diesel exhausts.

1	Acetaldehyde	21	Ethyl benzene
2	Acrolein	22	Fomaldehyde
3	Aniline	23	Inorganic- pb
4	Antimony compounds	24	Mn – compounds
5	Arsenic	25	Hg – compound
6	Benzene	26	Methanol
7	Beryllium compounds	27	Methyl ethyl ketone
8	Biphenyl	28	napthalene
9	Bis (2- ethylhexyl) phthalate	29	Nickel
10	1,3 – butadiene	30	3 – nitrobenzene anthrone
11	Cadmium	31	4 – nitrodiphenyl
12	Chlorine	32	Phenol
13	Chlorobenzene	33	Phosphorus
14	Chromium compounds	34	Polycyclic organic matter including (PAHS)
15	Co- compounds	35	Propionaldehyde
16	Cresol isomers	36	Selenium compound
17	Cyanidine compounds	37	Styrene
18	Dibutyl phthalate	38	Toluene
19	1,8 – dinitropyrene	39	Xylene isomer and mixture
20	Dioxins and dibenzofuran		

Table 4. Correlation between root length, shoot length, carotenoid, phenol, sugar and protein

	RL	SL	T.Chl	CAR	PRO	SUG	PHE
SL	-0.388						
T.Chl	0.200	-0,127					
CAR	-0.584	0.109	0.113				
PRO	-0.271	-0.078	0.750	0.114			
SUG	0.148	-0.831*	0.144	0.156	-0.038		
PHE	0.493	-0.194	0.782*	0.145	0.186	0.368	
PRO	0.026	-0.295	0.838*	0.278	0.858*	0.113	0.457

*P<0.05; **p<0.01; Note: RL (Root length), SL (Shoot length), CAR (Carotenoid), PRO (Protein), SUG (Sugar), PHE (Phenol) and T.Chl (Total chlorophyll).

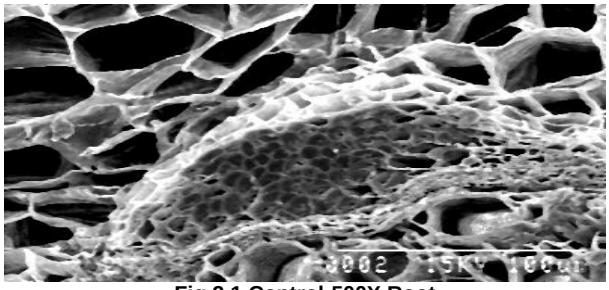
Fig 2.1 Control-500X Root

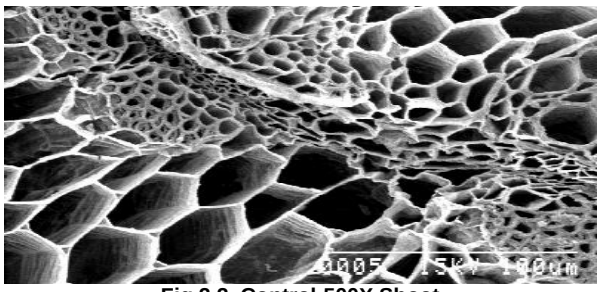
Fig 2.2 Control-500X Shoot

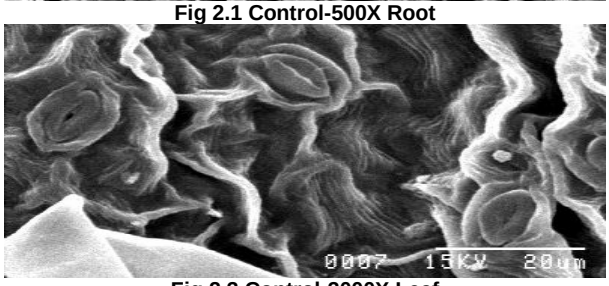
Fig 2.3 Control-3000X Leaf

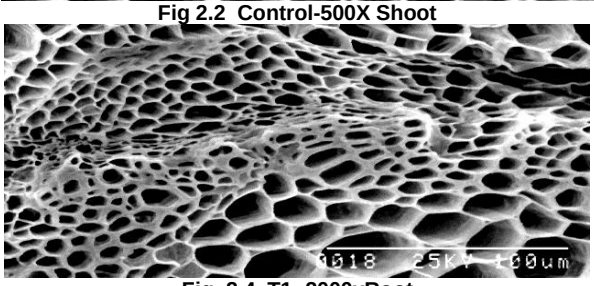
Fig. 2.4 T1: 2000xRoot

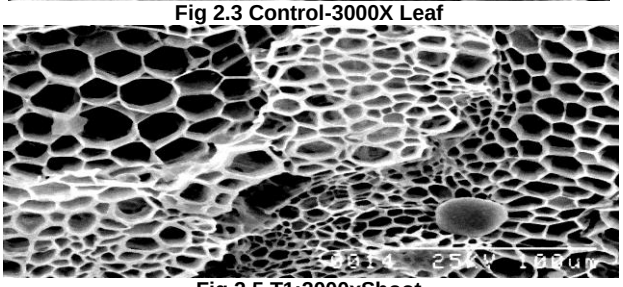
Fig.2.5 T1:2000xShoot

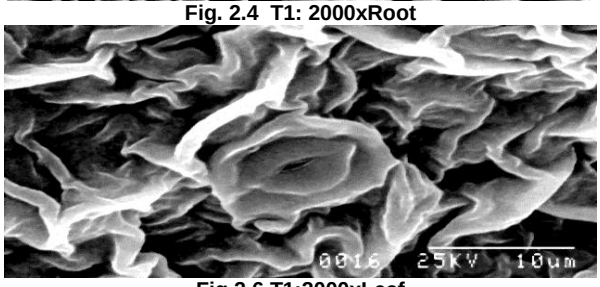
Fig.2.6 T1:2000xLeaf

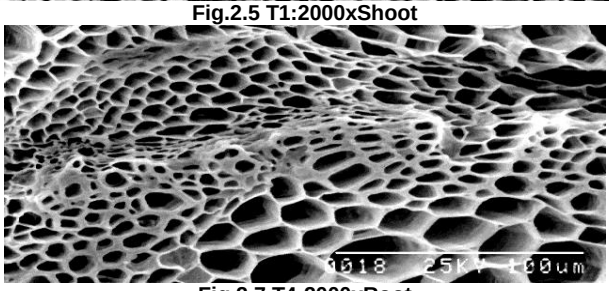
Fig.2.7 T4:2000xRoot

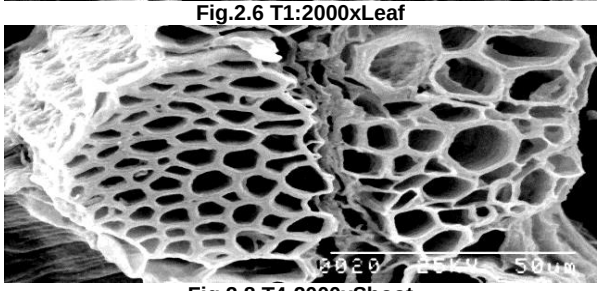
Fig.2.8 T4:2000xShoot

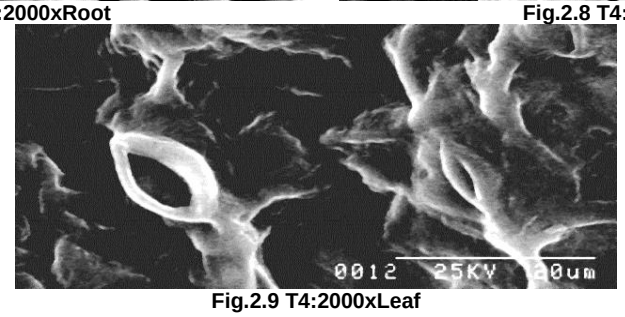

Fig.2.9 T4:2000xLeaf

Figure 2. Scanning Electron Micrograph study of seedling (root, shoot and leaf) under different treatment.

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