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Response of *Cymbopogon martinii* to the combined effect of auto exhaust pollution and herbicide (2,4-D sodium salt) : A spectrophotometric study

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

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

ABSTRACT

The present study reveals the degree of antioxidant response stimulated in Cymbopogon martinii as a result of combined effect of herbicide (2, 4-Dichlorophenoxy acetic acid) and air pollution (auto exhaust). Two indigenously developed varieties of Cymbopogon martinii, namely Trishna and PRC-1, commonly grown in Lucknow were selected as the target plant. Two sites, 1: BBAU campus, as control site (less polluted) and 2: NBRI, Sikanderbagh crossing (one of the highly polluted site of Lucknow city were selected for the transfer experiment study. Results of the study very vividly revealed that the herbicide 2, 4-D enhanced the generation of antioxidant quite significantly in Cymbopogon martinii and works in synergies the effect of auto exhaust pollution.

Keywords: Cymbopogon martini, 2,4-D, antioxidant, pollution

INTRODUCTION

Being rooted to soil, plants are handicapped and thus receive the brunt of the exposure around them be it any pollutant/contaminant, disaster (natural or anthropogenic) or any other activity which is deleterious. The level of auto exhaust air pollutants are on the rise throughout the world, in developed, developing and even in under-developed countries, albeit their rate of increase differ. In India, the level of auto exhaust air pollutants has increased alarmingly in the past three decades and at present it constitutes around 65% of the total air pollutants of the lower troposphere or within trans boundary layer, which encompasses the life on this planet.

Air pollutants cause injury to vegetation and reduce crop yields [37, 38, 45]. Studies by several researchers vividly indicate the impact of automobile exhaust on roadside vegetation either in the form of visible (morphological changes, which are actually the manifestations of changes that are occurring at biochemical and physiological levels) or invisible effects [2, 12, 29, 33, 52]. Besides air pollutants to which plants are continuously exposed in today's urban environment, they under many circumstances receive the brunt of inclemency of other pollutants/contaminants also, like herbicides, the use of which has become an integral part of present day agricultural/horticultural practice.

Albeit studies on response of herbicides and air pollutants have been carried out, which indicate that herbicide and air pollutants do stimulate a series of responses within the plant [1, 14, 21, 22, 23, 37]. Our study is explicitly targeted to understand the joint effect of auto exhaust pollutants and a herbicide (sodium salt of 2, 4-D) in the two strains of *Cymbopogon martinii* through Transfer Experiment Method.

EXPERIMENTAL SECTION

Study site

Lucknow, the capital city of Uttar Pradesh, India is located between 26.50^{o}N and 80.50^{o}E along the banks of River Gomti. Around 12, 09,745 vehicles had been registered by RTO (Road Transport Organisation), Lucknow till March, 2011 for this city. For our Transfer Experiment study, two sites were selected on the basis of the number of auto-mobile vehicles plying there; Site1: control site, the campus of Babasaheb Bhimrao Ambedkar University located about 1.5 km off Raebareli Road (very low number of auto-mobiles, 410.13 h^{-1}) and Site 2: the highly polluted site, the campus of CSIR-NBRI at Sikunderbagh crossing, one of the busiest crossings of Lucknow (a very large number of auto-mobiles, 6632.67 h^{-1}).

Air pollution monitoring

Air pollutants (SPM, RSPM, SO_2 and NO_X) at both the selected sites were measured using a dichotomous high volume sampler (Envirotech make-APM 460). SPM and RSPM were measured by sucking air through a whatman glass fiber filter paper of 45µm pore size. SPM was measured by weighing the amount of dust collected over the filter paper for 6 h and RSPM was measured by weighing the dust collected at the bottom of the vortex of the high volume sampler. SO_2 and NO_2 were measured by West and Gaeke [36] and Jacobs and Hochheiser [30] method, respectively.

Ozone concentration of ambient air was monitored by using an ozone analyser (2B Technologies 106 L). The values of the oxides of nitrogen, sulphur and ozone are represented in microgram per meter cube.

Soil assay

Garden soil from Babasaheb Bhimrao University, Lucknow was used for this study. Soil pH was measured using a pH meter (CAT No. CL-54). The conductivity was measured in ds m^{-1} using a Toshnival (TCM 15) conductivity meter by following the same method. Total organic carbon was measured by following the method of Walkely and Black [3] using ferrous ammonium sulphate (0.5 N) as titrant. Available nitrogen was estimated using a nitrogen analyser and HCl as titrant. The metals were estimated using an atomic absorption spectrophotometer (Varian AA240FS). The available phosphorus was measured by following the method of Olsen and Sommers [43] using a UVKON Spectrophotometer.

Experimental set -up

Seeds of two varieties of *Cymbopgon martinii* Trishna and PRC-1 were obtained from CIMAP- a CSIR Laboratory at Lucknow. Seeds were grown in earthen pots of 12" filled with garden soil and cow dung manure. Equal numbers of saplings (5 in each pot) were allowed to grow to keep a rough check on the nutrient availability meant to be almost the same. After 30 days when the saplings were quite established, one set of pots (30) was placed at Site 2 while the other set was allowed to grow at Site1 for 45 days (1November to 15December, 2011).

Both the sets of plants were exposed to the ambient levels of air pollutants, which differed significantly at Site 1 and 2. Three concentrations of herbicide (2,4 D sodium salt) – 30 mgl^{-1}, 100 mgl^{-1} and 300 mgl^{-1} were sprayed on plants when they were 8-10" of height. Thus, five spraying treatments viz. T1(treatment 1) -no spraying, T2 (treatment 2)-spraying with distilled water, T3(treatment 3)-30mg/l of 2,4-D sodium salt, T4 (treatment 4) -100mg/l of 2,4-D sodium salt and T5 (treatment 5)-300mg/l of 2,4-D sodium salt were undertaken. The volume of herbicide sprayed on each plant was based on the count of leaves on each plant. The volume of herbicide was kept 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, washed in distilled water and analysed for various stress parameters.

Biochemical assay

Equal number of leaves was sampled randomly from all the sides of each plant for each pot. Leave samples were harvested in triplicate and weighed separately. Leaves sampled from each plant were sealed in labelled polythene bags and kept in a container that filled with liquid nitrogen and brought to lab for further analysis.

Non protein thiol was estimated using Ellman's [13] method. Total flavonoid was determined as per the method described by Chang *et al.* [8]. Total phenolic content was estimated by using the method of Mc Donald *et al.* [42]. Super oxide dismutase (SOD) was analysed by using Scandalios [18] method.

Statistical analysis

Mathematical values of all the assays were represented as the mean of three replicates with their standard deviation. Interrelationship between the means of vehicles plying at each site and stress indicators was deduced using rank correlation method. Two way ANOVA (Analysis of Variance) was performed to find the significance in the

differences between values at the two Sites at 5% significance level. T- Test at 5% significance level was performed to find out the significance in differences between the values in the five treatments. The values of the various air pollutants have been averaged out of the total values obtained over a 6 hour period in every season.

RESULTS

Air pollution monitoring

The level of various air pollutants at the two sites are tabulated in table 1. It was clear that the level of pollutants in the ambient air at site 2 was remarkably higher than site 1. The gaseous pollutants i.e. SO_2, NO_2 and ozone were found within permissible limit over a six hour period for both selected site (Site 1 and 2). However, the SPM (738.79 ± 8.5 µg m^{-3}) was recorded beyond the permissible limit (200 µg m^{-3} for residential areas) set by the Central Pollution Control Board (CPCB) [9]. Similarly, RSPM (397.89 ± 4.5 µg m^{-3}) was also found beyond the permissible limit of CPCB (100 µg m^{-3} for residential areas).

Soil characterization

The pH of amended soil was found alkaline (8.24 ± 0.089) and electrical conductivity was 154.64 ± 20.39 µS cm^{-1}. The metal content of the amended soil was recorded in the range of 1.73 - 18.29 µg g^{-1} as shown in table 2.

Change in morphological characteristics of plant

The fresh weight of plants (Table 3) was recorded maximum (9.88 ± 0.82g per plantlet) in Trishna variety at the control site. This value showed a sequential decrease from T1 to T5 and reached as low as 6.28 ± 0.34 g in T5 per plantlet for the same variety. Similarly, in PRC-1 the fresh weight of plants was maximum (9.18±0.64g per plantlet) in T1 which decreased sequentially in the later treatments and was minimum (5.21 ± 0.25g) per plantlet in T5. Similar decreasing trend upon exposure to 2, 4 D in plant fresh weight was recorded for Site 2. Maximum fresh weights for Trishna and PRC-1 varieties at Site 2 were recorded as 8.41 ± 0.31 and 8.10 ± 0.27 g per plantlet, respectively whereas least weights were 4.74 ± 0.33 and 4.10 ± 0.20 for these varieties. A decrease of 14.87% and 11.76% in fresh weight of Trishna and PRC-1 varieties, respectively were recorded at the polluted site i.e. Site 2 in comparison to the control Site i.e. Site 1. Similarly, in T3, T4 and T5 the plant fresh weight further decreased by 19.5%, 21.83% and 24.52%, respectively for Trishna and by 13.85%, 16.07% and 21.3%, respectively in PRC-1. These values were statistically significant (p value=8.7331E-06 for differences between treatments, 2.4972E-05 for differences between sites in Trishna variety and p value=5.8634E-08 for differences between treatments, 7.365E-07 for differences between sites in PRC-1 variety).

The biomass of plants in T1 of Trishna variety at Site 1 was 5.35±0.39 g per plantlet which decreased in the subsequent treatments. In T5 it was as low as 3.22±0.09 g per plantlet. The decrease in biomass in T5 in comparison to T1 was 39.81% and 60.71% for Trishna and PRC-1varieties at the Site. At Site 2, the biomass was 4.42±0.08 g per plantlet in Trishna variety in T1. It decreased by 52.71% and 74.02% in T5 in comparison to T1 in Trishna and PRC-1 varieties respectively. In comparison to Site 1, the biomass of plants of Trishna variety decreased by 17.38%, 15.07%, 23.51% , 28.45% and 35.09% in T1, T2, T3, T4 and T5, respectively at the Site 2. From these findings, it was evident that the fresh weight and dry weight (biomass) of plants witness a gradual decrease with an increase in the concentration of herbicide sprayed. This decrease was more conspicuous at the polluted site (Sikanderbagh crossing). The dry weight of PRC-1 variety exhibited a sharper decrease as compared to Trishna variety at both sites i.e. the control and polluted site. The differences in biomass were found to be statistically significant between the treatments as well as between the two sites(p value= 1.44E-05 for differences between treatments, p value= 3.12E-05 for differences between sites in Trishna variety and p value=8.92E-05 for differences between treatments, p value=0.000473 for differences between sites).

Unlike the fresh weight and biomass the total ash content recorded a significant increase from T1 to T5 in both Trishna and PRC-1 varieties at both the sites p value= 0.00020249 for differences between treatments, p value =0.01629571 for differences beween sites for Trishna variety and p value=0.02349471 for differences between treatments and p value=0.02600636 for differences between sites for PRC-1 variety). The total ash content of plants from the polluted site was greater that those harvested from the control site. The total ash content at Site 2 was recorded 0.005, 0.003, 0.010, 0.014 and 0.016 g dw (dry weight) higher than Site1 for T1, T2, T3, T4 and T5, respectively. This increase was found to be statistically significant.

A similar trend was observed in the water soluble ash and acid soluble ash and both of these parameters increase with an increase in the concentration of 2, 4-D sodium salt sprayed. Here also the values at Site 2 were higher than those at Site 1.

Biochemical changes in plant

There was an increase in the level of total flavonoid content, total phenolics, non-protein thiol and SOD from T1 to T5. The level of these secondary metabolites (Table 4) was higher at the polluted site as compared to the control site for both the varieties.

The total flavonoid content in T1 in Trishna variety at Site 1 was 126.42±1.05 mg g^{-1} dw. This value increased sequentially from T1 to T5 and rose to177.21±1.12 mg g^{-1} dw in T5. Similarly, the total flavonoid content rose from 228.15±0.74 mg g^{-1} dw in T1 to 283.93±4.46 in T5 in PRC-1 at Site 1. The total flavonoid content was found 59.28, 57.34, 42.11, 53.79 and 55.59% higher at Site 2 as compared to Site 1 for treatments T1, T2, T3, T4 and T5, respectively for Trishna variety. In case of PRC-1 variety, it was recorded as 212.06, 214.06, 180.94, 187.43, 180.96% higher at Site 2 in T1, T2, T3, T4 andT5 respectively in comparison to Site 1.

The total phenolic content was recorded 174.86%, 179.72%, 139.18%, 129.81% and 132.15% higher in treatments T1, T2, T3, T4 and T5 of Trishna variety at Site 2 in comparison to Site 1. Similarly it was 89.13%, 88.60%, 118.56%, 111.52% and 116.88% higher in treatments T1, T2, T3, T4 and T5 of PRC-1 variety at Site 2 than Site 1.

Similarly, the foliar SOD content recorded an increase upon exposure to 2, 4-D at both the sites. Higher levels of SOD were recorded at Site 2 in comparison to Site 1. There was 69.95% increase in the SOD content between T1 (no spraying) at the control Site and T1 (no spraying) at the polluted Site in Trishna. This difference was 86.43% PRC-1 between the two sites.

The non-protein thiol content was recorded minimum 5.34±0.07 mg g^{-1} fw(fresh weight) in Trishna variety in T1 at Site 1. This increased upon exposure to herbicide and rose to 8.08±0.58 mg g^{-1} fw in T5. In case of PRC-1, the non-protein thiol content increased from 4.93±0.05 mg g^{-1} fw in T1 to7.15±0.59 mg g^{-1} fw in T5. Similar observations were made at Site 2. Here, the non-protein thiol content increased from 9.05 ± 0.75 mg g^{-1} fw in T1 to15.68±0.96 mg g^{-1} fw in T5 in Trishna variety and from 6.70 ± 0.75 mg g^{-1} fw in T1 to 11.64±0.49 mg g^{-1} fw in T5 in PRC-1 variety.

Thus there was a positive correlation (0.96, 0.90 for total flavonoid content, 0.79,0.89 for phenolic content, 0.97, 0.98 for non-protein thiol and 0.99, 0.99 for SOD at sites 1 and 2 respectively for Trishna variety and 0.98,0.86 for total flavonoid content, 0.93, 0.99 for phenolic content, 0.89, 0.96 for non-protein thiol and 0.98, 0.98 for SOD at sites 1 and 2 respectively for PRC-1 variety) between the concentration of 2,4-D sodium salt used and secondary metabolites produced within the plant. Also, in both the varieties at Sites 1 and 2 for each dose i.e. treatments 1,2,3,4 and 5 the values were found to be significantly different from each other for all the four secondary metabolites assayed.

DISCUSSION

The effect of both toxicants i.e. air pollutants and herbicide was reflected upon the growth parameters of the plants. A decrease in the fresh weight and biomass of plants with an increasing concentration of 2, 4-D in this study represented a detrimental effect of the herbicide on the two varieties. There was a negative correlation between the concentration of 2,4-D sprayed and the growth parameters (Table1). The herbicide 2,4D has earlier been found to cause a decrease in biomass in grasses [11]. A sharper decrease of biomass and fresh weight of selected varieties at the polluted site in this study showed that air pollution further aggravated the problem. This can be explained by the fact that air pollution stress led to stomatal closure, which reduced CO_2 availability in leaves and inhibited carbon fixation. Besides, air pollutants like SO_2 are known to decrease the pH of the cell sap leading to the destruction of the photosynthetic pigments [34, 41, 47]. Thus, a reduction in photosynthetic pigments and partial closure of stomata lead to reduced rate of carbohydrate formation hence lower biomass of the plants at the polluted site. Reduction in biomass of plants of the poaceae family due to SO_2, NO_2 and O_3 in laboratory conditions has earlier been reported by Ashenden *et al.* [49]. The reduction in above ground biomass due to urban air pollution in the ambient environment has also been reported by Dineva [45], Agrawal *et al.* [24], Tiwari *et al.* [44] and Rai and Agrawal [38].

Trag [5] reported an increase in the level of total ash content in *Azadirachta indica* and *Dalbergia sissoo* due to air pollution as in present study the total ash content has been found to increase at Site 2 the polluted site in Trishna and PRC-1 varieties of *Cymbopogon martinii*. The positive correlation between ash content and concentration of herbicide may be due the sodium content in the herbicide. Sodium salts have been found to increase the accumulation of acid soluble ash and water soluble ash in plants *Suaeda fruticosa*(L.) and *Spartina patens* [17, 26]. The increase in acid soluble ash and the water soluble ash due to the spraying of 2, 4 D together increased the total ash content in both the species. Thus there was an increase in the inorganic content in plants due to the effect of air pollution and 2,4-D Sodium salt.

The statistically significant differences between the recorded values at the two sites indicates that the impact on the parameters was actually due to differences between ambient air pollution levels between sites and not just a coincidence.

Flavonoids and phenolic compounds are produced by plant cell in response to injury or stress [7, 25, 35]. According to Yu [47], SO_2 is metabolized in the plant cells to HSO^-_3 and O_2^- (superoxide radical) are generated in this process. O_2^- is a reactive oxygen species known to cause lipid peroxidation in the cell membranes. The production of reactive oxygen species upon exposure to 2, 4-D in pea plants has been reported by Pazmino *et al.* [10].This is combated by the production of flavonoids. Induction of oxidative stress upon exposure to air pollution followed by an increase in foliar flavonoid content has been reported by Mir [4] in *Catharanthus roseus* L. and *Oscimum santum* L. Nikolova and Ivancheva [31] have reported an increase in total flavonoids in *Artemisia vulgaris* L. and *Veronica chamaedrys* L. in response to air pollution stress. Similar observations were made by Loponen *et al*. [16-17] in *Betula pubescens* and white birch leaves.

Kumar and Kumar [19] in their experiments with *Nosctoc muscorum* suggested that phenols could act as protectants for the organisms under oxidative stress or drought conditions. An increase in the accumulation phenolic compounds upon exposure to 2,4-Dwas reported by Kumar *et al.* [20] in three different cyanobacterial species – *Anabaena fertilissima* Rao, *Aulosira fertilissima* Ghose and *Westiellopsis prolifica* Janet, as in this study an increase in this metabolite is recorded upon exposure to 2,4-D. An increase in the production of phenolic compounds upon exposure to vehicular air pollution at Site 2 was also recorded. Similar rise in phenolic compounds in has been documented by Mir [4] in *Catharanthus roseus* L. and *Oscimum santum* L. and by Kanoun *et al*. [27] in *Phaseolus vulgaris* L.cv. It was also observed that the concentration of phenolic compounds increased cumulatively with an increase in the concentration of herbicide at Site 2. Thus it was evident that herbicide 2, 4-D led an increase in the phenolic content in *Cymbopogon martinii* and the effect is further aggravated in the presence of auto exhaust pollution. An increase in the production of flavonoids and total phenolic compounds in the leaves in this study described a scavenging mechanism adopted by *Cymbopogon martinii* to the damage done to them by automobile exhausts and 2, 4-D.

SOD is a very important enzymatic antioxidant produced by the cell during oxidative damage. It catalyses the dismutation of highly reactive superoxide (O_2^-) produced in the cell through incomplete reduction of oxygen to less reactive hydrogen peroxide (H_2O_2). This radical is produced in the photosynthetic organisms at low concentration of CO_2 and high concentration of O_2. The production of SOD during oxidative stress and its ability to combat the same has been well documented by Kresger *et al.* [32]. A significant increase in the foliar SOD content was observed in T3, T4 and T5 upon exposure to herbicides in both the varieties at both the Sites. This may be related to the resistance posed by *Cymbopogon martinii* to the oxidative stress caused by herbicide 2, 4-D. The foliar SOD content increased further at Site 2 upon exposure to air pollution in each treatment in comparison to Site 1. An increase in the foliar SOD content due to air pollution has earlier been reported by Mir [4] *Catharanthus roseus* L. and *Oscimum santum* L and Dixon *et al.* [14] in sugar beet. An increase in the super oxide dismutase activity in the leaves upon exposure to 2,4D and auto exhaust denoted the cellular antioxidant response against the cumulative stress generated by vehicular exhausts and herbicide.

Non-protein thiols refer to a group of low molecular weight compounds produced by the cell to mitigate oxidative stress [6, 35]. This is the second line of defence adopted by the cell. It scavenges the hydrogen peroxide formed in the cell due to the interaction of SOD and O_2^-. An increase in the concentration of non-protein thiol with increase in the concentration of herbicidal spray indicates a similar defensive response of the plant to combat damage by the toxicants. Similar response was reported in the leaves of *C. aerientinum* against oxidative stress induced by sodium chloride treatment. Treatment of *C. arientinum* plants with sodium chloride led to the production of reactive oxygen species i.e. O_2^- and H_2O_2.There was a sequential increase in non-protein thiol content with increase in sodium chloride toxicity. This increase was suggested to be part of the antioxidant defence mechanism adopted by the plant [28]. Oxidative stress induced by heavy metals like cadmium in *Zea mays* of poaceae family have been reported to increase the accumulation of glutathione; a non-protein thiol [40].Similar response was reported by Zeng *et al*. [50-51] in roots of *Arabis paniculata* where an increase in cadmium accumulation in the roots was found to be positively correlated to cysteine and glutathione.

A high foliar content of all these compounds i.e. flavonoids, phenolic compounds, super oxide dismutase (enzymatic antioxidant) and non protein-thiol(non-enzymatic antioxidants) in the plants upon exposure to 2,4-D indicates a certain scavenging mechanism adopted by the plants in response to 2,4-D sodium salt. It was observed that each treatment induced a significant change in the foliar content of these secondary metabolites in comparison to the previous treatment (Table 4). This indicated that each treatment was capable of significantly affecting the physiology of the plants belonging to both Trishna and PRC-1 varieties. The concentration of these metabolites further increased at Site 2 where the ambient air was charged with pollutants from auto exhaust. Vehicular air

pollution has earlier been found to induce biochemical changes in *Erythrina orientalis* [48], *Abelmoschus esculentus, Celosia cristata, Coleus blumei, Cyamopsis tetragonolobus, Gomphrena globosa, Impatiens balsamina, Ocimum sanctum, Phaseolus vulgaris, Solanum melongena*, and *Zinnia elegans* [2].

Table 1: Chemical properties of garden soil used in 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/gm)	
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: Average concentration of air pollutants at the control and polluted site during the study period

Pollutants (in µg/m^3)	Site 1	Site 2	CPCB standards 24hrs(in µg/m^3)
SO_2	11.82±0.14	38.95±0.52	80.00
NO_2	13.1±0.21	55.39±9.64	80.00
SPM	200.65±3.62	738.79±8.5	200.00
RSPM	106.56±2.10	397.89±4.5	100.00
Ozone	50.87±3.45	64.75±3.75	100.00(1hr)

Table 3: Changes in plant parameters of Trishna and PRC-1 varieties of *Cymbopogon martinii* in various treatments at the two sites

Treatments	Fresh weight (gm/plantlet)		Dry weight (gm/plantlet)		Total ash (gm/gm d w of plant material)		Water soluble ash (gm/gm total ash)		Acid insoluble ash (gm/gm total ash)	
	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2
Trishna T1	9.88±0.82*	8.41±0.31*	5.35±0.17*	4.42±0.14*	0.080±0.001*	0.85±0.002*	0.436±0.004*	0.469±0.018*	0.68±0.01*	0.73±0.015*
Trishna T2	10.04±0.26*	8.86±0.54*	5.64±0.14*	4.79±0.53*	0.078±0.001*	0.082±0.002*	0.39±0.018*	0.424±0.015*	0.647±0.025*	0.715±0.015*
Trishna T3	7.69±0.48*	6.19±0.33*	4.21±0.10*	3.22±0.11*	0.108±0.0005*	0.118±0.008*	0.452±0.003*	0.49±0.020*	0.71±0.02*	0.767±0.021*
Trishna T4	6.96±0.22*	5.44±0.18*	3.55±0.14*	2.54±0.35*	0.130±0.001*	0.144±0.008*	0.475±0.002*	0.517±0.025*	0.74±0.01*	0.806±0.035*
Trishna T5	6.28±0.34*	4.74±0.33*	3.22±0.09*	2.09±0.11*	0.138±0.001*	0.1533±0.004*	0.487±0.008*	0.534±0.025*	0.763±0.025*	0.843±0.035*
PRC-1 T1	9.18±0.64*	8.10±0.27*	4.81±0.09*	3.81±0.30*	0.082±0.0009*	0.0856±0.001*	0.463±0.02*	0.484±0.010*	0.67±0.020*	0.713±0.025*
PRC-1 T2	9.81±0.67*	8.82±0.79*	5.13±0.06*	4.05±0.50*	0.079±0.0014*	0.0843±0.002*	0.459±0.005*	0.479±0.011*	0.643±0.035*	0.693±0.015*
PRC-1 T3	7.58±0.22*	6.53±0.96*	3.98±0.12*	2.89±0.18*	0.090±0.0004*	0.096±0.001*	0.483±0.012*	0.51±0.040*	0.713±0.004*	0.76±0.010*
PRC-1 T4	6.72±0.18*	5.64±0.54*	2.56±0.32*	1.89±0.32*	0.091±0.0005*	0.1012±0.006*	0.51±0.02*	0.540±0.010*	0.75±0.02*	0.81±0.020*
PRC-1 T5	5.21±0.25*	4.10±0.20*	1.89±0.08*	1.14±0.14*	0.094±0.005*	0.113±0.015*	0.534±0.005*	0.567±0.025*	0.777±0.012*	0.843±0.031*

Table 4: Changes in the level of antioxidant compounds in Trishna and PRC-1 varieties of *Cymbopogon martinii* in various treatments at the two sites

Treatments	Total flavonoid content (µg/gm d. W.)		Total phenolic content (µg/gm d. W.)		Non protein thiol (µmole/gm f. W.)		Super oxide dismutase ((µg/gm protein)	
	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2
Trishna T1	126.42±1.05a	201.36±1.5a	127.63±1.49a	350.81±2.39a	5.34±0.07a	9.05±0.75a	4.06±0.06a	8.00±0.17a
Trishna T2	121.22±1.41b	190.73±1.3b	121.83±1.12b	340.78±3.38b	5.29±0.03b	8.03±0.03b	3.51±0.31b	7.27±0.41b
Trishna T3	157.88±1.07c	224.36±1.89c	161.69±1.20c	386.73±3.07c	6.21±0.51c	10.03±0.02c	5.37±0.21c	9.31±0.65c
Trishna T4	167.44±0.57d	257.51±10.95d	177.78±1.02d	408.56±7.98d	7.09±0.01d	12.28±0.97d	6.03±0.23d	10.70±0.20d
Trishna T5	177.21±1.12e	275.73±9.18e	180.66±2.28e	419.42±5.74e	8.08±0.58e	15.68±0.96e	7.15±0.24e	13.17±0.64e
PRC-1 T1	228.15±0.74a	711.96±1.56a	121.37±0.66a	229.36±1.56a	4.93±0.05a	6.70±0.75a	3.83±0.14a	7.48±0.42a
PRC-1 T2	223.29±1.68b	701.28±0.65b	116.69±5.72b	220.08±2.31b	4.29±0.25b	6.06±0.42b	3.08±0.06b	6.85±0.34b
PRC-1 T3	262.08±0.55c	736.29±1.87c	161.28±2.11c	352.49±1.09c	5.18±0.07c	7.50±0.49c	4.66±0.13c	9.03±0.11c
PRC-1 T4	271.88±2.39d	781.46±2.07d	173.18±2.70d	366.32±1.22d	6.49±0.43d	9.61±0.40d	5.49±0.25d	10.51±0.30d
PRC-1 T5	283.93±4.46e	797.73±10.44e	181.15±3.44e	392.88±1.03e	7.15±0.59e	11.64±0.49e	6.58±0.37e	12.47±0.75e

CONCLUSION

Given its medicinal importance *Cymbopogon martinii* is largely cultivated in Southeast Asia. This study represents a clear evidence of significant reduction in biomass of its varieties Trishna and PRC-1 due to use of herbicide. This problem is further exaggerated upon exposure to auto exhaust, a ubiquitous menace. The cumulative impact of herbicide (2,4-D sodium salt) and air pollution plays an important role to significantly enhance the production of secondary metabolites i.e. flavonoids, phenolic compounds, SOD and non-protein thiols which are directly involved in various physiological process and growth of the plant and are indicators of a plant's resistance towards abiotic stress.

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