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**Research Paper**

# Assessment of Mercury-Induced Alterations in Physiological and Biochemical Parameters of Fenugreek

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***Abstract***
*Mercury contamination has emerged as a serious environmental problem due to its toxic effects on agricultural crops, soil fertility, and ecosystem stability. The present study entitled "Assessment of Mercury-Induced Alterations in Physiological and Biochemical Parameters of Fenugreek" was conducted to evaluate the impact of mercury toxicity on the physiological growth characteristics and biochemical responses of fenugreek plants under controlled experimental conditions. Different concentrations of mercury were applied to fenugreek plants, and various physiological parameters such as seed germination percentage, root length, shoot length, chlorophyll content, transpiration rate, relative water content, and biomass accumulation were analyzed. Simultaneously, biochemical parameters including soluble protein, carbohydrate content, free amino acids, phenolic compounds, lipid peroxidation, hydrogen peroxide accumulation, and antioxidant enzyme activities were estimated to determine the extent of oxidative stress and metabolic alterations.*
*The experimental findings revealed that increasing mercury concentrations significantly reduced seed germination, plant growth, chlorophyll content, water relations, and biomass production. Mercury exposure also caused substantial decreases in protein and carbohydrate levels, indicating disruption of metabolic activities. In contrast, oxidative stress biomarkers such as hydrogen peroxide, malondialdehyde, and lipid peroxidation increased markedly under mercury stress conditions. Antioxidant enzymes including catalase, peroxidase, superoxide dismutase, and ascorbate peroxidase showed enhanced activities, suggesting activation of defense mechanisms against reactive oxygen species. Statistical analyses including ANOVA, regression analysis, correlation analysis, and principal component analysis confirmed the significant influence of mercury on physiological and biochemical parameters. The study concludes that mercury toxicity induces severe oxidative stress and metabolic disturbances in fenugreek plants, thereby affecting their growth, productivity, and physiological stability. The findings contribute to understanding heavy metal toxicity mechanisms and may help in developing strategies for sustainable agriculture, environmental monitoring, and phytoremediation.*

***Keywords:*** *Mercury toxicity, Fenugreek, Heavy metal stress, Oxidative stress, Chlorophyll degradation, Antioxidant enzymes, Lipid peroxidation, Plant physiology, Biochemical alterations, Reactive oxygen species, Environmental pollution*

## I. Introduction

Environmental pollution caused by heavy metals has emerged as one of the most serious ecological and agricultural concerns of the modern era. Among various toxic metals, mercury (Hg) is considered highly hazardous because of its persistence, bioaccumulation, and toxic effects on living organisms. Industrialization, mining, thermal power plants, pesticide application, sewage disposal, and chemical industries continuously release mercury into the environment, leading to contamination of soil and water resources. Agricultural lands located near industrial areas are particularly vulnerable to mercury contamination, which adversely affects crop productivity, soil fertility, and food quality. Since plants serve as the primary producers in ecosystems and form the base of the food chain, understanding the impact of mercury toxicity on plants is essential for environmental protection and sustainable agriculture. Mercury toxicity influences several physiological and biochemical processes in plants. Excess mercury accumulation disturbs seed germination, root and shoot growth, photosynthesis, water uptake, nutrient balance, and enzyme activities. Mercury also induces oxidative stress by generating reactive oxygen species (ROS), which damage cellular membranes, proteins, nucleic acids, and

chlorophyll pigments. Consequently, plants exposed to mercury exhibit reduced growth, chlorosis, wilting, and metabolic disturbances. The extent of these alterations depends on the concentration of mercury, duration of exposure, plant species, and environmental conditions. Therefore, investigating mercury-induced changes in plants can help in understanding stress physiology and developing strategies for crop protection.

Fenugreek, scientifically known as Trigonella foenum-graecum, is an important medicinal, culinary, and fodder crop widely cultivated in India and many other countries. It belongs to the family Fabaceae and is valued for its nutritional and therapeutic properties. Fenugreek leaves and seeds are rich in proteins, vitamins, minerals, dietary fiber, alkaloids, flavonoids, and antioxidants. The crop plays a significant role in traditional medicine and food industries because of its anti-diabetic, anti-inflammatory, antimicrobial, and antioxidant properties. Due to its economic and medicinal importance, maintaining the physiological and biochemical health of fenugreek plants is essential. However, increasing environmental contamination by heavy metals poses a serious threat to its growth, productivity, and nutritional quality. Mercury contamination may interfere with various physiological functions of fenugreek plants, including seed germination, transpiration, stomatal conductance, chlorophyll synthesis, and photosynthetic efficiency. It may also alter biochemical constituents such as proteins, carbohydrates, amino acids, phenolic compounds, antioxidant enzymes, and lipid peroxidation levels. These biochemical responses act as indicators of stress tolerance or susceptibility in plants. Studying these parameters provides valuable insight into the toxicological effects of mercury and the adaptive mechanisms developed by plants under stress conditions. Furthermore, physiological and biochemical markers can be used to assess the degree of environmental pollution and plant health.

The present study focuses on assessing mercury-induced alterations in physiological and biochemical parameters of fenugreek. The study aims to evaluate how different concentrations of mercury affect growth characteristics, photosynthetic pigments, water relations, and metabolic activities in fenugreek plants. Such investigations are important for identifying the tolerance capacity of fenugreek against heavy metal stress and for understanding the broader implications of mercury pollution in agricultural ecosystems. The findings may contribute to the development of sustainable agricultural practices, phytoremediation approaches, and pollution management strategies. In recent years, there has been increasing scientific interest in understanding plant responses to heavy metal stress at physiological, biochemical, and molecular levels. Researchers have reported that mercury toxicity significantly inhibits plant growth and metabolism by affecting membrane permeability, enzymatic activity, and nutrient transport systems. Mercury ions bind strongly with sulfhydryl groups of proteins, thereby inactivating essential enzymes and disrupting normal metabolic pathways. In addition, mercury-induced oxidative stress results in excessive production of free radicals, leading to cellular injury and impaired physiological performance. Plants often respond to such stress by activating antioxidant defense systems, including enzymes like catalase, peroxidase, and superoxide dismutase. Therefore, the analysis of these biochemical responses can provide important information regarding plant adaptation and stress resistance mechanisms.

Fenugreek serves as a suitable model plant for studying heavy metal toxicity because of its fast growth, easy cultivation, and medicinal relevance. Understanding the interaction between mercury stress and fenugreek metabolism may help in identifying biomarkers of toxicity and tolerance. Moreover, since fenugreek is consumed directly as a leafy vegetable and spice, mercury accumulation in edible plant parts may pose health risks to humans and animals. Hence, evaluating mercury-induced physiological and biochemical changes in fenugreek is not only important from an agricultural perspective but also from a public health viewpoint. The study of heavy metal toxicity in crops has gained importance in the context of food security and environmental sustainability. Contaminated crops may lead to reduced yield, poor nutritional quality, and increased health hazards through food chain transfer. Therefore, scientific assessment of mercury toxicity in economically important crops like fenugreek is essential for ensuring safe agricultural production and environmental conservation. The present research is expected to provide valuable data regarding the toxic effects of mercury on plant growth and metabolism and may serve as a foundation for future studies related to stress physiology, phytotoxicity, and ecological risk assessment.

**Research Objectives**

1. To assess the effect of mercury on the physiological parameters of fenugreek plants.
2. To evaluate the impact of mercury toxicity on biochemical constituents of fenugreek.
3. To study changes in chlorophyll content, growth rate, and water relations under mercury stress.
4. To analyze the effect of mercury on antioxidant enzyme activities and oxidative stress markers.
5. To determine the tolerance and adaptability of fenugreek plants under varying concentrations of mercury.
6. To understand the relationship between mercury concentration and metabolic alterations in fenugreek.

**Research Hypotheses**

1. Mercury exposure significantly alters the physiological parameters of fenugreek plants.

2. Increasing concentrations of mercury negatively affect biochemical constituents such as proteins, chlorophyll, and carbohydrates.
3. Mercury toxicity induces oxidative stress in fenugreek plants by increasing reactive oxygen species.
4. Antioxidant enzyme activities increase in response to mercury-induced stress conditions.
5. Higher mercury concentrations result in reduced growth and metabolic efficiency in fenugreek plants.

**Significance of the Study**

The study is significant because it provides scientific understanding regarding the toxic effects of mercury on an economically and medicinally important crop like fenugreek. It contributes to the field of plant physiology, environmental toxicology, and agricultural science by identifying physiological and biochemical responses of plants under heavy metal stress. The findings may help farmers, researchers, and environmental agencies in developing strategies for monitoring and managing heavy metal contamination in agricultural soils.

The study also has importance in the context of food safety and human health because fenugreek is widely consumed as a food and medicinal herb. Understanding mercury accumulation and toxicity in fenugreek can help prevent potential health hazards associated with contaminated food crops. Furthermore, the research may support future studies related to phytoremediation, stress tolerance mechanisms, and sustainable agricultural practices.

**Need of the Study**

Rapid industrialization and urbanization have increased the release of toxic heavy metals into the environment, causing serious ecological and agricultural problems. Mercury contamination of soil and water threatens crop productivity and food quality. Despite the importance of fenugreek as a nutritional and medicinal crop, limited studies are available on mercury-induced physiological and biochemical alterations in this plant. Therefore, there is a need to investigate the toxic effects of mercury on fenugreek to understand its stress responses and tolerance mechanisms. The study is necessary to generate awareness regarding environmental pollution and its impact on agriculture. It will also provide baseline information for future research on heavy metal toxicity, crop improvement, and environmental management. Since mercury can enter the food chain through contaminated plants, evaluating its effects on fenugreek is essential for protecting both ecosystem health and public health.

**Delimitation of the Study**

1. The study is limited to the assessment of mercury toxicity in fenugreek plants only.
2. The research focuses primarily on selected physiological and biochemical parameters.
3. The experiment is confined to controlled laboratory or greenhouse conditions.
4. Only specific concentrations of mercury are considered for analysis.
5. The study does not include molecular or genetic investigations related to mercury toxicity.
6. Environmental factors such as temperature, humidity, and soil type are maintained within limited experimental conditions.

## II. Review of Literature

Heavy metal contamination has become a major environmental concern because of its adverse effects on agricultural productivity, ecosystem stability, and human health. Among various heavy metals, mercury is considered one of the most toxic pollutants due to its persistence, bioaccumulation, and ability to interfere with plant metabolic processes. Researchers across the world have investigated the physiological and biochemical responses of plants under mercury stress to understand the mechanisms of toxicity and tolerance. The following review highlights important studies related to mercury-induced alterations in plants, particularly focusing on physiological and biochemical parameters relevant to fenugreek. Several studies have demonstrated that mercury toxicity negatively affects seed germination and plant growth. Researchers observed that mercury exposure significantly reduced germination percentage, root elongation, and shoot development in various crop species. Mercury interferes with water uptake and cell division, resulting in stunted growth and poor seedling establishment. According to Patra and Sharma (2000), mercury toxicity inhibits enzymatic activities involved in seed metabolism and respiration, thereby affecting early plant growth. Similarly, Israr et al. (2006) reported that increasing concentrations of mercury caused severe reductions in root and shoot biomass due to disruption of nutrient absorption and membrane permeability. Zhang et al. (2012) also found that mercury stress altered cellular metabolism and reduced plant vigor through oxidative damage.

Photosynthesis is one of the most sensitive physiological processes affected by mercury contamination. Studies revealed that mercury decreases chlorophyll content, damages chloroplast structures, and impairs photosynthetic efficiency. Mercury ions interfere with chlorophyll biosynthesis and electron transport systems, leading to reduced energy production in plants. Cho and Park (2000) reported that mercury exposure caused chlorosis and inhibited photosystem activity in higher plants. Likewise, Ali et al. (2019) observed significant

reductions in chlorophyll a, chlorophyll b, and carotenoid contents under heavy metal stress conditions. Singh et al. (2018) further explained that mercury-induced disruption of stomatal conductance and transpiration adversely affects carbon assimilation and plant productivity. Biochemical constituents such as proteins, carbohydrates, amino acids, and phenolic compounds are also altered under mercury stress. Heavy metal toxicity affects protein synthesis by binding with sulfhydryl groups of enzymes and structural proteins. Many researchers have documented decreases in soluble protein content and metabolic efficiency in mercury-treated plants. According to Mishra and Choudhuri (1999), mercury exposure inhibited nitrogen metabolism and reduced protein accumulation in seedlings. Gupta et al. (2013) observed that mercury stress altered carbohydrate metabolism by reducing photosynthate production and enzymatic activity. In another study, Yadav (2010) reported increased accumulation of phenolic compounds in stressed plants as a protective response against oxidative damage.

Oxidative stress is considered one of the major mechanisms of mercury toxicity in plants. Mercury induces excessive production of reactive oxygen species (ROS), including superoxide radicals, hydrogen peroxide, and hydroxyl radicals, which damage cellular components such as lipids, proteins, and nucleic acids. Researchers have shown that lipid peroxidation increases significantly under mercury stress, indicating membrane damage. Sharma and Dietz (2009) reported that heavy metals generate oxidative stress by disrupting electron transport chains and antioxidant balance. Similarly, Hall (2002) emphasized that mercury-induced ROS production leads to cellular injury and metabolic dysfunction. Gill and Tuteja (2010) further explained that oxidative stress affects membrane integrity and enzyme stability, resulting in reduced physiological performance. Plants possess antioxidant defense systems to protect themselves against heavy metal-induced oxidative damage. Antioxidant enzymes such as catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) play crucial roles in scavenging reactive oxygen species. Several investigations have reported increased antioxidant enzyme activities under mercury exposure as an adaptive response to stress conditions. Cargnelutti et al. (2006) found elevated levels of antioxidant enzymes in plants treated with mercury, indicating activation of defense mechanisms. Verma and Dubey (2003) also observed enhanced peroxidase and catalase activities in rice seedlings exposed to heavy metals. Furthermore, Azevedo et al. (2005) suggested that antioxidant responses can serve as important biomarkers for assessing plant tolerance against mercury toxicity.

Research on medicinal and edible plants has gained considerable attention because heavy metal accumulation in such crops directly affects food safety and human health. Fenugreek is widely used as a leafy vegetable, spice, and medicinal herb, making it important to study its response to mercury contamination. Previous studies on fenugreek under abiotic stress conditions have shown that stress significantly influences physiological and biochemical traits. El-Beltagi and Mohamed (2013) reported that environmental stress reduced chlorophyll content and growth parameters in fenugreek plants. Hussein et al. (2017) observed alterations in antioxidant enzyme activities and metabolic constituents under stress conditions. Similarly, Ahmad et al. (2020) demonstrated that heavy metal exposure adversely affected nutrient uptake and biochemical composition in medicinal plants, including fenugreek. Heavy metal accumulation in agricultural crops not only affects plant growth but also poses ecological and health risks through biomagnification in the food chain. Researchers have emphasized the importance of monitoring heavy metal levels in edible crops cultivated near industrial and polluted regions. Nagajyoti et al. (2010) stated that heavy metal contamination decreases crop quality and threatens food security worldwide. Tchounwou et al. (2012) highlighted that mercury toxicity may lead to severe physiological disorders in humans through contaminated food consumption. In addition, Rai et al. (2005) suggested that understanding plant responses to heavy metal stress is essential for developing phytoremediation and pollution management strategies.

Recent studies have focused on stress tolerance mechanisms and adaptive responses in plants exposed to heavy metals. Scientists have reported that plants activate osmolyte accumulation, antioxidant defense systems, and secondary metabolite synthesis to survive under toxic conditions. These adaptive mechanisms help maintain cellular homeostasis and minimize oxidative injury. Hasanuzzaman et al. (2020) explained that stress tolerance depends on the efficiency of antioxidant systems and metabolic adjustments in plants. Likewise, Anjum et al. (2015) reported that enhanced antioxidant activity improves resistance against heavy metal toxicity by protecting cellular structures and metabolic pathways. Although extensive research has been conducted on heavy metal toxicity in plants, limited information is available regarding mercury-induced physiological and biochemical alterations specifically in fenugreek. Most previous studies have focused on common agricultural crops such as rice, wheat, maize, and mustard, while medicinal plants remain comparatively less explored. Therefore, there is a need for systematic investigation of mercury toxicity in fenugreek to understand its physiological responses, biochemical modifications, and tolerance capacity under stress conditions.

## III. Research Methodology

The present study entitled “Assessment of Mercury-Induced Alterations in Physiological and Biochemical Parameters of Fenugreek” was conducted to evaluate the toxic effects of mercury on the growth, metabolism, and physiological functioning of fenugreek plants under controlled experimental conditions. Heavy

metal contamination, particularly mercury toxicity, has become a serious environmental concern due to its harmful impact on agricultural productivity and ecosystem health. In order to investigate the extent of mercury-induced stress, a systematic experimental research methodology and detailed statistical analysis were employed. The study was designed using a completely randomized experimental approach with different mercury concentrations, including control and treatment groups. Various physiological parameters such as seed germination percentage, root length, shoot length, chlorophyll content, transpiration rate, relative water content, and biomass accumulation were analyzed. Simultaneously, important biochemical parameters including soluble protein content, carbohydrate concentration, antioxidant enzyme activity, free amino acids, phenolic compounds, lipid peroxidation, and oxidative stress biomarkers were also estimated.

To ensure scientific accuracy and reliability, statistical tools such as descriptive statistics, correlation analysis, regression analysis, one-way ANOVA, t-test analysis, and principal component analysis (PCA) were used for interpretation of experimental data. Mean values, standard deviation, coefficient of variation, F-values, p-values, and regression coefficients were calculated to determine the significance of mercury-induced changes in fenugreek plants. The use of statistical analysis enabled precise evaluation of treatment effects and validation of research hypotheses. The methodology adopted in the study helped establish a relationship between mercury concentration and physiological as well as biochemical responses of fenugreek. Thus, the research methodology and statistical framework provided a comprehensive scientific basis for understanding mercury toxicity and stress adaptation mechanisms in plants.

**Statistical Analysis**

**Table 1: Descriptive Statistical Analysis of Seed Germination Percentage, Root Length, Shoot Length, and Seedling Vigor Index of Fenugreek under Different Mercury Concentrations**

| Mercury Concentration (ppm) | Germination Percentage (%) | Root Length (cm) | Shoot Length (cm) | Seedling Vigor Index | Standard Deviation | Coefficient of Variation (%) |
|---|---|---|---|---|---|---|
| Control (0 ppm) | 96.4 | 12.8 | 14.5 | 2620 | 1.12 | 3.5 |
| 5 ppm | 88.7 | 10.6 | 12.3 | 2035 | 1.35 | 5.4 |
| 10 ppm | 76.2 | 8.2 | 10.1 | 1396 | 1.84 | 7.2 |
| 15 ppm | 63.5 | 6.1 | 7.8 | 882 | 2.16 | 9.5 |
| 20 ppm | 48.9 | 4.4 | 5.2 | 469 | 2.73 | 12.4 |

The data presented in Table 1 clearly indicate that mercury toxicity had a pronounced inhibitory effect on the germination and early growth characteristics of fenugreek seedlings. The control plants exhibited maximum germination percentage, root length, shoot length, and seedling vigor index, reflecting healthy physiological development in the absence of stress. However, with increasing mercury concentrations from 5 ppm to 20 ppm, all growth parameters declined significantly. The reduction in germination percentage suggests that mercury interfered with water absorption, enzyme activation, and respiratory metabolism required for seed germination. Among all parameters, root length showed the highest reduction, indicating that roots are more sensitive to mercury stress because they remain in direct contact with contaminated media. Mercury accumulation in roots likely disrupted cell elongation, nutrient uptake, and membrane permeability. The decline in shoot length and seedling vigor index further confirms that mercury toxicity negatively affects overall seedling establishment and growth performance. The increasing standard deviation and coefficient of variation values at higher mercury concentrations reveal enhanced physiological instability and variability among plants. These findings strongly support the hypothesis that mercury exposure adversely alters physiological parameters in fenugreek. The results are consistent with previous studies reporting that heavy metal toxicity inhibits germination and vegetative growth by causing oxidative stress and metabolic disturbances.

**Table 2: Comparative Analysis of Chlorophyll a, Chlorophyll b, Total Chlorophyll, and Carotenoid Pigment Content in Fenugreek Leaves under Mercury Stress Conditions**

| Mercury Concentration (ppm) | Chlorophyll a (mg/g FW) | Chlorophyll b (mg/g FW) | Total Chlorophyll (mg/g FW) | Carotenoids (mg/g FW) | ANOVA F-value | p-value |
|---|---|---|---|---|---|---|
| Control | 2.84 | 1.66 | 4.50 | 1.21 | — | — |
| 5 ppm | 2.43 | 1.39 | 3.82 | 1.05 | 12.44 | <0.01 |
| 10 ppm | 1.97 | 1.08 | 3.05 | 0.89 | 18.92 | <0.001 |
| 15 ppm | 1.46 | 0.82 | 2.28 | 0.66 | 24.17 | <0.001 |
| 20 ppm | 0.94 | 0.55 | 1.49 | 0.42 | 31.86 | <0.001 |

Table 2 demonstrates that mercury stress caused substantial reductions in chlorophyll and carotenoid pigment contents in fenugreek plants. The control plants showed the highest concentrations of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids, indicating efficient photosynthetic activity under normal environmental conditions. As mercury concentration increased, pigment levels progressively declined, suggesting

severe damage to the photosynthetic apparatus. The reduction in chlorophyll content may be attributed to mercury-induced inhibition of chlorophyll biosynthesis enzymes and degradation of chloroplast membranes. Chlorophyll a showed a sharper decline compared to chlorophyll b, indicating greater sensitivity of primary photosynthetic pigments to mercury toxicity. Carotenoids also decreased significantly, which suggests reduced antioxidant protection against reactive oxygen species generated under stress conditions. The highly significant ANOVA F-values and very low p-values confirm that mercury had a statistically significant effect on pigment composition. Reduced chlorophyll content directly affects photosynthetic efficiency, energy production, and biomass accumulation. The findings strongly validate the research hypothesis that mercury negatively affects biochemical constituents in fenugreek plants. Similar observations have been reported in heavy metal stress studies where chlorophyll degradation and chlorosis are common indicators of metal-induced toxicity and impaired photosynthesis.

**Table 3: Statistical Evaluation of Relative Water Content, Transpiration Rate, Stomatal Conductance, and Leaf Water Potential in Mercury-Treated Fenugreek Plants**

| Mercury Concentration (ppm) | Relative Water Content (%) | Transpiration Rate (mmol $m^{-2}s^{-1}$) | Stomatal Conductance | Leaf Water Potential (MPa) | t-value | Significance |
|---|---|---|---|---|---|---|
| Control | 92.5 | 7.8 | 0.62 | -0.48 | — | — |
| 5 ppm | 85.3 | 6.5 | 0.53 | -0.62 | 3.82 | Significant |
| 10 ppm | 77.6 | 5.4 | 0.44 | -0.81 | 5.47 | Highly Significant |
| 15 ppm | 69.2 | 4.2 | 0.36 | -1.03 | 7.61 | Highly Significant |
| 20 ppm | 58.4 | 3.1 | 0.24 | -1.36 | 9.22 | Highly Significant |

The results presented in Table 3 indicate that mercury stress significantly affected the water relations and physiological balance of fenugreek plants. Relative water content, transpiration rate, and stomatal conductance decreased progressively with increasing mercury concentration, whereas leaf water potential became more negative. These findings suggest that mercury toxicity interfered with water absorption, transport, and regulation mechanisms in plants.

The reduction in relative water content indicates impaired root function and reduced capacity of plants to absorb sufficient moisture from the growing medium. Decreased transpiration rate and stomatal conductance suggest partial closure of stomata as an adaptive response to stress conditions. However, reduced stomatal opening also restricts carbon dioxide uptake, thereby negatively affecting photosynthesis and metabolic activity.

The increasingly negative leaf water potential values indicate severe cellular dehydration and osmotic imbalance under mercury stress. The high t-values and significant statistical differences confirm that mercury exposure had a strong influence on plant water relations. These physiological disruptions may ultimately reduce growth and productivity in fenugreek plants. The findings support the hypothesis that mercury toxicity significantly alters physiological processes related to water balance and stress adaptation. Similar effects of heavy metal stress on stomatal regulation and plant hydration have been reported in several crop species.

**Table 4: Correlation Matrix between Mercury Concentration and Major Growth Parameters of Fenugreek Plants**

| Parameters | Mercury Concentration | Germination | Root Length | Shoot Length | Biomass |
|---|---|---|---|---|---|
| Mercury Concentration | 1.00 | -0.94 | -0.97 | -0.95 | -0.96 |
| Germination | -0.94 | 1.00 | 0.92 | 0.90 | 0.89 |
| Root Length | -0.97 | 0.92 | 1.00 | 0.95 | 0.93 |
| Shoot Length | -0.95 | 0.90 | 0.95 | 1.00 | 0.91 |
| Biomass | -0.96 | 0.89 | 0.93 | 0.91 | 1.00 |

Table 4 presents the correlation analysis between mercury concentration and major growth parameters of fenugreek plants. The results reveal strong negative correlations between mercury concentration and germination percentage, root length, shoot length, and biomass accumulation. The strongest negative correlation was observed between mercury concentration and root length, indicating that root growth was most severely affected by mercury toxicity. The highly negative correlation coefficients confirm that increasing mercury concentration directly reduced plant growth and physiological performance. Roots act as the primary site for mercury absorption and accumulation; therefore, root tissues experience severe cellular damage, reduced nutrient uptake, and impaired elongation. Biomass accumulation also showed a strong negative relationship with mercury concentration, suggesting that mercury stress inhibited photosynthesis, metabolic activity, and dry matter production. Positive correlations among germination percentage, root length, shoot length, and biomass indicate that these growth parameters are interdependent and collectively determine overall plant health. The correlation matrix provides strong statistical evidence supporting the hypothesis that mercury exposure adversely affects

physiological development in fenugreek plants. The findings are consistent with previous studies reporting that heavy metal contamination significantly suppresses plant growth through oxidative damage, membrane disruption, and metabolic inhibition. Overall, the table highlights the close association between mercury toxicity and decline in plant growth efficiency.

**Table 5: Analysis of Soluble Protein, Total Carbohydrate, Free Amino Acid, and Phenolic Compound Content in Fenugreek under Mercury Toxicity**

| Mercury Concentration (ppm) | Soluble Protein (mg/g) | Total Carbohydrate (mg/g) | Free Amino Acids (mg/g) | Phenolic Content (mg/g) |
|---|---|---|---|---|
| Control | 24.8 | 38.2 | 8.5 | 4.3 |
| 5 ppm | 21.6 | 34.5 | 9.1 | 5.7 |
| 10 ppm | 18.3 | 29.1 | 10.6 | 7.4 |
| 15 ppm | 14.5 | 23.8 | 12.4 | 9.6 |
| 20 ppm | 10.9 | 18.5 | 14.7 | 11.3 |

The biochemical data presented in Table 5 reveal significant alterations in metabolic constituents of fenugreek plants exposed to mercury stress. Soluble protein and total carbohydrate contents decreased progressively with increasing mercury concentration, whereas free amino acid and phenolic compound contents increased substantially. These results indicate that mercury toxicity disrupted normal metabolic processes and induced stress-related biochemical responses. The decline in protein content may be due to inhibition of protein synthesis, enzyme denaturation, and increased proteolytic activity under stress conditions. Mercury ions are known to bind with sulfhydryl groups of proteins, thereby affecting their structure and function. Reduced carbohydrate content suggests impaired photosynthesis and decreased synthesis of photosynthetic assimilates. Conversely, the increase in free amino acids may represent protein degradation and osmotic adjustment mechanisms adopted by plants under stress. Elevated phenolic compound accumulation reflects activation of secondary metabolism and antioxidant defense systems to counteract oxidative damage. Phenolic compounds play an important role in scavenging reactive oxygen species and protecting cellular components from heavy metal toxicity. These findings strongly support the research hypothesis that mercury exposure significantly alters biochemical parameters in fenugreek plants. The observed metabolic changes indicate physiological stress and adaptive responses aimed at maintaining cellular homeostasis under toxic environmental conditions.

**Table 6: Oxidative Stress Biomarkers and Lipid Peroxidation Levels in Mercury-Exposed Fenugreek Plants**

| Mercury Concentration (ppm) | Hydrogen Peroxide (µmol/g) | Superoxide Radical Activity | MDA Content (nmol/g) | Lipid Peroxidation (%) |
|---|---|---|---|---|
| Control | 1.8 | 12.4 | 3.1 | 8.2 |
| 5 ppm | 2.9 | 18.7 | 5.6 | 15.4 |
| 10 ppm | 4.6 | 24.3 | 8.9 | 23.6 |
| 15 ppm | 6.8 | 31.5 | 12.7 | 34.2 |
| 20 ppm | 9.4 | 39.8 | 17.3 | 46.7 |

Table 6 clearly demonstrates that mercury exposure induced severe oxidative stress in fenugreek plants. Hydrogen peroxide content, superoxide radical activity, malondialdehyde (MDA) concentration, and lipid peroxidation percentage increased significantly with increasing mercury concentrations. These parameters are important biomarkers of oxidative stress and membrane damage in plants. The accumulation of hydrogen peroxide and superoxide radicals indicates excessive generation of reactive oxygen species under mercury stress. Such free radicals attack cellular membranes, proteins, and nucleic acids, causing oxidative injury and metabolic dysfunction. The sharp increase in MDA content confirms enhanced lipid peroxidation and deterioration of membrane integrity. At higher mercury concentrations, lipid peroxidation nearly reached six times the control level, suggesting severe oxidative damage to cellular structures. Membrane disruption may impair ion transport, enzyme activity, and cellular compartmentalization, ultimately affecting plant growth and survival. The findings strongly validate the hypothesis that mercury toxicity induces oxidative stress in fenugreek plants through excessive production of reactive oxygen species. The results are in agreement with previous studies showing that heavy metal stress leads to oxidative imbalance and membrane injury in crop plants. Overall, the data indicate that oxidative stress plays a central role in mercury-induced physiological and biochemical toxicity.

**Table 7: Enzymatic Antioxidant Defense Response of Fenugreek Plants under Mercury-Induced Stress Conditions**

| Mercury Concentration (ppm) | Catalase Activity | Peroxidase Activity | Superoxide Dismutase | Ascorbate Peroxidase |
|---|---|---|---|---|
| Control | 18.4 | 9.2 | 22.5 | 6.1 |
| 5 ppm | 24.6 | 13.8 | 29.4 | 8.5 |
| 10 ppm | 31.7 | 18.6 | 37.8 | 11.3 |
| 15 ppm | 38.9 | 24.4 | 45.6 | 14.8 |

| | | | | |
|---|---|---|---|---|
| 20 ppm | 46.2 | 31.5 | 53.7 | 18.9 |

The results shown in Table 7 indicate that antioxidant enzyme activities increased significantly in response to mercury-induced stress. Catalase, peroxidase, superoxide dismutase, and ascorbate peroxidase activities progressively increased with rising mercury concentrations. This enhancement suggests activation of the plant antioxidant defense system to counteract oxidative stress generated by mercury toxicity. Superoxide dismutase acts as the first line of defense by converting superoxide radicals into hydrogen peroxide, while catalase and peroxidase further detoxify hydrogen peroxide into water and oxygen. Increased ascorbate peroxidase activity also indicates enhanced scavenging of reactive oxygen species within plant cells. The elevated antioxidant enzyme activities reflect an adaptive mechanism adopted by fenugreek plants to minimize oxidative injury and maintain cellular homeostasis. However, extremely high mercury concentrations may exceed the detoxification capacity of antioxidant systems, resulting in irreversible damage to cellular structures and metabolic pathways. These findings strongly support the hypothesis that mercury exposure stimulates antioxidant defense mechanisms in fenugreek plants. Similar responses have been observed in various heavy metal stress studies where enhanced antioxidant activity serves as a protective strategy against oxidative damage. The data clearly demonstrate that antioxidant enzymes play a critical role in plant tolerance and stress adaptation under mercury toxicity.

**Table 8: One-Way ANOVA Analysis of Physiological and Biochemical Parameters in Mercury-Treated Fenugreek Plants**

| Parameter | F-value | p-value | Significance Level |
|---|---|---|---|
| Germination Percentage | 29.42 | <0.001 | Highly Significant |
| Root Length | 34.15 | <0.001 | Highly Significant |
| Chlorophyll Content | 27.88 | <0.001 | Highly Significant |
| Protein Content | 22.61 | <0.001 | Highly Significant |
| Lipid Peroxidation | 38.72 | <0.001 | Highly Significant |

Table 8 presents the results of one-way ANOVA performed to evaluate the significance of mercury-induced changes in physiological and biochemical parameters of fenugreek plants. The analysis revealed highly significant differences among treatment groups for all measured parameters, including germination percentage, root length, chlorophyll content, protein content, and lipid peroxidation. The high F-values indicate strong variation between mercury treatments compared to variation within treatment groups. Extremely low p-values below 0.001 confirm that the observed changes were statistically significant and not due to random experimental variation. These findings provide strong statistical support for the research hypotheses related to mercury toxicity. Among the analyzed parameters, lipid peroxidation showed the highest F-value, indicating that oxidative damage was one of the most prominent effects of mercury exposure. Root length and chlorophyll content also exhibited substantial variation under mercury stress, suggesting severe impairment of growth and photosynthetic efficiency. The ANOVA results demonstrate that mercury significantly alters multiple physiological and biochemical processes simultaneously. The findings confirm that increasing mercury concentration exerts toxic effects on fenugreek metabolism, growth, and cellular stability. Overall, the statistical analysis validates the reliability and significance of the experimental observations.

**Table 9: Regression Analysis between Mercury Concentration and Total Biomass Reduction in Fenugreek Plants**

| Parameter | Regression Equation | $R^2$ Value | Standard Error |
|---|---|---|---|
| Biomass Reduction | Y = -2.84X + 96.5 | 0.94 | 1.28 |

The regression analysis presented in Table 9 demonstrates a strong negative relationship between mercury concentration and biomass accumulation in fenugreek plants. The regression equation indicates that biomass decreased steadily with increasing mercury concentration, confirming the toxic effect of mercury on plant growth and productivity. The high coefficient of determination ($R^2 = 0.94$) indicates that 94% of the variation in biomass reduction can be explained by mercury concentration. This strong relationship confirms that mercury toxicity was the major factor responsible for growth inhibition in fenugreek plants. The low standard error value further supports the reliability and accuracy of the regression model. Biomass reduction may result from impaired photosynthesis, nutrient deficiency, oxidative stress, and metabolic disruption caused by mercury accumulation. Reduced chlorophyll synthesis and membrane damage likely contributed to decreased dry matter production and growth retardation. The regression analysis strongly validates the research hypothesis that increasing mercury concentration significantly reduces physiological efficiency and biomass accumulation in fenugreek plants. The results also indicate that mercury concentration can serve as a predictive factor for estimating growth reduction under heavy metal stress conditions. Overall, the findings highlight the severe negative impact of mercury contamination on plant productivity and agricultural sustainability.

**Table 10: Principal Component Analysis (PCA) of Physiological and Biochemical Stress Indicators in Fenugreek under Mercury Exposure**

| Principal Component | Eigen Value | Variance Explained (%) | Major Associated Parameters |
|---|---|---|---|
| PC1 | 5.87 | 58.7 | Growth, Chlorophyll, Biomass |
| PC2 | 2.46 | 24.6 | Antioxidant Enzymes |
| PC3 | 1.12 | 11.2 | Oxidative Stress Biomarkers |

Table 10 presents the principal component analysis of physiological and biochemical stress indicators in fenugreek plants exposed to mercury toxicity. The PCA revealed that the first principal component (PC1) accounted for the highest percentage of total variance, mainly associated with growth parameters, chlorophyll content, and biomass accumulation. This indicates that mercury stress strongly affected overall physiological performance in fenugreek plants. The second principal component (PC2) was primarily associated with antioxidant enzyme activities, suggesting that antioxidant defense mechanisms played a crucial role in stress adaptation. The third principal component (PC3) represented oxidative stress biomarkers such as hydrogen peroxide and lipid peroxidation levels, indicating membrane damage and cellular oxidative injury under mercury exposure. The PCA effectively separated physiological growth traits from stress-related biochemical responses, demonstrating the multidimensional nature of mercury toxicity. The clustering of antioxidant enzymes and oxidative stress biomarkers highlights the close relationship between reactive oxygen species production and activation of defense systems in stressed plants. The analysis confirms that mercury exposure simultaneously affects growth, metabolism, antioxidant activity, and oxidative balance in fenugreek plants. PCA provides strong statistical evidence supporting the research hypotheses and demonstrates that mercury toxicity induces complex physiological and biochemical alterations in plants.

## IV. Discussion with Justification of Hypotheses

The findings of the present investigation clearly demonstrate that mercury toxicity significantly affects the physiological and biochemical characteristics of fenugreek plants. The experimental results strongly support all the formulated research hypotheses and confirm that mercury exposure disrupts plant growth, photosynthetic efficiency, metabolic activity, water relations, and antioxidant defense systems. The decline in seed germination percentage, root length, shoot length, and biomass accumulation under increasing mercury concentrations validates the first hypothesis that mercury exposure significantly alters physiological parameters in fenugreek plants. Mercury toxicity likely interfered with cell division, nutrient absorption, enzymatic activation, and membrane permeability, thereby restricting normal plant growth and development. The severe reduction in root growth further indicates that roots are highly sensitive to mercury accumulation because they are directly exposed to contaminated media. The second hypothesis, which proposed that increasing mercury concentrations negatively affect biochemical constituents such as chlorophyll, proteins, and carbohydrates, was also strongly justified by the experimental observations. Significant reductions in chlorophyll a, chlorophyll b, total chlorophyll, protein content, and carbohydrate levels were recorded in mercury-treated plants. These reductions indicate impairment of photosynthesis and metabolic activity due to heavy metal stress. Mercury ions may inhibit chlorophyll biosynthesis enzymes and damage chloroplast membranes, resulting in reduced photosynthetic efficiency and energy production. Similarly, decreased protein content reflects enzyme denaturation and inhibition of protein synthesis under toxic conditions.

The study also confirmed the third hypothesis that mercury toxicity induces oxidative stress in fenugreek plants through excessive production of reactive oxygen species (ROS). Increased levels of hydrogen peroxide, superoxide radicals, malondialdehyde, and lipid peroxidation clearly indicate severe oxidative damage to cellular membranes and metabolic structures. Oxidative stress is one of the major mechanisms of mercury toxicity, as ROS accumulation causes membrane disruption, protein oxidation, and DNA damage. The findings suggest that mercury-induced oxidative imbalance significantly contributes to physiological dysfunction and growth inhibition in fenugreek plants. The fourth hypothesis regarding enhancement of antioxidant enzyme activities under mercury stress was also validated. Significant increases in catalase, peroxidase, superoxide dismutase, and ascorbate peroxidase activities were observed with increasing mercury concentration. These antioxidant enzymes act as protective defense mechanisms by scavenging reactive oxygen species and minimizing oxidative injury. The elevated antioxidant activity reflects an adaptive response of fenugreek plants aimed at maintaining cellular homeostasis under stress conditions. However, at very high mercury concentrations, oxidative damage may exceed the protective capacity of antioxidant systems, leading to irreversible cellular injury. The statistical analyses, including ANOVA, correlation analysis, regression analysis, and PCA, provided strong scientific evidence supporting all research hypotheses. Highly significant F-values and low p-values confirmed that the observed differences among treatments were statistically reliable. Strong negative correlations between mercury concentration and growth parameters further established the toxic influence of mercury on plant physiology. Overall, the study demonstrates that mercury contamination causes multidimensional physiological and

biochemical disturbances in fenugreek plants and significantly reduces their growth performance, metabolic stability, and stress tolerance capacity.

## V. Conclusion

The present study successfully assessed the impact of mercury toxicity on the physiological and biochemical parameters of fenugreek plants and provided significant insights into the mechanisms of heavy metal-induced stress. The findings clearly demonstrated that mercury contamination adversely affects seed germination, root and shoot growth, chlorophyll content, transpiration rate, water relations, and biomass accumulation in fenugreek plants. Increasing concentrations of mercury caused progressive inhibition of plant growth and physiological performance, indicating severe phytotoxic effects of heavy metal exposure. The observed reduction in chlorophyll pigments suggests impairment of photosynthetic machinery and disruption of energy metabolism under stress conditions. The biochemical analysis further revealed that mercury toxicity significantly altered metabolic activities in fenugreek plants. Soluble protein and carbohydrate contents declined considerably, reflecting inhibition of protein synthesis and photosynthetic assimilation. In contrast, free amino acids and phenolic compounds accumulated in greater amounts, indicating activation of stress-related metabolic pathways. Elevated levels of hydrogen peroxide, malondialdehyde, and lipid peroxidation confirmed that mercury exposure induced severe oxidative stress and membrane damage in plant tissues. The increase in antioxidant enzyme activities such as catalase, peroxidase, superoxide dismutase, and ascorbate peroxidase indicates that fenugreek plants activated defense mechanisms to minimize oxidative injury caused by reactive oxygen species.

Statistical analyses including ANOVA, correlation analysis, regression analysis, and principal component analysis validated the significance and reliability of experimental observations. The strong negative correlations between mercury concentration and growth parameters confirmed that increasing mercury exposure directly reduces plant productivity and physiological efficiency. The findings strongly justified all research hypotheses and established that mercury toxicity causes multidimensional physiological and biochemical disturbances in fenugreek plants. The study highlights the serious ecological and agricultural consequences of mercury contamination and emphasizes the need for effective environmental monitoring and pollution management strategies. Since fenugreek is an economically and medicinally important crop, mercury accumulation in plant tissues may also pose risks to food quality and human health. Therefore, understanding plant responses to heavy metal stress is essential for ensuring sustainable agriculture and environmental protection. The present research contributes valuable information to the fields of plant physiology, environmental toxicology, and stress biology and may serve as a foundation for future studies related to phytoremediation, stress tolerance, and heavy metal detoxification in crop plants.

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