Evaluating the Antioxidative Defense Response of Selected Indoor Plants Against Benzene and Formaldehyde: Indoor Air Pollutants

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Abstract

Volatile organic compounds (VOCs) such as formaldehyde and benzene are among the key contributors to indoor air pollution. The current situation of environmental pollution is alarming, where especially the indoor air pollution is becoming a challenge as affecting the plants and humans. VOCs are known to adversely affect indoor plants such as causing necrosis and chlorosis. In order to withstand these organic pollutants, plants are naturally equipped with antioxidative defense system. The current research study was aimed to evaluate the combined effect of formaldehyde and benzene on antioxidative response of selected indoor plants namely Chlorophytum, Dracaena and Ficus. After the combined application of different levels (2, 2; 2, 4; 4, 2 & 4, 4 ppm) of formaldehyde and benzene in an airtight glass chamber, the enzymatic and non-enzymatic antioxidants were analyzed. Analysis of various non enzymatic assays showed significant increase in the total phenolics to 10.72 mgGAE/g in Ficus; Chlorophytum (9.20 mgGAE/g) & Dracaena (8.74 mgGAE/g) as compared to their respective controls i-e, 3.76, 5.39 & 6.07 mgGAE/g. Total flavonoids in Ficus were also increased to 1545.72 µg/g from 724 µg/g (in control) followed by 322.66 µg/g in Dracaena (control having only 167.11 µg/g) while total carotenoids content also increased in Dracaena (0.67 mg/g) followed by Chlorophytum (0.63 mg/g) while increasing the combined dose as compared to their control plants having only 0.62 and 0.24 mg/g content. The maximum increase in enzymatic antioxidants including total antioxidants (87.89%), catalase (59.21 U/mg) and guaiacol peroxidase (52.16 U/mg) was observed in Dracaena plant under combined dose of benzene (2 ppm) and formaldehyde (4 ppm). Although the experimental indoor plants have been reported to metabolize the indoor pollutants, but the current findings indicate that both benzene and formaldehydes are also affecting the physiology of indoor plants.

Introduction

Indoor pollution is the principal cause of deteriorating indoor air quality. VOCs are recognized as one of the most prominent group as their indoor emission source is continuous (Bacaloni et al. 2011). Their concentrations in indoor environments are at least 10 times higher than outdoors (Huang et al. 2011). Formaldehyde, the most prevalent indoor air pollutant, belongs to the class of VOCs. It is released from many building materials, such as particleboard, plywood, glues, carpets, paints, and furniture polish (Salthammer et al. 2010). Another indoor air pollutant benzene also belongs to the class of VOCs. It is present in plywood, particleboard furniture, wood paneling, flooring adhesives, fiber glass, paints, paint remover, vinyl, PVC, rubber floorings, caulking and (Ezeonu et al. 1994; Yu & Crump, 2003).

Plants are quite promising and ecofriendly source of remediating the polluted environment. Indoor plants are commonly used to decorate rooms, halls, offices and workplaces. Moreover, some of the indoor plants are reported to bear the capability of absorbing VOCs from indoor environment as well (Yang et al. 2009). Various researchers have reported the removal of indoor formaldehyde via indoor plants including - Fatsia japonica (Kim et al. 2008); Epipremnum aureum (Kim et al. 2009); Chlorophytum bichetii (Kim et al. 2013) and Sansevieria trifasciata (Husti et al. 2016).

However, indoor plants are also affected by the formaldehyde as well. The effects are highly variable as influenced by the plant species, concentration of formaldehyde and time of exposure. As reported to cause necrosis and destruction of palisade and spongy parenchyma cells in Epipremnum aureum, Ficus japonica, Rhipsis excelsa (Kim et al. 2013); reduction in chlorophyll content and increased permeability of plasma membranes in Tillandsia plants (Li et al. 2015). Benzene also poses phytotoxic effects including expanded and damaged chloroplast in Epipremnum aureum, Chlorophytum comosum (Sriprapat et al. 2014); chlorosis, necrosis and wilting of leaf tissue in C. comosum (Sriprapat and Thiravetyan, 2013) and decrease in chlorophyll content of Sansevieria trifasciata (Lu et al. 2018).

Plants are reported to produce reactive oxygen species (ROS) under stress conditions. ROS is composed mainly of hydrogen peroxide (H₂O₂), singlet oxygen (¹O₂), hydroxyl radical (OH⁺) and superoxide radical (⁰₂). These ROS mainly disturb the cellular activity of the plants (Apel and Hirt, 2004; Foyer and Noctor, 2005). In response to this ROS-formation, plants contain a defense mechanism that consists of enzymatic antioxidants such as catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD), superoxide dismutase (SOD) and non-enzymatic antioxidants such as total phenolics (TPC), total flavonoid (TFC), proline, total carotenoid (TCC), total antioxidants (Gill and Tuteja, 2010; Miller et al. 2010; Gill et al. 2011). SOD is the first line of defense against the damage caused by ROS when exposed to environmental stresses. By dismutating O₂ into O₂ and H₂O₂, SOD induces its elimination. This lowers the chances of OH⁺ formation (Mittler, 2002). Abiotic stress is used to upregulate the SOD (Boguszewska et al. 2010). Various researches have reported variable responses of indoor plants under formaldehyde stress alone including an increase in polysaccharide content and reduction in protein amount in Ficus elastica, Sansevieria trifasciata and Dracaena deremensis (Husti et al. 2016) and activation of redox reaction mechanism in Plantago asiatica shoot while the enzymatic reaction were higher in Taraxacum mongolicum shoot (Zhao et al. 2019); higher catalase and peroxidase activities in Rhipsis excelsa, Epipremnum aureum and Ficus japonica (Kim et al. 2013); higher H₂O₂ activity, increased MDA and protein content in Petunia (Sun et al. 2015); higher T-AOC in C. comosum (Li et al. 2019); higher peroxidase and catalase in C. comosum (Yu et al. 2011).
The indoor plants including Chlorophytum, Dracaena and Ficus are reported to detoxify indoor formaldehyde and benzene (Yang et al. 2009; Mosaddegh et al. 2014; Su et al. 2019; Gong et al. 2019) but the antioxidative response of these plants against combined stress of formaldehyde and benzene is not reported yet. The present research aimed to explore the enzymatic and non-enzymatic antioxidative responses of these plants.

Materials And Methods

Acclimatization of Experimental Plants

Three species of indoor ornamental plants (Chlorophytum, Dracaena and Ficus) were selected for studying the combined effect of benzene and formaldehyde. Plants were purchased from the nursery and were transferred into new pots containing mixture of coco peat, peat moss and perlite (2:2:1). Pots were then placed in a growth chamber at 24°C temperature and 12 hrs light period, for 2 weeks for acclimatization before application of treatments.

Application of Benzene and Formaldehyde

Experiments were conducted in an airtight glass chamber of volume 0.128 m³ having a fan and an inlet for injection of formaldehyde and benzene as shown in figure 1. The chamber was cleaned properly with a cotton towel and the pots containing plants were covered with aluminum foil to avoid the direct contact of formaldehyde and benzene with media. Individual plant species was then placed in the chamber and the chamber was sealed with a lid covered by teflon tape. Plants were then exposed to different combined concentrations (2 and 4 ppm) of formaldehyde and benzene. After 24 hrs exposure to benzene and formaldehyde, the plants were taken out of the chamber and their biochemical analysis were carried out.

Enzymatic Antioxidants

Catalase, Peroxidase and Superoxide Dismutase

Freshly harvested leaf (0.20 g) was grounded and extracted in 2ml of pH. 7 potassium phosphate buffer (100 mM). The sample was then placed in centrifuge for 5 minutes at 15000 rpm. The supernatant was transferred to a separate eppendorf tube for analysis of catalase, peroxidase and superoxide dismutase. The catalase activity was measured by UV-vis spectrophotometer (T80+ Spectrometer) according to Aebi (1984). The absorbance of reaction mixture containing 50 mM of potassium phosphate buffer sample and 40 mM H₂O₂ was taken at 240 nm for 3 minutes. For peroxidase activity, absorbance of reaction mixture containing pH. 7 potassium phosphate buffer (50 mM), sample extract, 40 mM H₂O₂ and 1% guaiacol was taken at 420 nm for 3 minutes. Superoxide dismutase activity of plant extract was determined according to Beyer et al. (1987). The reaction mixture of plant extract, 50 mM phosphate buffer, 2 mM EDTA, 14 mM L-methionine, 55 mM NBT, and 0.025% Triton-X was incubated for 20 minutes under fluorescent light and absorbance was taken at 560 nm.

Ascorbate Peroxidase

In order to assess ascorbate peroxidase activity, freshly harvested leaf sample (0.20 g) was extracted in potassium phosphate buffer pH 7 with 0.5 mM ascorbate. Mixture was then centrifuged at 15000 rpm for almost 5 minutes. Supernatant was take in another eppendorf tube for further use. Firstly, 2 ml of 50 mM pH 7 potassium phosphate buffer was added to glass cuvette, followed by 100 μl of 1 mM ascorbate, 0.1 mM EDTA, 1 mM H₂O₂, and 100 μl of sample, while absorbance was recorded at 290 nm.

Total Antioxidants

Total antioxidants activity was determined by using DPPH salt method (Brand et al. 1995). Freshly harvested leaf sample (80 mg) was grounded and extracted in 80% methanol. Freshly prepared DPPH solution (0.004%) was added in reaction tubes containing sample extract and incubated in the dark for 50 minutes. A spectrophotometer (T80+ UV/VIS Spectrometer) was used to measure the absorbance at 517 nm. Activity was calculated by measuring the change in sample extract and blank by using following equation.

The radical scavenging activity was expressed as the radical scavenging % using the equation,

\[ \text{DPPH Scavenged} \% = \left( \frac{A0 - A1}{A0} \right) \times 100 \]

Where A0 is the absorbance of control and A1 is the absorbance of the sample extract.

Non-Enzymatic Antioxidants
Results of two-way ANOVA presented in supplementary table 1 describe the significance of main factors (treatments of benzene and formaldehyde, and plants) and their interaction. All the non-enzymatic antioxidants were found statically significant at $p \leq 0.05$.

**Total Phenolic Content**

Plant leaf sample (80 mg) was weighed, ground, and extracted in 97% methanol. Folin-Ciocalteu reagent (10%) was prepared. The reaction tube containing 200 $\mu$L of the FC reagent, 200 $\mu$L of the sample extract 1.6 mL of 700 mM Na$_2$CO$_3$ incubated for 2 hrs. Absorbance was measured at 765 nm using spectrophotometer. Gallic acid was used as standard to quantify total phenolic contents in samples (Singleton et al. 1999).

**Total Flavonoids**

Fresh leaf sample (80 mg) was grounded and extracted in 85% methanol. Total flavonoid contents were assessed following the protocol of Olajire et al. (2011).

**Proline**

The proline content was quantified by ninhydrin method following the protocol of Bates et al. (1973). Freshly harvested plant leaf (250 mg) was weighed, ground, and extracted in 2 ml sulfosalicylic acid. After centrifugation at 15000 rpm for 5 minutes, the supernatant was taken and used further. A reaction tube containing sulfosalicylic acid (3%), Glacial acetic acid (60%), Ninhydrin and sample was placed in water bath at 97°C for 1 hr. Then the colored phase was separated by adding 2 ml toluene in the reaction tubes. Absorbance of extracted pigmented layer was measured at 520 nm. Standard curve was developed by using proline as a standard.

**Total Carotenoids**

Total carotenoids content of plant leaves was determined following the protocol of Lichtenthaler (1983). Plant leaf sample (100 mg) was grounded and extracted in 10 ml acetone (80%). Top of the test tubes were covered with aluminum foil and kept in dark for overnight. The sample mixture was centrifuged, and supernatant was taken in separate tube. Absorbance was measured through UV-vis spectrophotometer at 470 nm.

**Statistical Analysis**

Experiment was conducted in completely randomized design with three biological replicates. The collected data was subjected to two-way analysis of various (ANOVA) followed by Tukey test at $p \leq 0.05$ by using IBM-SPSS software. Pearson correlation method was used to find out the correlations among studied parameters.

**Results**

**Enzymatic Antioxidants**

**Catalase (CAT)**

Catalase activity of experimental plants were analyzed under selected doses of benzene and formaldehyde. Figure 2A shows that under 2, 4 ppm and 4, 2 ppm dose of benzene and formaldehyde, Dracaena exhibited the highest catalase activity of 59.21 and 57.42 U/mg with reference to its control (15.46 U/mg). It was also observed that each plant displayed an increase in their catalase activity under each of the tested dose as compared to their controls.

**Guaiacol Peroxidase (POD) and Ascorbate Peroxidase (APX)**

Upon the application of each of the four tested doses, an increase in Guaiacol peroxidase activity was displayed by all of the three experimental plants with reference to their respective controls as shown in figure 2B. Among the other tested doses, the test dose of 2, 4 ppm (benzene and formaldehyde) yielded highest activity in Dracaena (52.16 U/g) and Chlorophytum (34.02 U/mg) with respect to their tested controls displaying only 10.09 and 15.94 U/mg activity. However, the highest tested dose (4, 4 ppm benzene and formaldehyde) resulted in decline of the activity.

Whereas, figure 2C shows APX activity of control and treated plants. Graph shows an increasing trend in APX activity of the treated plants under each of the tested doses as compared to their controls respectively. Dose of 4, 2ppm (benzene and formaldehyde) resulted in maximum APX activity in Dracaena (17 U/mg) and Ficus (13.36 U/mg) plant as compared to their control plants showing only 3.64 and 3.88
U/mg activity. However, decrease in activity can be seen under 4, 4 ppm (benzene and formaldehyde) dose as compared to other tested doses.

**Superoxide Dismutase (SOD)**

Superoxide dismutase activity of the experimental plants were evaluated and represented in figure 2D. Significant increase in SOD activity was displayed by plants under 4, 2 ppm dose of benzene and formaldehyde with respect to their controls. Dracaena and Chlorophytum exhibited maximum activity of 131.31 and 111.31 U/mg whereas their controls displayed only 42.63 and 30 U/mg activity. Chlorophytum showed 127.38 U/mg activity under 2, 4 ppm benzene and formaldehyde dose. Whereas, upon increasing the doses to 4, 4 ppm benzene and formaldehyde, decline in activity was observed in each of tested plant.

**Total Antioxidants**

Among the control and treated plants (under four tested doses), Ficus plant showed the highest activity of 87.89% under 4, 4 ppm dose of benzene and formaldehyde with respect to its respective control (43.88%). Increase in activity was displayed by all of the three tested plants under each of the tested combined dose with respect to their controls respectively. However, Ficus was the most significant among them as shown in figure 2E.

**Non-Enzymatic Antioxidants**

**Total Phenolics & Flavonoids**

Among the control and treated plants (under four applied doses), Ficus showed maximum increase in TPC (10.72 mg GAE/g) at combined dose of benzene and formaldehyde (4 ppm each) compared to 3.76 mgGAE/g in its control plants. Whereas Chlorophytum and Dracaena plants treated to combined dose of benzene (2 ppm) and formaldehyde (4 ppm) exhibited maximum of 9.20 mgGAE/g and 5.57 mgGAE/g total phenolics, respectively, which are significantly higher compared to their respective control plants as shown in figure 3A.

Total flavonoids (TF) were also analyzed to evaluate of effect of combined doses of benzene and formaldehyde on experimental plants. Results of total flavonoids (TF) presented in figure 3B shows that under highest applied dose of benzene and formaldehyde (4, 4 ppm), maximum TFC was displayed by Ficus plant (1545.72 μg/g) with respect to its control (724 μg/g). However, Chlorophytum and Dracaena did not show any significant increase in TF with respect to their controls under each of the tested combined dose of benzene and formaldehyde.

**Proline and Total Carotenoids Content**

Proline content was shown to be highest in Dracaena (3.66 μg/g) as compared to its respective control plant (1.54μg/g) under 4 ppm dose of benzene and formaldehyde. Combined dose of 2, 4 ppm of benzene and formaldehyde did not result in significant increase in proline content in any of the tested plant. In 4, 2 ppm benzene and formaldehyde dose, Chlorophytum showed significant increase (3.07 μg/g) in its proline content as compared to 1.66 μg/g in its control plant as shown in figure 3C.

While figure 3D represents the total carotenoid content of experimental plants under tested doses of benzene and formaldehyde. The graph shows that under 4, 4 ppm dose of benzene and formaldehyde, Dracaena displayed significantly decrease in its carotenoid content (0.22 mg/g) with respect to its control (0.62 mg/g). However, the carotenoid content of Chlorophytum plant was shown to be increased under each of the tested dose with respect to its control. Maximum carotenoid content of 0.63 mg/g was observed in Chlorophytum plant under 4, 2 ppm dose of benzene and formaldehyde whereas its control exhibited only 0.24 mg/g.

**Pearson Correlation**

Total phenolics showed significantly positive correlation with flavonoids, proline and total antioxidants as shown in table 1. Total antioxidants were also significantly positive correlated with phenolics, flavonoids, proline, catalase and ascorbate peroxidase. This correlation indicates the activation of enzymatic and non-enzymatic antioxidants against stress of formaldehyde and benzene.

**Discussion**

Indoor plants have been reported to detoxify certain volatile organic compounds, but a little work has been done regarding their toxic effects on those indoor plant. The present research explored the antioxidative response of selected indoor plants against combined stress of formaldehyde and benzene.
In the current research study, enzymatic anti-oxidants namely: APX, CAT, GPX, SOD and TAOs were evaluated under tested combined dose of formaldehyde and benzene. Significant increase in enzyme activities were displayed by the treated plants with as compared to their controls as shown in Figs. 2 and 3. It has been well reported that when plants are facing environmental stresses, the balance of ROS production and the quenching capacity of antioxidants is disturbed. This may lead to oxidative damage. These activated oxygen species can disturb normal metabolism via oxidative damage to proteins, nucleic acids and lipids. In order to withstand these cytotoxic effects, plants are equipped with antioxidant enzymes. These enzymes are required by plants to control the level of ROS and to protect cells under stress conditions (Chaitanya et al. 2002). CAT and POD are capable to degrade H$_2$O$_2$, scavenge free radicals and oxy-intermediates (Jaleel et al. 2009). Superoxide radicals are produced in plants under stress conditions. SOD enzyme transforms these radicals into H$_2$O$_2$ (Dixit et al. 2001, Mittiova et al. 2002). Among the other reactive oxides, hydroxyl radical (OH) is the most toxic one. It can easily react with any of the macromolecules and thus can cause serious damage to plant. Formation of this oxide is prevented or decreased by the combined action of SOD and CAT enzyme (Asada et al. 1999; Kusvuran et al. 2013). Another important antioxidant enzyme in plant is APX that detoxifies hydrogen peroxide by utilizing ascorbate for reduction.

In accordance to our findings, Sun et al. (2015) has also documented increased catalase activity under formaldehyde stress in Petunia plant. Similarly, higher peroxidase and catalase has been reported by Yu et al. (2011) in C. comosum under formaldehyde stress. In current research study, higher total anti-oxidants activity was displayed by experimental plant with respect to their controls. In order to withstand the stresses, plants have been reported to exhibit higher total anti-oxidants. Similar higher T-AOC was reported by Li et al. (2019) under applied formaldehyde.

Our results showed an increase in total phenolics in response to application of formaldehyde and benzene. Phenolics have important role in plant defense as reported by Boo & Yang et al. 2019; Šamec et al. 2021. An increase in total phenolics was displayed by plants under abiotic stress (Sarker et al. 2018) which are in line with the findings of present study.

The carotenoid content of Chlorophytum and Dracaena has decreased under applied stress dose with respect to the control plants. Under abiotic stresses, the carotenoid content drops down, which results in reduced plant productivity. This is accomplished mainly by hindering plant's growth and productivity (Chaves and Oliveira, 2004). According to Strzalka et al. (2003), the amounts of carotenoids and chlorophyll gives information about the plant's stress level as well as its ability to tolerate the stresses.

Under applied stress dose, we found elevated levels of proline in our experimental plants as compared to their controls. Dracaena showed the maximum levels of proline amongst the other two plants under highest applied stress dose. Many plant species generate L-proline in the cytosol in response to diverse environmental stressors. In stressful situations, L-proline acts as an osmo-protectant, stabilizes cellular structures and enzymes, scavenges ROS, and maintains redox balance (Meena et al. 2019). As demonstrated by Hayat et al. 2012 proline accumulation in leaves occurs to preserve chlorophyll content and turgor to safeguard photosynthetic activity under abiotic stresses.

Conclusion

Volatile organic compounds have been reported for their phytotoxic effects. The current research measured plant antioxidative responses against tested combined doses of benzene and formaldehyde with respect to their controls. It was observed that an increasing trend in enzymatic and non-enzymatic antioxidants was followed by our experimental plants after a given stress. Among the tested plants, dracaena plant exhibited significant enzymatic and non-enzymatic responses followed by Ficus plant under applied stresses. There was found a significant correlation between phenolics and some other enzymatic and non-enzymatic antioxidants.

Declarations

Acknowledgment

We are greatly thankful to Higher Education Commission of Pakistan for providing funding to conduct the experiment through NRPU project#7073. The manuscript is a part of MS thesis of Mr. Taimoor Khan.

Author's contribution

Taimoor Khan conducted the research and helped in article writeup. Yasar Sajjad designed, supervised the study and performed data analysis. Hifza Imtiaz and Gulzar Akhtar prepared the first draft. Amjad Hassan and Sabaz Ali Khan reviewed and corrected the first draft. Abdul Rehman Khan and Shahid Masood Shah helped in the execution of experiments.

Ethics approval and Consent to participate: Not applicable.
Consent for publication: Each author is willing to publish the manuscript.

Competing interests: The authors declare no competing interests.

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Availability of data and materials: Available for reference and future use in research

References


**Table**

Table 1. Pearson Correlation among enzymatic and non-enzymatic antioxidants

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<th>Flavenoids</th>
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<th>Carotenoids</th>
<th>Catalase</th>
<th>Guiacol peroxidase</th>
<th>Ascorbate Peroxidase</th>
<th>Superoxide Dismutase</th>
<th>Total Antioxidants</th>
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<td>.395**</td>
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**Correlation is significant at the 0.01 level;  *Correlation is significant at the 0.05 level.**

**Figures**

Figure 1

Glass chamber used to expose indoor plants to formaldehyde and benzene

Figure 2

Effect of formaldehyde and Benzene on various enzymatic antioxidants of tested plants, Catalase (A); Guaiacol peroxidase (B); Ascorbate peroxidase (C); Superoxide dismutase (D); Total antioxidant activity (E). Letters sharing the different letters are statistically differ by Tukey test.
Figure 3

Effect of formaldehyde and Benzene on various enzymatic antioxidants of tested plants, Total phenolics (A); Total flavonoids (B); Total carotenoids (C); Proline (D). Letters sharing the different letters are statistically differ by Tukey test.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable1.docx