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Assessment of Oxidative Stress Markers in Some Common Plants

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Abstract
Oxidative stress is a pathological condition that arises due to an imbalance between reactive oxygen species produced and the body's antioxidant defense to neutralize them. This stress has been linked to the pathogenesis of several diseases in animals as well as plants. The present study was designed with the aim of investigating oxidative stress markers in some common indigenous plants of Prayagraj, Uttar Pradesh, under natural environmental conditions. Catalase activity and lipid peroxidation levels were assessed in the fresh leaves of Azadirachta indica (Neem), Ficus religiosa (Peepal), Ocimum tenuiflorum (Tulsi) and Mentha spicata (Pudina/Mint). The results were comparatively analyzed. High catalase activity and lipid peroxidation, in the leaves of Azadirachta indica (Neem), Ficus religiosa (Peepal) reflect a stress-induced physiological state. Ocimum tenuiflorum (Tulsi) and Mentha spicata (Mint) displayed better oxidative stress homeostasis.

Keywords: *Oxidative stress, plants, antioxidants, catalase.*

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I. Introduction

Oxidative stress is caused by an imbalance between the production of reactive oxygen species and the biological system's ability to readily detoxify the reactive intermediates. Every living organism maintains a reducing environment within its cells. This reducing environment is conserved by enzymes that preserve the reduced state through a constant input of metabolic energy. Disorder in this normal redox state can lead to the production of peroxides and free radicals that can damage components of the cell, including proteins, lipids, and DNA. The reactive oxygen species, also called pro-oxidants, can be classified into two groups of compounds: radicals and non-radical derivatives. Figure 1 shows these radical and non-radical species with symbols. These species are called radicals because they contain, as a minimum, one unpaired electron in the shells around the atomic nucleus and can exist independently. The presence of one unpaired electron results in the high reactivity of the species due to their affinity to donate or obtain another electron to attain stability [1].

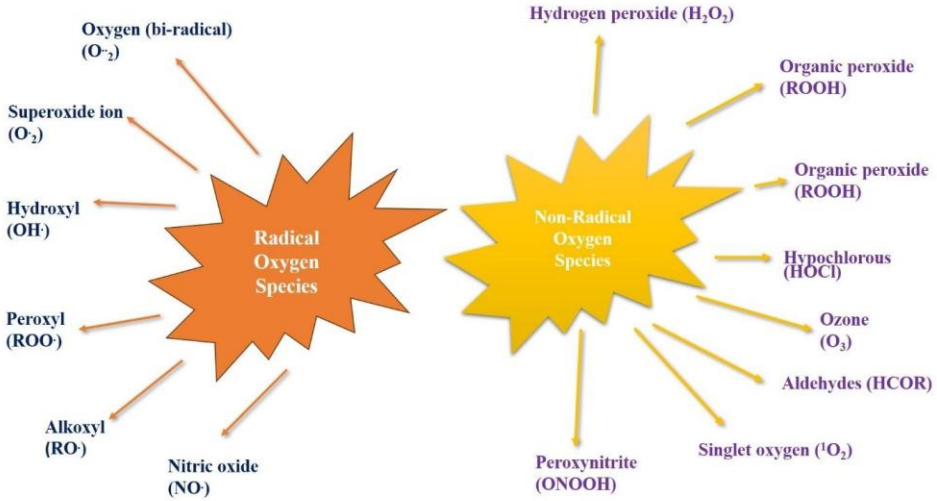

Figure 1: Radical and non-radical metabolites of oxygen

In plants, the reactive oxygen species (ROS, when overproduced, can damage cellular components and cause cell dysfunction [2]. Environmental stressors, including high temperatures, salt stress, drought, or heavy metal toxicity, may cause oxidative stress in plants, which can result in plant cell death [3]. The reactive intermediates, such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($HO\cdot$) are formed during the electron transport chain in photosynthesis and cellular respiration, as well as during photorespiration and other metabolism [4].

Lipid peroxidation (LPO) is a well-established mechanism of cellular injury in plants and is used as an indicator of oxidative stress in cells and tissues. It refers to the oxidative degradation of lipids such as polyunsaturated fatty acids, phospholipids, glycolipids, cholesterol esters and cholesterol. In this process, free radicals seize electrons from lipid molecules, leading to the formation of lipid peroxides. Being unstable, lipid peroxides decompose to form a complex series of compounds, including reactive carbonyl compounds. Polyunsaturated fatty acid peroxides generate malondialdehyde (MDA) and 4-hydroxyalkenals (HAE) upon decomposition. Measurement of malondialdehyde (MDA) and 4-hydroxy alkenals has been used as an indicator of lipid peroxidation [5].

Catalase is found in nearly all organisms exposed to oxygen, where it catalyzes the decomposition of hydrogen peroxide into water and oxygen. Catalase has one of the highest turnover numbers of all enzymes; one catalase molecule can convert 40 million molecules of hydrogen peroxide to water and oxygen each second [6]. Each molecule of catalase has four polypeptide chains, each composed of more than 500 amino acids, and four porphyrin heme groups are placed within this tetrad. The structure is very much like the familiar hemoglobin, cytochromes, chlorophyll and nitrogen-fixing enzymes in legumes. Catalases are divided into three forms: the most abundant heme-containing monofunctional enzymes, bifunctional catalase-peroxidases and manganese-containing catalases [7].

Reactive oxygen species (ROS) production is a normal component of plant physiology that promotes cellular proliferation, physiological function, and viability [8]. However, when overproduced due to stress, ROS may cause cellular damage. ROS play a role as signaling molecules that trigger apoptosis in cells [8]. Climate change–induced abiotic stress has a detrimental effect on crop productivity [9]. ROS are generated in large amounts during abiotic stress in different organelles such as mitochondria, chloroplast and peroxisomes. These are the organelles with a highly oxidizing metabolic activity or an intense rate of electron flow and, hence, are the major source of ROS production in plants [9]. Other sources of ROS in plants are plasma membrane-bound NADPH oxidase and cell wall-bound enzymes such as peroxidases, and amine oxidases [9]. According to Medina *et al.* 2021, the accumulation of reactive intermediates has positive as well as negative effects on plant development [10].

Superoxide dismutase, peroxidase, polyphenol oxidase, catalase, lipoxygenase, ascorbate peroxidase, glutathione reductase, chitinase, and 1, 3 glucanase are some of the enzymatic antioxidants that protect the plant from the detrimental effects of excessive ROS by neutralising them [11]. While ascorbic acid, glutathione and phenols are some examples of non-enzymatic antioxidants.

Understanding the mechanisms and effects of oxidative stress in plants is crucial for comprehending their responses to environmental challenges. However, there is a dearth of data regarding oxidative stress markers in different plant species under natural conditions. By investigating oxidative stress markers in local plant species, we aim to contribute valuable knowledge that can enhance our understanding of plant resilience and inform agricultural practices in the face of increasing environmental stressors.

II. Methodology

Chemicals

All the chemicals used in the present study were of analytical grade and were purchased from SISCO Research Laboratories, Loba Chemicals, India and Sigma Chemical Co., USA. The Bradford reagent for protein assay was purchased from Sigma-Aldrich.

Collection of plant samples: Fresh leaves of *Azadirachta indica* (Neem), *Ficus religiosa* (Peepal), *Ocimum tenuiflorum* (Tulsi), and *Mentha spicata* (Pudina/Mint) were collected from Prayagraj, located at 25.45°N 81.84°E in southern Uttar Pradesh, Northern India, between February and March. To maintain consistency, only leaves from plants at similar phenological stages were chosen. We specifically focused on collecting fully mature leaves to reduce variability caused by developmental differences. The sampling process was conducted at regular intervals to account for any seasonal or phenological influences on oxidative stress markers. Additionally, the sampling protocol, including factors such as time of day and leaf age, was carefully standardized to ensure accurate comparisons across species.

Plant sample preparation for oxidative stress studies

One gram of leaves were weighed and rinsed 3-4 times with tap water and then with deionized water. The leaf material was then crushed for 5 minutes in 3 ml of ice-cold 100 mm K-phosphate buffer pH 6.8 with 0.1 mm EDTA using a mortar and pestle. The homogenate was filtered through Whatman filter paper and centrifuged at 16000g for 15 minutes before being used as an enzyme source [12].

Stock Phosphate buffer saline (PBS) preparation: 10 X Phosphate buffer saline (PBS), pH 7.4 was prepared by dissolving 80g NaCl, 2 gm KCl, 14.4 g Na_2HPO_4, 2.4g KH_2PO_4; dissolved in DDW and volume was made up to 1000 ml.

Measurement of Catalase activity

Catalase activity was determined in the sample using the Luck method [13]. This assay is based on the decomposition of hydrogen peroxide into water and oxygen by Catalase. The rate of decomposition of H2O2 is assessed spectrophotometrically at 240 nm. The reagent was prepared by adding 12.5 mM H_2O_2 in 0.067 M Phosphate buffer; to this, 50µL plant sample was added and thoroughly mixed. The decrease in absorbance was immediately followed at 240 nm for 3 minutes at 30-second intervals. The activity of the enzyme was expressed as µ moles of H_2O_2 decomposed per minute per milligram of protein, using a molar extinction coefficient of H_2O_2 ($71M^{-1}cm^{-1}$).

Lipid peroxidation

Lipid peroxidation (LPO) was measured by the method of Buege and Aust [14]. Malondialdehyde (MDA), the by-product of LPO, forms an adduct with thiobarbituric acid (TBA). On boiling, a pink- colored complex is formed, which absorbs maximally at 532 nm. Reagents used were 150 mM Tris-HCl buffer (pH 7.1), 1.5 mM Ascorbic acid, 1.0 mM Ferrous sulphate ($FeSO_4$), 10% Trichloroacetic acid (TCA), 0.375 g% thiobarbituric acid (TBA). Briefly; 0.1 ml Tris-HCl buffer, 0.1 ml $FeSO_4$, 0.1 ml Ascorbic acid and 0.1 ml sample were added to the test tubes and the volume was made up to 1.0 ml with DDW. The test tubes were then incubated at 37ºC for 15 min and then 1.0 ml TCA and 2 ml TBA were added. Tubes were plugged and incubated for 15 min. in a boiling water bath. After incubation, tubes were centrifuged at 3000 rpm for 10 min. The supernatant was read at 532 nm. The concentration of MDA was calculated using extinction coefficient of MDA-TBA complex, which is 1.56×10^5 M^{-1} cm^{-1} and the results are expressed as n moles MDA/mg protein.

Bradford assay for protein estimation

The assay is based on the observation that the absorbance maximum for an acidic solution of Coomassie Brilliant Blue G-250 shifts from 465 nm to 595 nm when binding to protein occurs. Bovine serum Albumin (BSA) (1 mg/ml) was used as standard. Briefly, the standard curve of BSA was plotted with five standards with amounts ranging from 10 µg/ml to 50 µg/ml; Plant samples were diluted 10X with PBS before the assay. Two milliliters Bradford reagent (Sigma Aldrich) was added to each tube; readings were taken after 5 minutes at 595 nm. The standard curve of BSA was used for calculating concentrations of proteins in samples. The concentration was expressed in mg/ml.

Statistical analysis

All tests were conducted in triplicates, with samples collected from three independent plants of each species to ensure biological replication. Each sample was analyzed in technical triplicates to ensure precision, and the entire experiment was repeated thrice for reproducibility. The results were expressed as mean ± SD. GraphPad Prism 9.5.1 was used for statistical analysis. Catalase activity and LPO among different test samples were analyzed using a one-way ANOVA followed by a post hoc test (Tukey's HSD). P values less than or equal to 0.05 were considered significant. Linear correlation between Catalase activity and LPO was analyzed using the Pearson correlation coefficient.

III. Results and Discussion

Catalase is an antioxidant enzyme that decomposes hydrogen peroxide, a non-radical reactive oxygen species, into water molecules. The specific activity of catalase was measured in leaves of *Azadirachta indica*, commonly known as Neem, *Ficus religiosa*, commonly known as Peepal, *Ocimum tenuiflorum*, commonly known as Tulsi, and *Mentha spicata*, commonly known as Mint/pudina. The results are shown in Table 1. Extremely significant ($p < 0.0001$) differences in the catalase activity were observed in different plant samples. Peepal showed the highest activity, followed by Neem, and the activities in Mint and Tulsi were less as compared to Peepal and Neem, activity was least in Tulsi.

Table 1: Specific activity of catalase (µmol H_2O_2/min./ mg protein) in leaves of *Azadirachta indica, Ficus religiosa, Ocimum tenuiflorum* and *Mentha spicata*.

Plant tissue	**Specific activity (µmol H_2O_2/min/ mg protein)**
Azadirachta indica	16.72 ± 0.27*
Ficus religiosa	22.77 ± 0.95*
Ocimum tenuiflorum	0.145 ± 0.007*
Mentha spicata	1.855 ± 0.02*

Values are expressed as Mean ± S.D.; n=3; * represents an extremely significant ($p<0.0001$) difference among the groups as analyzed by a one-way ANOVA followed by Tukey's HSD test.

In a 2012 study conducted by Goud and Kachole, mature Neem leaves exhibited notably low catalase specific activity, and young leaves showed an absence of it [15]. This contrasts with our current findings. There is a dearth of data on oxidative stress and antioxidant enzymes, particularly on catalase activity and LPO in the plants selected for the current study. The results obtained in this study, which indicate elevated catalase activity in Neem and Peepal, suggest a potent antioxidant profile in these plants. The elevated catalase activity could be attributed to either stress response or as a natural part of the physiological response.

Lipid peroxidation (LPO) is a marker of oxidative stress and is measured as nanomoles of MDA formed per mg of protein. Table 2 displays LPO measured in Neem, Peepal, Tulsi and Mint. The difference among the tissues was significant, with the highest LPO found in Neem leaves followed by Peepal. Like catalase activity, lipid peroxidation (LPO) was observed to be elevated in Neem and Peepal leaves in comparison to the other samples. This suggests that these plants may have been experiencing oxidative stress, leading to an increase in catalase levels as a response to manage it. In contrast, low LPO in Tulsi and Mint reflect better homeostasis of ROS. In plants, lipid peroxidation is a consequence of oxidative stress caused by various biotic and abiotic stress factors [16].

Table 2: Malondialdehyde levels (nmoles MDA/mg protein) in leaves of *Azadirachta indica, Ficus religiosa, Ocimum tenuiflorum* and *Mentha spicata*

Plant sample	nmoles MDA/mg protein
Azadirachta indica	54.4± 0.11*
Ficus religiosa	22.2± 0.85*
Ocimum tenuiflorum	3.4 ± 0.34*
Mentha spicata	3.2± 0.11*

Values are expressed as Mean ± S.D.; n=3; * represents extremely significant ($p<0.0001$) difference among the groups as analyzed by a one-way ANOVA followed by Tukey's HSD test.

A positive correlation was observed between catalase activity and LPO, as depicted in Figure 2. This finding reflects that increased catalase activity was not enough to contain oxidative stress in the leaves.

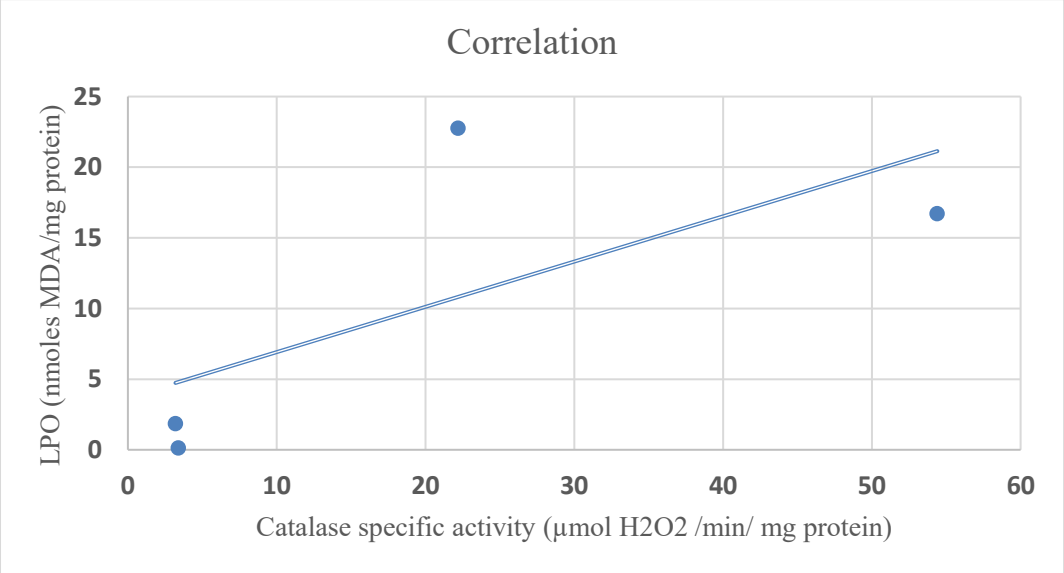

Figure 2: Graphical representation of correlation (Pearson's correlation coefficient (r) =0.694) between catalase activity and LPO.

IV. Conclusion

Oxidative stress is a concern for humans, animals, and plants. It is linked to the pathogenesis of several diseases. In plants, reactive oxygen species (ROS), when overproduced, can damage cellular components and cause cell dysfunction. Environmental stressors, such as high temperatures, salt stress, drought, or heavy metal toxicity, may induce oxidative stress in plants, potentially leading to plant cell death. This oxidative stress can have a detrimental effect on crop productivity.

In the present study, we hypothesized that there is significant variation in oxidative stress markers across different plant species under natural environmental conditions. Our findings showed high catalase activity and lipid peroxidation, markers of oxidative stress, in the leaves of *Azadirachta indica* (Neem) and *Ficus religiosa* (Peepal), reflecting a stress-induced physiological state. In contrast, *Ocimum tenuiflorum* (Tulsi) and *Mentha spicata* (Mint) displayed better oxidative stress homeostasis. These results suggest that there is indeed variation in oxidative stress markers across the different plant species. This was a brief report; a more comprehensive examination, involving additional variables, is required for further investigation.

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Ethical statement

The collected plants were authenticated by the competent Botanist. The collection of the plants used in the study complies with local or national guidelines with no need for further affirmation.

Ethics, Consent to Participate, and Consent to Publish declarations: not applicable

Competing Interest declaration: The authors declare that there are no competing interests.

Data Availability statement

Data is provided within the manuscript.

References

[1]. Kohen R, Nyska A. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicol Pathol. 2002 Nov-Dec; 30(6):620-50. Doi: 10.1080/01926230290166724. PMID: 12512863

[2]. Demidchik, Vadim. "Mechanisms of oxidative stress in plants: from classical chemistry to cell biology." Environmental and experimental botany. 2015, 109:212-228.

[3]. Chaki M, Begara-Morales JC, Barroso JB. Oxidative Stress in Plants. Antioxidants (Basel). 2020 Jun 3;9(6):481. doi: 10.3390/antiox9060481. PMID: 32503179; PMCID: PMC7346199.

[4]. Lorin MS, Enjun X, Niina I, Frank AH, Per M, Mikael B, Frank VB, Jaakko K. Spreading the news: subcellular and organellar reactive oxygen species production and signalling. Journal of Experimental Botany 2016, 67:13, 3831-3844.
[5]. Esterbaur H, Schaur RJ, Zollner H. Chemistry and Biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic Bio Med. 1991; 11(1):81-128. Doi: 10.1016/0891-5849(91)90192-6. PMID:1937131
[6]. Smejkal, G. B., & Kakumanu, S. (2019). Enzymes and their turnover numbers. Expert Review of Proteomics, 16(7), 543–544.
[7]. Chelikani P, Fita I, Loewen, PC. Diversity of structures and properties among catalases. Cell. Mol. Life Sci. 2004, 61 (2):192–208.
[8]. Mittler R. ROS Are Good. Trends Plant Sci. 2017 Jan;22(1):11-19. doi: 10.1016/j.tplants.2016.08.002. Epub 2016 Sep 23. PMID:27666517.
[9]. Nadarajah KK. ROS Homeostasis in Abiotic Stress Tolerance in Plants. Int J Mol Sci. 2020 Jul 23;21(15):5208. doi: 10.3390/ijms21155208. PMID: 32717820; PMCID: PMC7432042.
[10]. Medina E, Kim SH, Yun M, Choi WG. Recapitulation of the Function and Role of ROS Generated in Response to Heat Stress in Plants. Plants. 2021; 10(2):371.https://doi.org/10.3390/plants10020371
[11]. Sofy AR, Dawoud RA, Sofy MR, Mohamed HI, Hmed AA, El-Dougdoug NK. Improving Regulation of Enzymatic and Non-Enzymatic Antioxidants and Stress-Related Gene Stimulation in Cucumber mosaic cucumo virus-Infected Cucumber Plants Treated with Glycine Betaine, Chitosan and Combination. Molecules. 2020 May 17; 25(10):2341. doi: 10.3390/molecules25102341. PMID: 32429524; PMCID: PMC7288169.
[12]. Cavalcanti FR, Oliveira JTA, Martins-Miranda AS, Viégas RA, Silveira JAG. Superoxide dismutase, catalase and peroxidase activities do not confer protection against oxidative damage in salt-stressed cowpea leaves. New Phytol. 2004 Sep;163(3):563-571. doi: 10.1111/j.1469-8137.2004.01139.x. PMID:33873746.
[13]. Luck H. In: Methods in enzymatic analysis. Ed Bergmeyer HU vol: III Academic press New York, 1971, 279.
[14]. Buege JA and Aust SD. Microsomal lipid peroxidation. Methods Enzymol. 1978, 52:302- 310.
[15]. Goud PB, Kachole MS. Antioxidant enzyme changes in neem, pigeonpea and mulberry leaves in two stages of maturity. Plant Signal Behav. 2012 Oct 1;7(10):1258-62. doi: 10.4161/psb.21584. Epub 2012 Aug 20. PMID: 22895104; PMCID: PMC3493408.
[16]. Awasthi JP, Saha B, Chowardhara B, Devi SS, Borgohain P, Panda SK. Qualitative Analysis of Lipid Peroxidation in Plants under Multiple Stress Through Schiff's Reagent: A Histochemical Approach. Bio Protoc. 2018 Apr 20; 8(8): e2807. doi: 10.21769/BioProtoc.2807. PMID: 34286024; PMCID: PMC8275295.