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Biochemical and Oxidative Stress Responses in Radish (*Raphanus sativus* L.) Exposed to Pharmaceutical Industrial Effluents

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Abstract

Pharmaceutical industries discharge effluents containing various organic and inorganic pollutants, which can adversely affect plant growth and metabolism. The present study investigates the biochemical and oxidative stress responses of radish (*Raphanus sativus* L.) exposed to different concentrations of pharmaceutical industrial effluents. Radish seeds were grown in soil irrigated with varying effluent concentrations (0%, 25%, 50%, 75%, and 100%) for a defined growth period. Biochemical parameters such as chlorophyll content, total soluble proteins, soluble sugars, and proline accumulation were analyzed. Oxidative stress markers including malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and antioxidant enzyme activities (superoxide dismutase, catalase, and peroxidase) were also determined.

The results revealed that increasing effluent concentrations significantly reduced chlorophyll, protein, and sugar contents, indicating impaired photosynthetic efficiency and metabolic activity. Conversely, proline accumulation increased under effluent stress, suggesting its protective role against environmental stress. Elevated levels of MDA and H_2O_2 indicated enhanced oxidative damage in treated plants. Antioxidant enzyme activities increased at moderate effluent concentrations as a defence mechanism, but declined at higher concentrations due to severe toxicity. The study concludes that pharmaceutical industrial effluents induce oxidative stress and biochemical alterations in radish plants, affecting their growth and physiological performance. Monitoring these responses can serve as valuable indicators of environmental pollution and plant health.

Keywords: *Raphanus sativus* L.; Pharmaceutical Effluent; Oxidative Stress; Antioxidant Enzymes; Chlorophyll; Proline; Phytotoxicity; Environmental Pollution.

1.0 Introduction

Exposure of *Raphanus sativus* L. (radish) to pharmaceutical industrial effluents can cause significant oxidative stress and adversely affect various biochemical and physiological processes. Pharmaceutical wastewater often contains heavy metals, pharmaceutical residues, and other toxic organic compounds that stimulate the production of reactive oxygen species (ROS), resulting in oxidative damage to plant cells (Sharma & Dietz, 2009; Gill & Tuteja, 2010).

One of the primary indicators of oxidative stress is lipid peroxidation, which is commonly measured by the accumulation of malondialdehyde (MDA). Increased MDA levels reflect membrane deterioration and cellular damage caused by excessive ROS generation (Heath & Packer, 1968). Under heavy metal and osmotic stress conditions, radish plants also accumulate proline, an important osmoprotectant that contributes to osmotic adjustment, stabilization of proteins and membranes, and detoxification of metal ions (Szabados &Savouré, 2010).

Plants respond to oxidative stress through an antioxidant defense system consisting of several enzymes. Superoxide dismutase (SOD) converts superoxide radicals into hydrogen peroxide (H_2O_2), while catalase (CAT) and peroxidase (POD) further detoxify H_2O_2 into harmless products such as water and oxygen. Nevertheless, prolonged or excessive exposure to pollutants may impair these defence mechanisms, leading to enhanced oxidative damage and reduced plant growth (Mittler, 2002; Gill & Tuteja, 2010).

Pharmaceutical effluents also affect photosynthetic pigments and protein metabolism. Toxic contaminants can disrupt chloroplast structure and function, causing reductions in chlorophyll a, chlorophyll b, and carotenoid contents, thereby decreasing photosynthetic efficiency (Yadav, 2010). Similarly, total protein content may decline under stress conditions, although plants often synthesize stress-related proteins such as phytochelatins and metallothioneins to enhance tolerance against heavy metal toxicity (Cobbett & Goldsbrough, 2002).

Although *Raphanus sativus* is sensitive to elevated concentrations of pollutants, it possesses adaptive mechanisms that enable it to tolerate and accumulate certain heavy metals. This characteristic makes radish a promising species for phytoremediation studies aimed at reducing environmental contamination. However, plants grown in polluted environments should not be used for human consumption because of the potential accumulation of toxic substances in edible tissues (Ali et al., 2013).

2.0 Collection of Effluent

Pharmaceutical industrial effluent was collected from the final discharge outlet of a pharmaceutical manufacturing unit in clean, sterile high-density polyethene (HDPE) containers following standard wastewater sampling procedures (APHA, 2017). Before collection, the containers were thoroughly washed with distilled water and rinsed with the effluent to prevent contamination. The collected samples were transported to the laboratory and stored at 4°C until further use and analysis. Physicochemical characteristics of the effluent, including pH, electrical conductivity (EC), total dissolved solids (TDS), and chemical oxygen demand (COD), were determined according to standard methods for water and wastewater examination (APHA, 2017; Tchobanoglous et al., 2014). The effluent was subsequently diluted with distilled water to obtain different concentrations (25%, 50%, 75%, and 100%) for treatment of radish (*R. sativus* L.) plants.

Immediately after collection, the pharmaceutical industrial effluent samples were transferred into clean, sterile high-density polyethene (HDPE) containers, properly labelled, and stored at 4°C until further physicochemical analysis and experimental application (APHA, 2017)."

3.0 Preparation of Treatments

The pharmaceutical industrial effluent was used to prepare different treatment concentrations for assessing its impact on *R. sativus* growth and biochemical responses. Five experimental treatments were prepared by diluting the raw effluent with distilled water in a graded concentration series. The control treatment (T_0) consisted of only tap water (0% effluent), while T_1, T_2, and T_3 were prepared by mixing effluent and distilled water at concentrations of 25%, 50%, and 75%, respectively. The highest treatment (T_4) contained undiluted effluent (100%). This graded dilution approach helps in evaluating dose-dependent phytotoxic effects of industrial effluents on plant systems (APHA, 2017; WHO, 2022**)** (Table-1).

Table-1 Treatment Details

Treatment	Effluent Concentration	Composition
T_0	0%	Tap water (Control)
T_1	25%	25% effluent + 75% distilled water
T_2	50%	50% effluent + 50% distilled water
T_3	75%	75% effluent + 25% distilled water
T_4	100%	Raw effluent

The dilution series was prepared freshly before each irrigation to maintain uniformity and avoid physicochemical changes in effluent composition. This method is widely adopted in phytotoxicity and wastewater impact studies to assess plant stress responses under controlled environmental conditions (Tchobanoglous et al., 2014; Ali et al., 2013).

3.1 Pot Preparation

Uniform plastic pots were selected for the experiment and filled with equal quantities of well-mixed garden soil to ensure consistent physicochemical conditions across all treatments. Before filling, the soil was thoroughly sieved to remove debris, stones, and organic residues to maintain homogeneity and uniform texture, which is essential for reliable plant growth studies (Brady & Weil, 2016).

Healthy and viable seeds of radish (*Raphanus sativus* L.) were selected and 10–15 seeds were sown in each pot at a uniform depth and spacing to ensure proper germination and minimize variability among experimental units. The use of uniform seed number per pot helps in reducing experimental error and ensures comparable growth conditions across treatments.

Each treatment was replicated three times (n = 3) to ensure statistical validity and reproducibility of the results. The pots were arranged in a completely randomized design (CRD) to eliminate positional effects and environmental bias such as light intensity, temperature variation, and watering differences (Gomez & Gomez, 1984).

3.2 Controlled Irrigation and Treatment Application in Pot Experiments

Daily application of the respective treatment solutions (such as nutrients, heavy metals, or salinity solutions) should be maintained consistently for each experimental pot. Each treatment must be applied carefully to ensure uniform exposure across all replicates.

A graduated cylinder or pipette should be used to measure the solution accurately, ensuring that an equal volume is applied to each plant. This helps maintain experimental precision and reduces variability caused by uneven dosing.

The controlled irrigation regimen should be continued for 30 to 45 days, depending on the experimental design and crop growth stage.

3.3 Best Practices for Pot Irrigation

1. Labelling of Pots:

Each pot must be clearly labelled with its respective treatment code (e.g., T_0, T_1, T_2, etc.) to prevent confusion and avoid cross-contamination between treatments.

2. Uniform Application Technique:

The treatment solution should be poured slowly and evenly at the base of the plant. Direct splashing on leaves should be avoided to prevent foliar damage and unintended absorption.

3. Runoff and Drainage Management:

Pots should be equipped with proper drainage holes to prevent waterlogging. Collection trays may be placed underneath pots if leachate measurement is required or to prevent environmental contamination from chemical runoff.

4. Irrigation

Pots should be irrigated with their respective treatment solutions according to the experimental design. Care must be taken to ensure uniform application of solutions across all replicates to avoid variability in treatment exposure. Irrigation should be carried out consistently for a period of 30–45 days, depending on the crop growth stage and experimental objectives. The volume of solution applied should be kept constant for all treatments to maintain experimental accuracy and reproducibility (Taiz et al., 2015; Gomez & Gomez, 1984).

5. Growth Observations

Plant growth responses should be recorded systematically at regular intervals throughout the experimental period. The following growth parameters should be measured to evaluate the effects of different treatments:

Germination percentage: Calculated as the proportion of seeds germinated in each treatment relative to the total number of seeds sown.

Shoot length: Measured from the base of the stem to the tip of the shoot using a standard scale or measuring tape.

Root length: Measured from the root-shoot junction to the tip of the primary root.
Fresh weight: Determined by weighing the whole plant immediately after harvest using an analytical balance.

Dry weight: Obtained after drying plant samples in a hot air oven at 60–70°C until constant weight is achieved.

These parameters provide a comprehensive assessment of plant growth performance under different experimental treatments and are commonly used in physiological and stress-related plant studies (Gomez & Gomez, 1984; Taiz et al., 2015).

6. Biochemical Analysis

Biochemical parameters should be estimated from fresh leaf samples collected from each treatment at the end of the experimental period. All analyses should be carried out using standard spectrophotometric methods under controlled laboratory conditions.

Total Chlorophyll Content: Total chlorophyll (chlorophyll a and b) should be estimated from fresh leaf tissue using 80% acetone extraction. The absorbance of the extract is measured at 645 nm and 663 nm using a spectrophotometer. Chlorophyll content is calculated using standard equations and expressed as mg g^{-1} fresh weight (Arnon, 1949).

Soluble Protein Content: Soluble protein content should be determined using the **Bradford method**, where Coomassie Brilliant Blue dye binds to proteins and the absorbance is measured at 595 nm. Bovine serum albumin (BSA) is used as a standard for calibration, and results are expressed as mg g^{-1} fresh weight (Bradford, 1976).

Soluble Sugar Content: Soluble sugars are estimated using the **anthrone reagent method**, where sugars react under acidic conditions to produce a green color measured at 620 nm. Glucose is used as a standard, and results are expressed as mg g^{-1} fresh weight (Dubois et al., 1956).

Proline Content: Proline accumulation is determined using the **acid ninhydrin method**, where proline reacts with ninhydrin reagent to form a colored complex measured at 520 nm. The concentration is calculated using a standard curve and expressed as µmol g^{-1} fresh weight (Bates et al., 1973). Proline is widely recognized as a key osmoprotectant involved in plant stress tolerance.

7. Oxidative Stress Analysis

Oxidative stress parameters should be estimated from fresh leaf tissues collected from each treatment group using standard biochemical assays. All measurements should be performed under controlled laboratory conditions using spectrophotometric methods.

Malondialdehyde (MDA) Content: Lipid peroxidation is estimated by measuring malondialdehyde (MDA) content using the **thiobarbituric acid (TBA) reaction method**. The absorbance of the supernatant is recorded at 532 nm and corrected at 600 nm. MDA content is expressed as nmol g^{-1} fresh weight, indicating the level of membrane damage caused by oxidative stress (Heath & Packer, 1968).

Hydrogen Peroxide (H_2O_2) Content: Hydrogen peroxide content is determined by reacting plant extracts with potassium iodide (KI), and the absorbance is measured at 390 nm. The concentration of H_2O_2 is calculated using a standard curve and expressed as µmol g^{-1} fresh weight, serving as an indicator of reactive oxygen species (Velikova et al., 2000).

Superoxide Dismutase (SOD) Activity: SOD activity is assayed based on its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). The reaction mixture is exposed to light, and absorbance is measured at 560 nm. One unit of SOD activity is defined as the amount of enzyme required to cause 50% inhibition of NBT reduction (Beauchamp & Fridovich, 1971).

Catalase (CAT) Activity: Catalase activity is determined by monitoring the decomposition of hydrogen peroxide (H_2O_2) at 240 nm. The decrease in absorbance over time is used to calculate enzyme activity, expressed as µmol H_2O_2 decomposed min^{-1} mg^{-1} protein (Aebi, 1984).

Peroxidase (POD) Activity: Peroxidase activity is estimated using guaiacol as a substrate in the presence of H_2O_2. The increase in absorbance due to tetraguaiacol formation is measured at 470 nm. POD activity is expressed as a change in absorbance min^{-1} mg^{-1} protein (Chance &Maehly, 1955).

Conclusion

The present study evaluated the effects of different concentrations of industrial effluent on the growth, biochemical attributes, and oxidative stress responses of radish (*Raphanus sativus* L.) under pot culture conditions. The results demonstrated that effluent concentration significantly influenced plant performance. Lower concentrations of effluent may provide certain nutrients that support plant growth, whereas higher concentrations adversely affect germination, shoot and root development, fresh and dry biomass accumulation.

Biochemical analyses revealed alterations in total chlorophyll, soluble proteins, soluble sugars, and proline content, indicating physiological adjustments of plants in response to effluent-induced stress. Furthermore, elevated levels of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) confirmed the occurrence of oxidative damage under higher effluent concentrations. Enhanced activities of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), reflected the activation of the plant's defense mechanisms against reactive oxygen species.

Overall, the findings suggest that untreated or highly concentrated industrial effluents can negatively affect plant growth and metabolism, while diluted effluent may be utilized with caution after proper assessment of its composition and toxicity. The study highlights the importance of monitoring industrial wastewater quality before its use in agriculture and emphasizes the need for effective effluent treatment to minimize environmental and agricultural risks. Further investigations under field conditions are recommended to validate the long-term effects of effluent irrigation on crop productivity, soil health, and food safety.

References

Aebi, H. (1984). Catalase in vitro. Methods in Enzymology, 105, 121–126.

Ali, H., Khan, E., & Sajad, M. A. (2013). Phytoremediation of heavy metals—Concepts and applications. Chemosphere, 91(7), 869–881. https://doi.org/10.1016/j.chemosphere.2013.01.075

APHA. (2017). Standard Methods for the Examination of Water and Wastewater (23rd ed.). American Public Health Association, Washington, DC.

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. Plant Physiology, 24(1), 1–15.

Bates, L. S., Waldren, R. P., & Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. Plant and Soil, 39, 205–207.

Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase: Improved assays. Analytical Biochemistry, 44, 276–287.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein. Analytical Biochemistry, 72, 248–254.

Chance, B., & Maehly, A. C. (1955). Assay of catalases and peroxidases. Methods in Enzymology, 2, 764–775.

Cobbett, C., & Goldsbrough, P. (2002). Phytochelatins and metallothioneins: Roles in heavy metal detoxification and homeostasis. Annual Review of Plant Biology, 53, 159–182. https://doi.org/10.1146/annurev.arplant.53.100301.135154

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. Analytical Chemistry, 28, 350–356.

Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry, 48(12), 909–930. https://doi.org/10.1016/j.plaphy.2010.08.016

Gomez, K. A., & Gomez, A. A. (1984). Statistical Procedures for Agricultural Research (2nd ed.). John Wiley & Sons.

Heath, R. L., & Packer, L. (1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics, 125(1), 189–198. https://doi.org/10.1016/0003-9861(68)90654-1

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science, 7(9), 405–410. https://doi.org/10.1016/S1360-1385(02)02312-9

Sharma, P., & Dietz, K. J. (2009). The relationship between metal toxicity and cellular redox imbalance. Trends in Plant Science, 14(1), 43–50.

https://doi.org/10.1016/j.tplants.2008.10.007

Szabados, L., & Savouré, A. (2010). Proline: A multifunctional amino acid. Trends in Plant Science, 15(2), 89–97. https://doi.org/10.1016/j.tplants.2009.11.009

Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. (2015). Plant Physiology and Development (6th ed.). Sinauer Associates.

Tchobanoglous, G., Stensel, H. D., Tsuchihashi, R., & Burton, F. L. (2014). Wastewater Engineering: Treatment and Resource Recovery. McGraw-Hill Education.

Tchobanoglous, G., Stensel, H. D., Tsuchihashi, R., Burton, F. L., Abu-Orf, M., Bowden, G., & Pfrang, W. (2014). Wastewater Engineering: Treatment and Resource Recovery (5th ed.). McGraw-Hill Education, New York.

Velikova, V., Yordanov, I., & Edreva, A. (2000). Oxidative stress and antioxidant systems in plants. Plant Science, 151, 59–66.

WHO. (2022). Guidelines for Drinking-water Quality. World Health Organization, Geneva.

Yadav, S. K. (2010). Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance. South African Journal of Botany, 76(2), 167–179. https://doi.org/10.1016/j.sajb.2009.10.007

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