

Effect of Air Pollutants on Enzyme Activity of Plants

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ABSTRACT: Air pollution is one of the severe problem world facing today. It deteriorates ecological condition and can be defined as the fluctuation in any atmospheric constituent from the value that would have existed without human activity. Hyderabad is continuously losing its grace and beauty under the growing pressure of densification of activities. The air is being continuously polluted in urban areas because of heavy traffic, industries. Three different sites of the city were selected for the study. The first site is industrial site its name is IDA Bollaram. At this site number of industries located and it is considered as polluted area. The second site is commercial site its name is Koti. Most of the Hyderabad business is done here and impact of vehicular number is high. The third site is residential site and its name is Shivam. In this study, the air pollution effects on the activity of antioxidant enzymes were investigated in 12 plant species named *Acacia nilotica*, *Azadirachta indica*, *Bauhinia variegata*.L, *Bougainvillea spectabilis*, *Cassipourea*.L, *Calotropis procera*, *Delonix regia*, *Ficus religiosa*, *Peltophorum ferrugineum*, *Pongamia pinnata*, *Polyalthia longifolia*, *Terminalia catappa*. The main objective of this study is to evaluate air quality by determination of the activity of detoxification enzymes such as catalase, polyphenol oxidase and peroxidase in the above mentioned plants in the polluted sites and less polluted areas. Based on our research we came to a conclusion that pollution may decrease the activity of enzyme. At site one the three enzymes activity are very low compared to the remaining two sites. *Ficus religiosa*, *Bauhinia variegata*.L, *Acacia nilotica* and *Peltophorum ferrugineum* are high tolerant to air pollution, compared to the remaining eight plant species. These tolerant plants can be used as bio-indicators to monitor the health of the environment.

KEY WORDS: Air pollution, Enzyme activity, Catalase, Peroxidase, Polyphenol oxidase.

I. INTRODUCTION

Air pollution is one of the severe problem world facing today. It deteriorates ecological condition and can be defined as the fluctuation in any atmospheric constituent from the value that would have existed without human activity [1]. The increasing anthropogenic activity intensifies the emission of various pollutants into the environment and introduces different types of harmful substances into the atmosphere. Air pollution is aesthetically unfriendly and can pose health hazards to human as well as plants. The major air pollutants are SO_x, NO_x, CO, particulate matter and sometimes heavy metals [2]. Considerable research has been reported on the impact of air pollutants on various plant enzyme systems. This has enabled elucidation of the mechanisms of pollutant toxicity in some cases along with the development of biochemical techniques to detect air pollution injury to vegetation prior to the development of visible symptoms of phytotoxicity [3]. Air pollutants such as ozone may enter through plant tissues via stomata and elevate the level of reactive oxygen species (ROS) causing serious damage to the DNA, proteins and lipids. For controlling air pollution, air needs first to be monitored by such as physico-chemical methods, biological methods, bioindicators and mathematical models. Among these methods, the biological method is known to be inexpensive for prolonged surveys of air quality due to integration of the effect of all the environmental factors [4]. It is well known that plant cells have several antioxidant defense mechanisms such as tocopherol, carotenoids, glutathione and glutathione reductase enzymes, superoxide dismutase, catalase, ascorbate peroxidase, polyphenol oxidase to protect plants against these oxidative stresses [5]. However, the activation of antioxidant defense mechanisms requires a high consumption of energy which may consequently inhibit the plants growth [6]. In this study, the air pollution effects on the activity of antioxidant enzymes were investigated in 12 plant species named *Acacia nilotica*, *Azadirachta indica*, *Bauhinia*

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variegata.L, Bougainvillea spectabilis, Cassiadora.L, Calotropisprocera, Delonixregia, Ficusreligiosa, Peltophorumferrugineum, Pongamiapinnata, Polyalthialongifolia, Terminaliacatappa. The main objective of this study is to evaluate air quality by determination of the activity of detoxification enzymes such as catalase, polyphenol oxidase and peroxidase in the above mentioned plants in the polluted sites and less polluted areas.

II. MATERIALS & METHODS

Site Selection: Hyderabad is continuously losing its grace and beauty under the growing pressure of densification of activities. The air is being continuously polluted in urban areas because of heavy traffic, industries. Three different sites of the city were selected for the study. The first site is industrial site its name is IDA Bollaram. At this site number of industries located and it is considered as polluted area. The second site is commercial site its name is Koti. Most of the Hyderabad business is done here and impact of vehicular number is high. The third site is residential site and its name is Shivam.

Parameters and sampling frequency: At the height of three to four meters, fully expanded mature leaves were collected from each plant in the polythene bags and transported to the laboratory. The leaf samples were collected on seasonal basis and this frequency was strictly maintained throughout the year.

Investigations of catalase, polyphenol oxidase and peroxidase activity were carried out in all the twelve plants (*Acacia nilotica, Azadirachtaindica, Bauhinia variegata.L, Bougainvillea spectabilis, Cassia tora.L, Calotropisprocera, Delonixregia, Ficusreligiosa, Peltophorumferrugineum, Pongamiapinnata, Polyalthialongifolia, Terminaliacatappa*).

Method for enzyme extraction: 20grams of plant material was grinded in 50ml phosphate buffer. The extract was centrifuge at 15,000 rpm for 20minutes at 4°C. Supernatant was used to study enzyme activity.

Measurement of Catalase activity (CAT) [7]:

The rate of H₂O₂ decomposition at 240 nm was measured spectrophotometrically and calculated using a molar extinction coefficient of 45.2mM⁻¹ cm⁻¹. The reaction mixture consisted of 50 mM phosphate buffer, 0.1mM H₂O₂ and enzyme extract. One unit of catalase activity was assumed as the amount of enzyme that decomposed 1 μmol of H₂O₂ per mg of soluble protein per minute at 30 °C.

Peroxidase (POD) [8]:

To 0.5 ml of enzyme extract, 2.5 ml of 0.1 M phosphate buffer (pH 7), 1 ml of 0.01 M pyrogallol and 1 ml of 0.005 M H₂O₂ were added. A blank was prepared with 0.5 ml of enzyme extract, 3.5 ml of 0.1 M phosphate buffer and 1 ml of 0.005 M H₂O₂. After 5 minutes of incubation at 25 °C, the reaction was stopped by adding 1 ml of 2.5 N H₂SO₄. The amount of purpurogallin formed was estimated by measuring the absorbance at 420 nm against a blank. The enzyme activity was expressed as Units mg⁻¹ protein.

Polyphenol Oxidase (PPO) [9]:

Extraction of PPO: Enzyme extraction was carried out in cold conditions (4°C). Five grams of plant leaves grinded in 25mL 0.1 M phosphate buffer pH 7. The homogenate was filtered through Whatman filter paper and then centrifuged at 5000 rpm for 20 min. The supernatant was filtered through Whatman filter paper and filtrate collected as crude enzyme extract. The filtrate was then subjected to ammonium sulphate fractionation. 20, 40, 60, 80 and 100% ammonium sulphate added sequentially. The protein fractions collected in each step were subjected to dialysis to remove excess ammonium sulphate.

Measurement of PPO activity: PPO activity was determined using a spectrophotometric method of Soliva et al. (2001). The reaction mixture composed of 1.95 ml 0.1M phosphate buffer (pH 7), 1mL of 0.1 M catechol and 50 μL of the enzyme solution. After incubation of 20 min the reaction was terminated by addition of 50 μl of H₂O₂ and absorbance at 410 nm was recorded. One unit of enzyme activity was defined as increase in absorbance at 410 nm by 0.01 per min under assay conditions.

Characterization of PPO: Effect of temperature, pH and substrate concentration on PPO activity was determined. For detection of effect of temperature on PPO the reaction mixture was incubated at different temperatures 20, 30, 40, 50, 60 and 70°C and fix pH (7). The reaction mixture components were equilibrated with specific temperature by prior incubation of 5 min at respective temperatures. To determine optimum pH the buffer in each reaction mixture was replaced with buffers of different pH. Citrate buffer (0.1M) was used for acidic pH range 3 to 5 and phosphate buffer (0.1M) was used for pH 6, 7 and 8. PPO activity with increasing catechol concentration was determined. For this catechol solution of 0.01, 0.02, 0.03.... 0.10 molarity was used.

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Vol. 5, Issue 8, August 2016

III. RESULTS & DISCUSSION

The 12 Plants analyzed site wise are arranged Tables- 1, 2 and 3. In the present study enzymes in leaf like catalase, peroxidase and polyphenol oxidase are used in evaluating their degree of tolerance to air pollution. In Table -1, it gives the values of Industrial site. Where catalase, peroxidase and polyphenol oxidase values are high in *Ficus religiosa* compared to the remaining 11 species. Simultaneously, *Pongamiapinnata* values are low compared to the other 11 species at all the three sites.

Table1. CAT, POD and PPO at IDA Bollaram(Industrial Site)

Plant Species	CAT (U μg^{-1} protein)	POD (U μg^{-1} protein)	PPO (U μg^{-1} protein)
<i>Acacia nilotica</i>	549.1	94.4	150.7
<i>Azadirachta indica</i>	313.6	102.7	267.1
<i>Bauhinia variegata.L</i>	522.7	88.1	154.6
<i>Bougainvillea spectabilis</i>	358.9	256.7	325.2
<i>Cassia tora.L</i>	295.2	65.3	94.5
<i>Calotropis procera</i>	300.9	115.0	227.4
<i>Delonix regia</i>	237.1	46.7	98.9
<i>Ficus religiosa</i>	607.8	321.5	474.8
<i>Peltophorum ferrugineum</i>	524.7	176.1	200.3
<i>Pongamiapinnata</i>	198.2	98.2	174.6
<i>Polyalthi alongifolia</i>	430.1	200.8	285.5
<i>Terminalia catappa</i>	331.7	51.1	200.4

Table2. CAT, POD and PPO at Koti (Commercial Site)

Plant Species	CAT (U μg^{-1} protein)	POD (U μg^{-1} protein)	PPO (U μg^{-1} protein)
<i>Acacia nilotica</i>	585.2	125.8	185.2
<i>Azadirachta indica</i>	379.5	198.2	300.5
<i>Bauhinia variegata.L</i>	599.9	126.1	201.9
<i>Bougainvillea spectabilis</i>	394.7	301.8	400.0
<i>Cassia tora.L</i>	363.6	102.5	184.6
<i>Calotropis procera</i>	378.1	199.9	295.6
<i>Delonix regia</i>	299.9	95.5	188.7
<i>Ficus religiosa</i>	668.5	382.4	502.4
<i>Peltophorum ferrugineum</i>	598.4	210.5	299.9
<i>Pongamiapinnata</i>	275.6	177.5	213.7
<i>Polyalthi alongifolia</i>	486.8	281.7	374.3
<i>Terminalia catappa</i>	395.5	94.2	293.8

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 8, August 2016

Table3. CAT, POD and PPO at Shivam(Residential Site)

Plant Species	CAT (U μg^{-1} protein)	POD (U μg^{-1} protein)	PPO (U μg^{-1} protein)
<i>Acacia nilotica</i>	633.6	151.9	226.8
<i>Azadirachta indica</i>	435.5	252.3	357.4
<i>Baauhiniavariegata.L.</i>	639.0	154.8	258.2
<i>Bouganvilleaspectabilis</i>	554.5	356.5	429.8
<i>Cassis tora.L.</i>	448.9	157.9	230.4
<i>Calotropisprocera</i>	447.9	259.0	352.2
<i>Delonixregia</i>	325.5	159.8	223.2
<i>Ficusreligiosa</i>	733.5	460.5	564.3
<i>Peltaforumferrigoenum</i>	633.1	260.3	353.6
<i>Pongamiapinnata</i>	333.5	260.1	293.2
<i>Polyalthialongifolia</i>	533.2	330.8	413.5
<i>Terminaliacatapa</i>	433.5	161.2	313.9

IV. CONCLUSION

Based on the above data we can conclude that pollution may decrease the activity of enzyme. At Site one the three enzymes activity are very low compared to the remaining two sites. *Ficusreligiosa*, *Baauhiniavariegata.L.*, *Acacia nilotica* and *Peltaforumferrigoenum* are high tolerant to air pollution, compared to the remaining eight plant species. These tolerant plants can be used as bio-indicators to monitor the health of the environment.

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