Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2012, 4(12):5114-5120

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Impact of 2,4-D sodium salt and automobile exhaust on the photosynthetic pigment and ascorbic acid content of *Cymbopogon martinii*

Annapurna Singh[1], Nandita Singh[2] and M. Yunus[1]*

[1]*Dept. of Environmental Science, Babasaheb Bhimrao Ambedkar (central) University, Lucknow 226 025. U.P. (INDIA)*
[2]*Eco- Auditing Group, National Botanical Research Institute-CSIR, Lucknow 226 001. U.P. (INDIA)*

ABSTRACT

Plants are exposed to a variety of anthropogenic gaseous and liquid pollutants which change the cellular biochemistry of plants directly or indirectly leading to reduced morphological growth. In this study we tried to correlate the combined impact of 2, 4-D sodium salt and automobile exhaust on the foliar chlorophyll and ascorbic acid content of Cymbopogon martinii. Two sites 1: B.B.A.U. campus as control site (less polluted) and 2: NBRI campus at Sekandarbagh crossing (one of the busiest crossings of Lucknow City) were selected based on the vehicular density. Three concentrations viz. 30mg/l, 100mg/l and 300mg/l of 2,4-D sodium salt were given to the plants. It was observed that exposure to 2, 4-D sodium salt led to an increase in ascorbic acid content from 4±1.0 µg/g f.w.in T3 (30mg/l of 2, 4-D) to 16.83±1.76 µg/g f.w.in T5 (300mg/l of 2,4-D) at the control site and from 22.23±3.35 µg/g f.w. in T3 to 38.20±1.31 µg/g f.w.in T5 at the polluted site. However, foliar pigment content decreased significantly which corroborated with the deterioration of other plant growth parameters like total photosynthetic area and stem girth. The ultra structural changes in foliar morphology revealed that these plants can be used as biomarkers for auto exhaust pollution.

Keywords: *Cymbopogon martinii*, chlorophyll, ascorbic acid, 2,4-D.

INTRODUCTION

The foliar parts of the plants are the initial receptors of the brunt of any sort of atmospheric pollutants/ contaminants. Automobile air pollution is a ubiquitous menace affecting almost every crop in today's world. The initial effect of this pollution is mostly visible on the leaves in the form of chlorosis, necrosis and tip burn etc. However atmospheric pollutants are not the only menace affecting plants. They often have to bear several other solid, liquid or gaseous pollutants [16,25]. Herbicides are one among them. 2, 4-D is the most widely used herbicide the world over. It is mainly used to kill broadleaf weeds which compete with monocotyledonous crops for food and nutrition[6]. Chlorophyll is found in the chloroplasts of green plants and is called photoreceptor. Chlorophyll itself is actually not a single molecule but a family of related molecules, designated as chlorophyll a, b, c, and d. Chlorophyll a and b are the major constituents of the photosynthetic apparatus [28]. Accessory pigments include xanthophylls and carotenoid. Carotenoids are a class of natural fat-soluble pigments found principally in plants, algae, and photosynthetic bacteria, where they play a critical role in the process of photosynthesis [8]. Chlorophyll is also one of the most sensitive biomolecules quite prone to degradation by light, heavy metal pollution, acid rain, air pollution and chemical exposure [20,24,30]. Any change in the chemistry of any of the macromolecules of this system leads to the destruction of chlorophyll molecules and ultimate loss of photosynthetic activity [38].

Ascorbic acid is a very important antioxidant compound synthesised in response to oxidative stress. It is thus regarded as an indicator of tolerance level of plant to oxidative damage [40].

Cymbopogon martinii is a perennial aromatic grass which is cultivated in India, Brazil, Paraguay, Madagascar, Guatemala and Indonesia for its essential oil rich in geraniol and is used in treating convalescence, gastrointestinal ailments and cough syrup formulations [9]. The present study elaborates the effect of 2,4-D sodium salt and automobile exhaust on the chlorophyll and ascorbic acid content of *Cymbopogon martinii* and its ultimate impact on plant health.

EXPERIMENTAL SECTION

Study site

Lucknow, the capital city of Uttar Pradesh, is located between 26.50^{o}N and 80.50^{o}E was chosen for this study. For our Transfer Experiment study, two sites were selected based on the average vehicular density. Site1: control site, the campus of Babasaheb Bhimrao Ambedkar University located about 1.5 km off Raebareli Road (very low number of auto-mobiles invariably petrol driven with vehicular density 410 automobiles/h) and Site 2: the highly polluted site, the Sikanderbagh crossing, one of the busiest crossings of Lucknow (with a large number of petrol, CNG and diesel driven auto-mobiles with vehicular density 6632 automobiles/h).

Experimental set up

Seeds of *Cymbopgon martinii* var. Trishna, procured from CSIR-CIMAP, Lucknow were grown in earthen pots 12"size filled with garden soil and cowdung manure in the ratio of 2:1. The physio-chemical characteristic of the soil is tabulated (table 1). Equal numbers of saplings (5 in each pot) were grown to keep the nutrient availability almost the same to each plant. After 30 days, when the saplings were quite established, one set of fifteen pots was placed in the campus of NBRI-a CSIR Lab at Sikunderbagh crossing (site 2), while the other set of fifteen pots was kept at BBAU campus (site1).

Both the sets of plants were left to get exposed to auto exhaust pollutants, which differed significantly at sites 1 and 2. After a pre exposure of 45days (1November-15December) were foliar sprayed with three concentrations of 2,4-D sodium salt (herbicide) T3-30mg l^{-1}, T4-100mg l^{-1} and T5-300mg l^{-1}. Treatments1 (T1) and 2 (T2) were kept as standard control (no spraying) and control sprayed with equal volumes of distilled water. The volume of herbicide sprayed on each plant was based on the count of leaves on each plant. The volume of herbicide was kept the same as the number of leaves on plant to ensure that almost 1ml of herbicide reached each leaf. After 48 hours of spray leaves were sampled, for laboratory analysis.

Physico-chemical characteristion of soil

Soil pH and conductivity were analyzed by following the method of Piper [11] whereas total organic carbon was determined by the method of Walkely and Black [5]. Available nitrogen in the soil was measured using a nitrogen analyser and HCl as titrant and available phosphorus [38] using a UVIKON Spectrophotometer. The metals e.g. zinc, iron and copper were estimated using an atomic absorption spectrophotometer (Varian AA240FS). The data are presented in Table 1.

Air pollution monitoring

Air pollutants (SPM, RSPM, SO_2, NO_X and Ozone) load at both the sites (Site 1 and Site 2) were measured using a dichotomous high volume sampler (Envirotech make-APM 460) and an ambient ozone analyzer. SPM and RSPM were measured by sucking air through a whatman glass fiber filter paper (45µm pore size). SPM was measured by weighing the amount of dust over the filter paper and RSPM was measured by weighing the dust collected at the bottom of the vortex of the high volume sampler. SO_2 was measured by West and Gaeke [26] method. Nitrogen oxide was measured using the method of Jacobs and Hochheiser [22]. 2B Technologies 106 L ozone monitor was used to measure the ozone concentration in ambient air. The values of the oxides of nitrogen and sulphur and ozone are represented in microgram per meter cube.

Biochemical assay

The photosynthetic pigments were extracted by the method of Arnon [14] using 80% cold acetone with a small amount of sodium carbonate. Equal number of leaves randomly from all the sides of the plant were harvested. 500 mg of leaf sample was crushed and the volume was made to 5 ml of acetone. This was then centrifuged at 4^{o}C at 5,000 x g. The optical density of clear pigment extract was recorded for chlorophyll a, chlorophyll b, total chlorophyll and carotenoid at 480 nm, 510 nm, 645 nm and 663 nm for all the three replicates for various treatments using a carry win spectrometer. All the experiment was carried in the dark to avoid the pigment degradation. Chlorophyll a and chlorophyll b was calculated using the formula given by Maclachlam and Zalik [29] and the amount of carotenoid was calculated using the formula given by Duxbury and Yentsch [7].

Ascorbic acid was estimated following the method proposed by Keller and Schwager [39]. 100 mg of plant material was homogenized in 3ml of extracting solution followed by its centrifugation at 5,000 rpm to get clear supernatant. 1ml of the extract thus prepared was added with 5ml of di chloro phenol indo phenol (DCPIP) to give a pink colour and the absorbance of the same is recorded at 520nm using a CARY UV-Visible spectrophotometer.

Sample preparation for scanning electron microscopy

In order to carry out Scanning Electron Microscopic studies (SEM), leaf samples of about same age were harvested and fixed in 2.5% of gluteraldehyde. Then the pieces were cut into squares of approximately 0.3 cm^2. These samples were fixed only under field condition to avoid any post plucking damage or changes. Later, these samples were brought to the laboratory and dehydrated in ethanol series (30–100%), and further drying was carried out in a critical point drier with liquid CO_2 at 1072-psi pressure and 31.4 ◦C temperature. Materials were then coated with thin conductive film of gold about 200 °A in thickness in an ion sputter coater and then examined under scanning electron microscope

Statistical analysis

Mathematical values of all the assays are based on the mean of three replicates. Results of all treatments at both the sites were expressed as mean (n) ± S.D. where n=3. For plant materials two way analysis of variance (ANOVA) was performed. Least Significant Difference was calculated at 0.05% level. The values of the various air pollutants have been averaged out of the total values obtained over 6 hour period in every season as per United Nations Environment Programme (UNEP).

RESULTS AND DISCUSSION

Air Quality

The ambient air quality data of selected sites are presented in Table 2. For all the comparisons the permissible limit laid down by the Central Pollution Control Board, New Delhi (CPCB) has been followed. Concentration of RSPM and SPM at site 2 were 397.89 ± µg $m^{-,3}$ and 738.79 ± µg m^{-3} which were higher than the permissible limit i.e. 150 µg m^{-3} for RSPM and 500 µg m^{-3} for SPM respectively. The concentration of SO_2 (38.95±0.52 µg m^{-3}) and NOx (55.39±9.64 µg m^{-3}) were also substantially higher at site 2 than at site 1, the control site (SO_2=11.82±0.14 µg m^{-3}), (NOx=13.1±0.21 µg m^{-3}). The values for ozone were also higher at site 2(64.75±3.75 µg m^{-3}) than at site 1(50.87±3.45 µg m^{-3}), however the concentrations of SO_2, NOx and O_3 were within the permissible limit. These results indicate that air pollution load at site 2 was substantially higher than the control site.

Photosynthetic pigments

The levels of photosynthetic pigments presented in Table 4 were found significantly lower for site 2 compared to site 1. Comparing each treatment from site 1 to site 2, Chl a at site 2 was recorded 0.23, 0.131 and 0.04 mg/g f.w. lower for the treatments with 30 , 100 and 300 mg/l of 2, 4 -D respectively. Similarly chl b at site 2 was recorded 0.055,0.068 and 0.083 mg/g f.w.lower than at site 2 for the treatments with 30 , 100 and 300 ppm of 2, 4 -D. Similar trends were observed for total chlorophyll and carotenoid as well. The differences between the two sites and different treatments are statistically significant at 0.05% significance level (F=15.176 and 22.265 for chlorophyll a, F=48.917 and 153.68 for chlorophyll b, F=34.082 and 64.2000 for total chlorophyll and F= 89.823 and 209.539 for carotenoid content).

This indicates that 2,4-D sodium salt adversely affects the chlorophyll content in the plants pre-exposed to automobile exhaust. High level of SPM and RSPM may be detrimental for the effect as these may block the stomatal pores and reduce the denovo synthesis of chlorophyll molecules by reducing the sunlight. Atmospheric dust has been well known for reducing the light required for photosynthesis and increase the leaf surface temperature due to obstructed evaporation [36]. Also, the particulate matter, moisture from transpiration and acidic gases like SO_2 and NOx tend to form sulphites and nitrites which enter the cell sap and degrade biomolecules [12]. Not only the pollutants from automobile exhaust but 2,4-D sodium salt also significantly alters the foliar biochemistry. Prajapati and Tripathi [32-35] have earlier reported that dusts that carry toxic soluble salts also have adverse effects on plants. Degradation of chlorophyll upon exposure to herbicide is indicated by a sequential decrease in chlorophyll pigments from T3 to T5 at both the sites. Chlorophyll degradation upon exposure to stress has earlier been reported [17-18]. The value of chlorophyll a is always higher than chlorophyll b in higher plants Mane *et al.* [3]. High value of this ratio indicates better tolerance to air pollution and *vice-versa* [22]. *Cymbopogon martinii* has chl a/chl b ratio higher than 1 showing an above average tolerance towards air pollution.

Ascorbic acid

Unlike chlorophyll ascorbic acid content (Table 5) increases with an increase in the concentration of the herbicide sprayed. Also, its concentration further shoots up in plants kept at the polluted site. Ascorbic acid, which is an

indicator of the tolerance index of plants [1, 39], shows an increase with the increase in 2,4-D and air pollutants. The differences in the amount of foliar ascorbic acid between the two sites are statistically significant at 0.05% significance level (F=4.266 and 14.022). This indicates the capacity of the plant to put up an antioxidant defense against chemical stress. Ascorbic acid is a potent scavenger of reactive oxygen species in plasma and extracellular compartments of the kidney. It scavenges and destroys free radicals in combination with vitamin E and glutathione [23].Similar results were reported by Zarzecka and Gugala [17] when potato tubers were exposed to herbicide. Despite of an increased production of ascorbic acid antioxidant the plant canopy and stem girth seem to decrease. This indicates the high intensity of stress imposed upon the plants due to the duel factors.

Plants treated with 2, 4-D show a sharp decrease in canopy (from 63.33±2.89cm in T1 to 50.00±5.0cm in T5) and stem girth (from 14±1.73mm in T1 to 12.33±0.58mm in T5) at the control site(Table 3). The decrease further aggravated at site 2 where air pollution acted along with 2,4-D sodium salt. At site 2 plant canopy decreased from 62.33±2.52cm in T1 to 46.0±1.73cm in T5. Whereas the stem girth decreased from 13.0±1.73mm T1 to 10.67±1.15mm in T5.

A reduction in stem girth and plant canopy is sure to result into less healthy plants which lead lower biomass. The decrease in the growth parameters at the polluted site may be due to high dust load. Similar findings have been reported by Tripathi and Prajapati [31], Rae et al. [2] and Saha and Padhy [13].

Table 1: Physico-chemical properties of garden soil used for the study

Ph	8.24±0.089
Electrical conductivity(µS cm^{-1})	154.64±20.39
Available nitrogen (%)	0.0336±0.0095
Available phosphorous(%)	0.00814±2.3
Total organic carbon (%)	0.448±0.7
Metals(µg/g)	
Sodium	18.29±2.48
Copper	2.7±0.2
Iron	9.62±0.143
Manganese	11.1±0.085
Zinc	1.73±0.2

Table 2: Ambient air quality at the selected sites

Pollutants (in µg/m^3)	Site 1	Site 2	CPCB standards (1995) 24hrs(in µg/m^3)
SO_2	11.82±0.14	38.95±0.52	120.00
NO_2	13.1±0.21	55.39±9.64	120.00
SPM	200.65±3.62	738.79±8.5	500.00
RSPM	106.56±2.10	397.89±4.5	150.00
Ozone	50.87±3.45	64.75±3.75	100.00(8 hr)

Table 3: Changes in morphological aspects at the selected sites

	Plant canopy(cm)		Stem girth(mm)	
TrishnaT1	63.33±2.89	59.67±0.58	14±1.53	12.67±1.53
TrishnaT2	64.33±2.08	60±1	14.67±0.53	13.34±1.55
TrishnaT3	60±1	56±1.73	12.83±2.5	11.33±0.58
TrishnaT4	56±1.73	49±1.73	12.33±1.52	10.67±1.0
TrishnaT5	50±2	45±1	11.33±1.15	10±0.58

Table 4 (a): Comparison of chlorophyll a and b content of leaves of *Cymbopogon martinii* at the two selected sites

Treatments	**Chlorophyll a(mg/g f.w.)**		**Chlorophyll b (mg/g f.w.)**	
	Site1	Site2	Site1	Site2
Tirshna T1	0.478±0.0036^{*0}	0.307±0.0008^{*0}	0.183±0.003^{*0}	0.137±0.035^{*0}
Tirshna T2	0.508±0.003^{*0}	0.339±0.033^{*0}	0.216±0.025^{*0}	0.141±0.0003^{*0}
Trishna T3	0.466±0.033^{*0}	0.236±0.0003^{*0}	0.144±0.0036^{*0}	0.089±0.001^{*0}
Trishna T4	0.265±0.0006^{*0}	0.133±9.75E-05^{*0}	0.124±0.0015^{*0}	0.0556±0.0001^{*0}
Trishna T5	0.124±0.001^{*0}	0.084±0.12^{*0}	0.120±0.056^{*0}	0.0375±0.00013^{*0}

Values expressed as mean± SD=Significant difference between rows (treatments) at 0.05% significance level.*
Values expressed as mean± SD^0=Significant difference between columns (sites) at 0.05% significance level.

An uneven epicuticular surface was observed in the leaf from control site (Figure 1), while at the polluted site, the cuticular surface was even and almost flat. In the leaf collected from site 2 the dust particles coming out of

automobile exhaust were embedded into the epicuticular wax and thus formed a crust on the foliar surface (Figure 2). As far as stomata are concerned, in control population, stomata are slightly sunken because of elevated outer stomatal edges and were widely open (Figure 1). While in polluted condition, stomata were severely damaged. The stomata were occluded by the particles on the leaves of polluted site. Similar disruption of foliar morphology due to air pollution was reported by Verma and Singh [4].

Table 4 (b): Comparison of chlorophyll a content of leaves of *Cymbopogon martinii* at the two selected sites

	Total chlorophyll (mg/g f.w.)		Carotenoid (mg/g f.w.)	
Treatments	Site1	Site2		
Tirshna T1	0.661±0.005^{*0}	0.44±0.06^{*0}	0.205±0.021^{*0}	0.158±0.009^{*0}
Tirshna T2	0.724±0.008^{*0}	0.48±0.0087^{*0}	0.218±0.008^{*0}	0.165±0.005^{*0}
Trishna T3	0.61±0.0071^{*0}	0.325±0.001^{*0}	0.176±0.006^{*0}	0.124±0.008^{*0}
Trishna T4	0.389±0.0021^{*0}	0.189±9.59E-05^{*0}	0.17±0.004^{*0}	0.109±0.007^{*0}
Trishna T5	0.244±0.25^{*0}	0.122±0.001^{*0}	0.15±0.002^{*0}	0.094±0.006^{*0}

Values expressed as mean± SD=Significant difference between rows (treatments) at 0.05% significance level.*
Values expressed as mean± SD^{0}=Significant difference between columns (sites) at 0.05% significance level.

Table 5: Comparison of ascorbic acid content of leaves of *Cymbopogon martinii* at the two selected sites

Ascorbic acid(µg/g f.w.)		
Treatments	Site 1	Site 2
Trishna T1	2.96±0.94*	9.57±3.76*
Trishna T2	1.0±0.50*	7.03±1.59*
Trishna T3	4±1.0*	22.23±3.35*
Trishna T4	7.07±1.10*	35.21±0.95*
Trishna T5	16.83±1.76*	38.20±1.31*

Values expressed as mean± SD=Significant difference between columns (sites) at 0.05% significance level.*

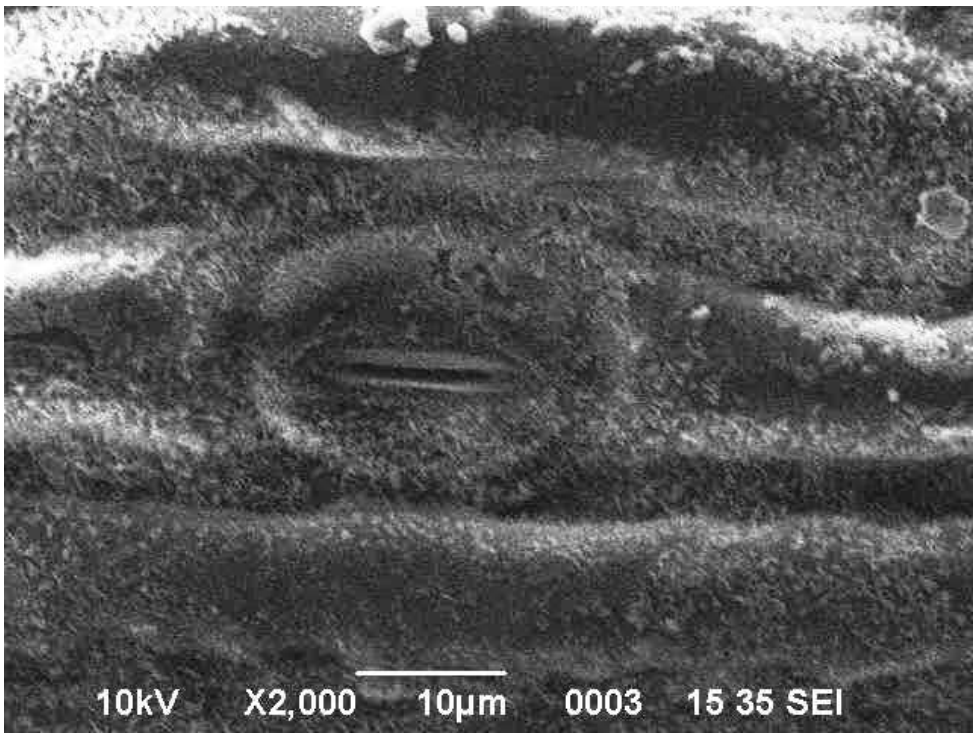

Fig.1 Scanning electron microscope view of a section of Trishna leaf at site 1

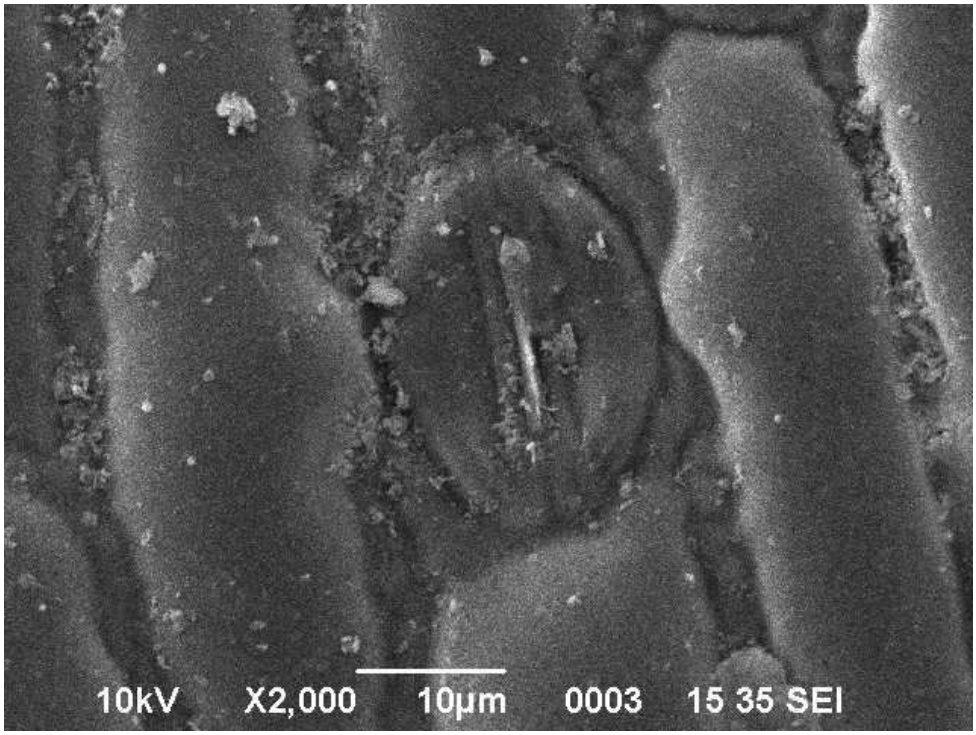

Fig.2 Scanning electron microscope view of a section of Trishna leaf at site 2

CONCLUSION

Thus from our study we conclude that pollutants from automobile exhaust exert a negative impact on the foliar content of photosynthetic pigments leading to its deterioration and this effect is further accelerated by 2,4-D sodium salt. Plant leaves however, produces ascorbic acid in order to combat the damage due to the combined effect of auto-exhaust and 2,4-D sodium salt. Still a decline in plant growth is observed, i.e., decline in stem girth and plant canopy. Thus, a decrease in chlorophyll and corresponding negative morphological changes and foliar surface aberrations show that this capacity of *Cymbopogon martinii* is insufficient compared to the quantum of damage done to it in the present scenario. Thus we conclude that the injury caused to plants due to the combined effect of air pollutants and herbicide surmounted the repair mechanism adopted by the plant *Cymbopogon martinii.*

Acknowledgements
The authors are thankful to the University Grants commission for providing fellowship to Ms. Annapurna Singh and Vice chancellor Babasaheb Bhimrao Ambedkar (central) university for providing the necessary space to carry out the experiments.

REFERENCES

[1] A Chauhan, and PC Joshi. *New York Sci. Journal*, **2010**, 3(2), 52-60.
[2] A Rai, K Kulshreshtha, PK Srivastava and CS Mohanty *Environmentalist*, **2010**, 30, 18-23.
[3] A V Mane, B A Karadge and J S Samant *J. Chem. Pharm. Res.*, **2010**, 2(3):338-347.
[4] A Verma and SN Singh. *Environ. Monit. Asses.*, **2006**, 120, 585–602.
[5] A Walkley and IA Black. *Soil Sci.*, **1934**, 37, 29-37.
[6] A Wise and A Senesac. Guidelines for application of 2,4-D and other phenoxy herbicides in proximity to grapes, **2004**.
[7] AC Duxbury and CS Yentsch. *J. Mar. Res.*, **1956**, 15, 19-101.
[8] ASH Ong and ES Tee. *Meth Enzymol*., **1992**, 213, 142 – 67.
[9] Central Institute of Medicinal and Aromatic plants, Lucknow. Palmarosa handbook. **2004**.
[10] Central Pollution Control Board. National Ambient Air Quality Sitandards, **1995**.
[11] CS Piper. Soil and plant analysis. Hans publication, Bombay, **1966**.
[12] DA Grantz, JHB Garner and DW Johnson. *Environ. Internat.*, **2003**, 29, 213-219.
[13] DC Saha and PK Padhy. *Atmos. Pollut. Res.*, **2011**, 2, 463-476.
[14] DI Arnon. *Plant Physiol.*, **1949**, 24, 1-15.
[15] DN Rao and F Leblanc. *Bryologist*, **1966**, 69, 69–75.

[16] HP Singh, DR Batish, S Kaur, RK Kohli and K Arora Zeitschrift für Naturforschung, **2006**, C, 61, 334-340.
[17] H Sixto; J M Grau ;N Alba; R Alia. *Forestry*, **2005**, 78, 93-104.
[18] K Abraham, K Raju, B Suresh and T Damodharam. *J. Chem. Parm.Res.,***2012**, 4(9),4112-4114.
[19] K Zarzecka and M Gugala. *Plant soil environ*. **2003**, 49(5), 237-240.
[20] KL Bignal, MR Ashmore and AD Headley., *Environ Pollut.,* **2008**,156**,** 332–340.
[10] M Agrawal, SK Singh, J Singh and DN Rao. *J. of Environ. Biol.,* **1991**, 211–222.
[22] MB Jacobs and S Hochheiser. *Anal. Chem.,* **1958**, 30, 426–428.
[23] M Inoue. Protective mechanisms against reactive oxygen species. In: Arias IM, Boyer, JL, Chisari FV, Fausto N, Schachter D, Shafritz DA, Editors, The liver: biology and pathobiology IV edn. Philadelphia; Lippincott Williams and Wilkins: **2001**; 282-290.
[24] MP Singh, SK Pandey, M Singh, PC Ram, and BB Singh. *Photosynthetica,* **1990**, 24, 623-627.
[25] N Kondo, Y Akiyama, M Fujiwara and K Sugahara. *Res. Rep.Nat. Environ. Study Jpn.,* **1980**, 11, 137–150.
[26] PW West and GC Gaeke. *Anal. Chem.,* **1956**, 28, 1816**–**1819.
[27] R Rai, M Agrawal and SB Agrawal. *Atmos. Environ.,* **2007**, 41, 9543-9554.
[28] R Tanaka and A Tanaka. *Biochem Biophys Acta.*, **2011**, (8), 968-976.
[29] S Maclachlam and S Zalik. *Can.J.Bot*., **1963**,41, 1053-62.
[30] S Oancea, N Foca, and A Airinei. *Analele Univ. Al. I. Cuza, Tom I, s, Biofizica, Fizică medicală şi Fizica mediului,* **2005**, 107-110.
[31] SK Prajapati and BD Tripathi. *Environ. Internat*., **2008**, 34(8), 1091-1096.
[32] SK Prajapati and BD Tripathi. *Environ. Monit. and Assess.*, **2008,** 139(1-3), 351-354.
[33] SK Prajapati and BD Tripathi. *Environ. Pollut*., **2008**, 151, 543-548.
[34] SK Prajapati and BD Tripathi. *J. Environ. Manag.*, **2008**, 88(4), 1343-1349.
[35] SK Prajapati and BD Tripathi. *J. Environ. Qual.*, **2008**, 37: 865-870.
[36] SK Prajapati. *Environ. Skeptics and Critics*, **2012**, 1(1), 12-22.
[37] S R Olsen and LE Sommers Phosphorus. In: *Methods of Soil Analysis.* Part 2: *Chemical and Microbiological Properties.* A.L. Page, R.H. Miller & D.R. Keeney (Eds). Madison, Wisconsin: American Society of Agronomy, **1982**, 403-427.
[38] SS Malhotra. *NewPhytol.,* **1977**,78(1), 101-109.
[39] T Keller and H Schwager. *Eur. J. Forestry Pathol.,* **1977**, 7, 338-350.
[40] YM Chen, PW Lucas and AR Wellburn. *Environ. Pollut.*, **1990**, 69, 1– 15.