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Bioaccumulation of Mercury and Its Consequences on Morphological and Biochemical Parameters in Fenugreek (*Trigonella Foenum-Graecum* L.)

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KEYWORDS

Fenugreek, Mercury, Germination, Heavy metal toxicity, Soil Pollution, Chlorophyll

ABSTRACT:

Introduction: Natural and anthropogenic sources of metals within the ecosystem have led to significant accumulation of heavy metals accumulation, which poses a serious environmental concern, affecting the food chains and cause various health issues due to their toxicity nature.

Objectives: The aim of this study was to evaluate the bioaccumulation of mercury and its consequences on morphological and biochemical parameters in Fenugreek *(Trigonella Foenum-Graecum* L.*)*.

Methods: Experimental plants were divided into 4 groups. Group 1 plants in bag served as control received normal nutritional and water support, Heavy metal toxicity was induced in Group 2, 3 and 4 by mercury treatment of 25, 50 and 100 mg throughout the experimental period.

Results: Results showed that mercury treated fenugreek plants exhibits significant reduction in germination percentage, root length, shoot length, fresh weight, dry weight, vigour index, chlorophyll a, b, total chlorophyll, carotenoids, carbohydrate, protein contents, catalase and super oxide dismutase.

Conclusions: Together results portray that mercury exhibits toxic effects in Fenugreek plants can significantly affect their growth and development. So effective management strategies are necessary in reducing the mercury toxicity and ensure protecting both the plant health as well as safety of foods.

1. INTRODUCTION

Heavy metals (HMs) are regarded as significant environmental contaminants and pose serious threats to the agricultural productivity. It can be more prevalent in upcoming years. HMs directly create impact on plant performance in a concentration dependent manner (Nagajyoti et al., 2010), when present in lower concentrations, macro and micronutrients maintain the important enzyme functions and regulating the metabolic pathways which includes photosynthesis, metabolism of carbohydrates, modifications of proteins, synthesis

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of DNA and redox homeostasis. Whereas HMs when present at higher concentrations exhibits severe toxicity and posing a risk to the environment, disturbing the soil's microbiological balance, thereby leads to reduction in soil fertility (Barbieri et al., 2016), contaminating food chains, and cause various health issues. Also, prolonged exposure of HMs in the environment is a serious threat to living things (Wieczorek-Dąbrowska et al., 2013). Moreover, HMs toxicity can also disrupt physiological processes, adversely impact growth, development, reproduction and survival rates across different species.

In soils, contamination of Mercury (Hg) considerably threatens the plant health and the surrounding ecosystem. As a heavy metal, Hg can enter the soil via different sources, which includes agricultural practices, industrial discharges and atmospheric deposition. It is well known that mercury inhibits the growth of plants leading to considerable reduction in root and shoot length and biomass of seedlings in a concentration dependent manner. Plants mostly that absorb Hg tend to accumulate initially on the roots (Lenka et al., 1992), while in shoots may be some moderate amounts may get accumulated (Dushenkov et al., 1995). The consequences of Hg on plants are quite complex, affecting nutrient uptake, growth and vitality. Since, in terrestrial ecosystems plants serve as the important foundation, any detrimental consequences on their health can have wide variety of implications for biodiversity and food webs.

In plants, seed germination and early seedling growth are the important stages of development that is most vulnerable to HMs toxicity (Seneviratne et al., 2019), provided that defence mechanisms of plant are not fully developed at those stages (Liu et al., 2005). Numerous studies have suggested that heavy metals toxicity leads to inhibition of seed germination and seedling development (Karmous et al, 2015; Adrees et al., 2015). Hg also disrupts the antioxidant defence mechanism by affecting the modulation of the nonenzymatic antioxidants and enzymatic antioxidants (Israr et al., 2006). Previous literature also reported that Hg has inhibitory effects on the growth of plants (Mishra et al., 1999), photosynthesis process (Assad et al., 2016) chlorophyll biosynthesis (Singh et al, 2012) and reactive oxygen species (ROS) generation (Kim et al., 2017).

Hg can interrupt metabolic pathways, potentially affecting carbohydrates processing in the body. So, when plants absorb Hg, it interferes with process of photosynthesis, initially by inhibiting synthesis of chlorophyll results in reduced yield and altered composition of carbohydrates. Additionally, Hg negatively affecting overall nutrition by inhibiting the activities of enzymes which is taking part in metabolism of carbohydrates. It can also induce changes in sequence of amino acids of proteins that is being synthesized (Zalups, 2000). Toxicity of Hg in plants occurs through the displacement of essential elements, its binding to thiol groups of proteins and the protein structure disruption (Safari et al., 2019).

Fenugreek (Trigonella Foenum-Graecum L.) is one of the rich protein sources and is widely used in the production of many medical and beauty products. It has been studied not only as a versatile medicinal herb that is used in pharmacological field (Xalxo and Keshavkant, 2020) but also in phytoremediation processes (Zayneb et al., 2015). Numerous studies have illustrated the biological actions of fenugreek which includes antimicrobial (Mawahib et al., 2015), hypocholesterolaemic, hepatoprotective, chemoprotective, antihypertensive, antidiabetic, antioxidative and immunostimulatory effects (Wannes and Tounsi, 2020). These biological effects justify our decision within the framework for choosing fenugreek plants for this research work.

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2. OBJECTIVES

Heavy metals pose substantial risks to environment and human health, making their study crucial across different fields which includes public health and environmental science. So, due to more prevalence of mercury pollution worldwide, this study was aimed to assess the bioaccumulation of mercury and its consequences on morphological and biochemical parameters in Fenugreek (Trigonella Foenum-Graecum L.). This investigation highlights the necessity of addressing mercury pollution to protect ecological integrity and plant life.

3. MATERIALS AND METHODS

The experimental protocol deduced in order to fulfil the objectives were carried out with standard procedures. Fenugreek obtained from agricultural shop, Puducherry. Mercuric Chloride was used to induce mercury toxicity.

Polyethylene bag experiment

Polyethylene bag culture experiments were conducted to study the effect of the heavy metal toxicity in Fenugreek. The growth medium in the polyethylene bags consist of artificially polluted soil at level of 25, 50 and 100 mg of Hg. By making 2cm deep holes with the wooden stick sowed seven sterilized seeds in each bag. Afterwards each seed was covered with a small amount of soil for proper supplement of germination factors. Soil moisture content was adjusted regularly by its water holding capacity with tap water.

Experimental design

After the preliminary phase, the fenugreek plants were categorized into four distinct treatment groups. Group 1 bag with soil served as control, not received any mercury treatment. In contrast, bags of groups 2, 3 and 4 were subjected to mercury treatments of 25 mg, 50 mg and 100 mg, respectively, over the experimental period. The plants were cultivated under conditions of relative humidity, average temperature and natural photoperiod.

Germination parameters

Germination percentage (%) was calculated by dividing the seed germination on each day by total number of seed × 100 and finally adding the total percentage.

Germination rate = No. of Seeds germination/Total number of seeds

Germination %= Germination rate × 100

Root length and Shoot length (in cm)

The root and shoot length from the ground level to the tip of the root and shoot is measured using standard centimetre scale.

Fresh Weight and dry weight (in gm)

Fresh weight and dry weight of the whole plant is determined using electronic balance.

Vigour Index

For Vigour index, value was recorded on germination basis. Using the mean value of root length and shoot length, Vigour index was calculated by the formula of Baki and Anderson, 1973.

Vigour Index = (Mean Shoot length + Mean root length) × Germination %

Biochemical Estimations

Estimation of Carbohydrate

The carbohydrate content was estimated by the method of Hedge and Hofreiter (1962). A volume ranging from 0.2 to 1 ml of working standard solutions were pipette out into different test tube, while 0.5 ml of the sample was placed in another test tube. Then all the test tubes are made upto 1 ml with distilled water. Following this, 4 ml of Anthrone reagent is added into each test tube. The contents of the test tubes were shaken and subsequently heated in a boiling water bath for a

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duration of 20 minutes, then it was cooled. The absorbance of the green coloured solution was measured at a wavelength of 640 nm.

Estimation of proteins

The protein content was estimated by Lowry's method (1951). A volume ranging from 0.2 ml to 1ml of the working standard is pipette out into a series of test tubes. Additionally, 0.2 ml of the sample extract is added on other test tube. Each of these tubes is then filled to a total volume of 1 ml with distilled water, with 0.5 ml of distilled water serving as a blank. The contents are mixed well and allowed to stand for 10 minutes. then 0.5 ml of Folin's cio calteau reagent is added to all the test tubes and mixed well. Then these test tubes are incubated at room temperature in a dark for 30 minutes. A blue colour develops, which is subsequently measured at a wavelength of 660 nm.

Photosynthetic pigments analysis

For the estimation of Chlorophyll, a, b, total chlorophyll, 1g of leaves was extracted with 20 ml of 80% acetone, centrifuge (5000 rpm) for 5 minutes and transferred the supernatant to a 100 ml volumetric flask. This procedure was repeated, until the residue become colourless. the supernatant was made up to 100 ml with 80% acetone. Chlorophyll content was estimated according to the method of Arnon,1949, the absorption of the solution was read at 645 nm and 663 against 80% acetone blank. The carotenoid content was estimated according to method of Lichtenthaler, 1987 and was measured at 473 nm.

Enzyme assays and analysis

Estimation of Catalase

The activity of catalase (CAT: EC 1.11.1.6) was assayed by the method of Sinha (1972). To 0.9 ml of phosphate buffer, 0.4 ml of hydrogen peroxide and 0.1 ml of sample were added. After intervals of 30 and 60 seconds, 2 ml of the dichromate mixture was added. The tubes were then placed in boiling water for a duration of 10 minutes, and the resulting colour was measured at 620 nm at 0 and 60 seconds. Standards ranging from 2-10 micromoles were taken and preceded as the test alongside a blank that contained only the reagent.

Estimation of Superoxide dismutase

Superoxide dismutase (SOD: EC 1.15.1.1) was assayed by the method of Kakkar et al., 1984. The assay solution contains 1.2 ml of sodium pyrophosphate buffer, 0.1 ml of phenazine methosulphate, 0.3 ml of nitroblue tetrazolium, 1ml of appropriately diluted enzyme preparation and water in a total volume of 3 ml. Initiate the reaction by adding 0.2 ml of NADH. Incubate the mixture at 30°C for 90 seconds. To terminate this reaction, added 1 ml of glacial acetic acid. Then this reaction mixture was shaken with 4 ml of n-butanol. Allow the mixture to stand for 10 minutes and then centrifuged. Measure the intensity if the chromogen in butanol layer at 560 nm again butanol as blank and system devoid of enzyme serves as control.

Statistical analysis

Results were expressed as means ± standard deviation of 6 plants per group. Data were analysed by oneway analysis of variance and any significant differences among treatment groups were evaluated using Duncan's multiple range test. Results were considered statistically significant when $P < 0.05$. All statistical analyses were performed using SPSS version 15.0 software package (SPSS, Tokyo, Japan).

4. RESULTS

Effect of mercury in Fenugreek plant on germination percentage, root length and shoot length

Table 1 represents the effect of Hg in fenugreek plant on germination percentage (%), root length and shoot length of various experimental groups. These observations were recorded at 30th day after sowing. Upon increasing the concentration of Hg

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for about 25 mg (T1), 50 mg (T2) and 100 mg (T3) resulted in reduced gemination percentage, root length and shoot length as compared to control plants.

Table 1: Effect of mercury in fenugreek plant on germination percentage (%), root length (in cm) and shoot length (in cm) of different experimental groups.

Groups	Germination percentage (%)	Root length (cm)	Shoot length (cm)
Control (C)	90	4.02±0.32	8.99±0.51
Test (T1)	60	2.89±0.23	5.82±0.46
Test (T2)	40	2.23±0.20	5.01±0.39
Test (T3)	30	1.2±0.08	4.48±0.36

Values are expressed as mean±SD. Groups not sharing a common superscript letter differ significantly at $p<0.05$. Duncan's multiple range test (DMRT).

Effect of Mercury in Fenugreek plant on Fresh weight, Dry weight and Vigour index

Table 2 shows the effect of Hg on fresh weight, dry weight and vigour index on various groups of fenugreek plant. These observations are recorded at 30^{th} day after sowing. Results indicates a significant decrease in fresh weight, dry weight and vigour index under Hg toxicity in fenugreek plant as compared to the control plants.

Table 2: Effect of mercury stress on fresh weight, dry weight and vigour index on different groups of fenugreek plant of different experimental groups.

Groups	Fresh weight (g)	Dry weight (g)	Vigour index
Control (C)	2.82±0.25	1.59±0.13	1170.9±80.92
Test (T1)	1.52±0.12	1.24±0.12	522.6±32.09
Test (T2)	1.36±0.10	1.12±0.11	289.60±12.04
Test (T3)	1.22±0.10	1.04±0.09	170.40±8.27

Values are expressed as mean±SD. Groups not sharing a common superscript letter differ significantly at $p<0.05$. Duncan's multiple range test (DMRT).

Effect of Mercury on carbohydrate and protein contents

Figure 1 depicts the effect of Hg on carbohydrate and protein levels in three different groups of tested plants, with observations recorded on the 30th day after sowing. Results revealed that significant reduction in carbohydrates and protein levels in Hg treated groups when compared to untreated control group.

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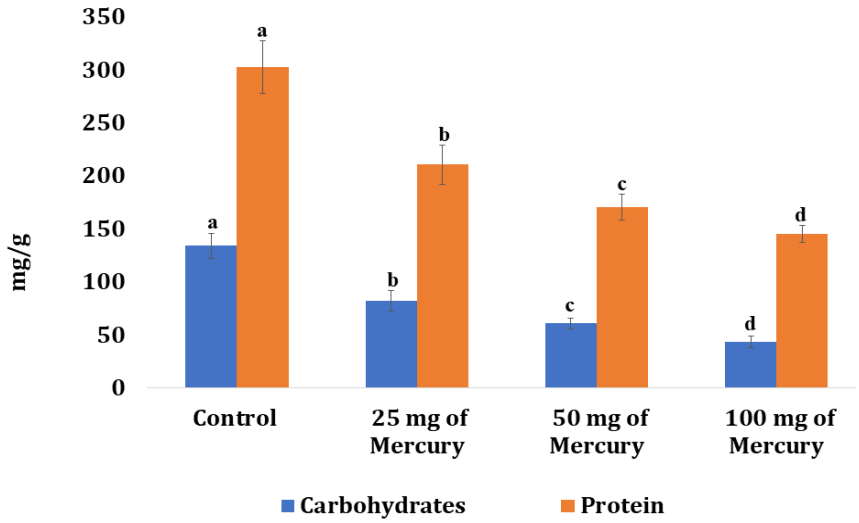
Figure 1: Effect of mercury stress on carbohydrate and protein contents on different groups of fenugreek plant of different experimental groups.

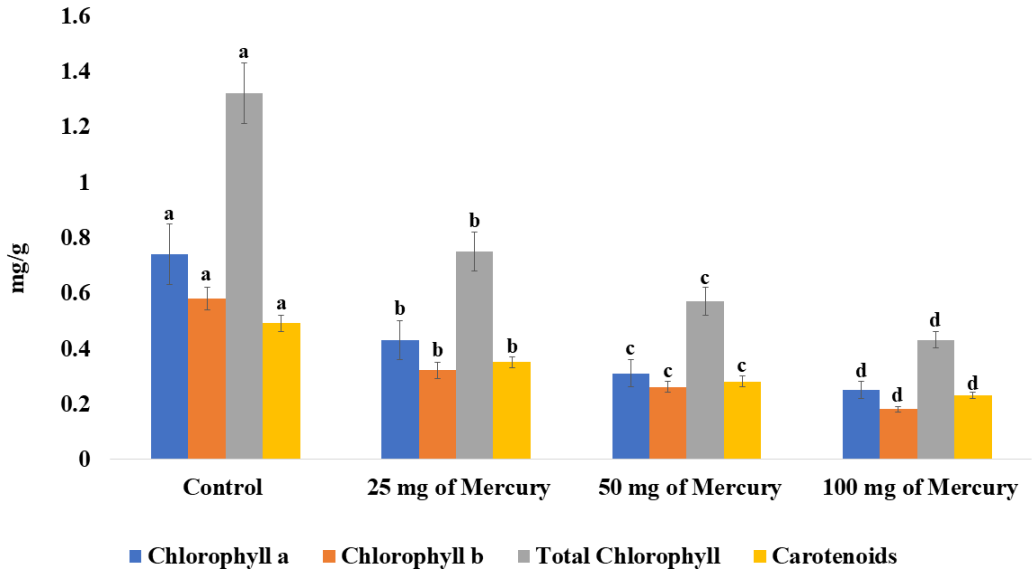


Values are expressed as mean±SD. Groups not sharing a common superscript letter differ significantly at $p<0.05$. Duncan's multiple range test (DMRT).

Effect of Mercury on Chlorophyll a, chlorophyll b, Total chlorophyll and Carotenoid contents

Figure 2 represents the effect of Hg on plant pigments such as chlorophyll a, chlorophyll b, total chlorophyll and carotenoids in fenugreek plant. Results showed that significant reduction in these plant pigments as compared to normal control plants.

Figure 2: Effect of mercury on plant pigments such as Chlorophyll a, Chlorophyll b, Total chlorophyll and Carotenoids on different groups of fenugreek plants of experimental groups.



Values are expressed as mean±SD. Groups not sharing a common superscript letter differ significantly at $p<0.05$. Duncan's multiple range test (DMRT).

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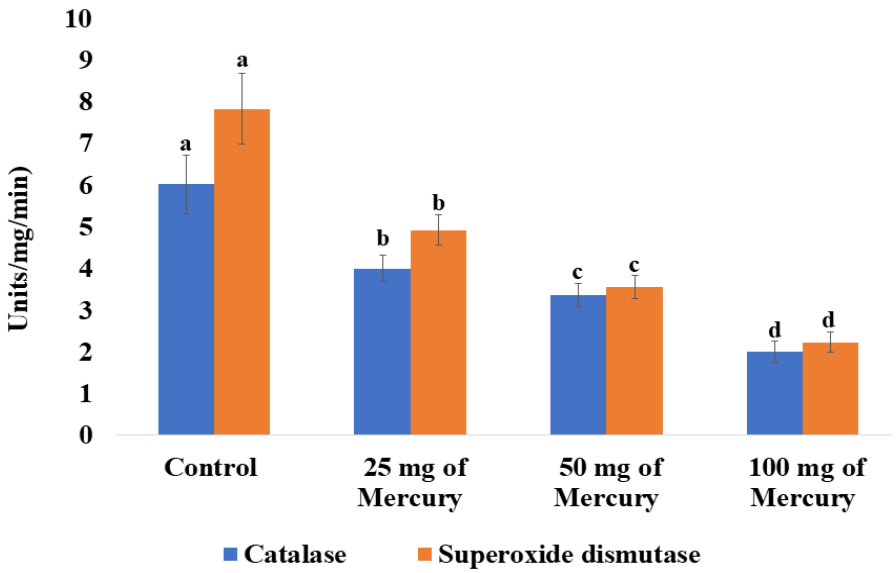
Effect of Mercury on Catalase and Superoxide dismutase

Figure 3 depicts the effect of Hg at different concentrations on enzymic antioxidants such as Catalase and Super oxide dismutase. Results showed that decreased levels of these enzymic antioxidants were observed in heavy metal stress in fenugreek plants.

Figure 3: Effect of mercury on Catalase and Superoxide dismutase on different groups of fenugreek plant of different experimental groups.



Values are expressed as mean±SD. Groups not sharing a common superscript letter differ significantly at $p<0.05$. Duncan's multiple range test (DMRT).

5. DISCUSSION

The presence of HMs in the ecosystem is primarily due to human activities, which is regarded as main sources for human exposure to these elements. (Verma et al., 2021). HMs toxicity is the major cause commonly reported for agricultural soils and the contamination rate is increasing nowadays (Kacholi and Sahu 2018). In soil, HMs are extensively recognized as an important constituent, but their elevated concentration in both soil and plants can have harmful effects on environment (Alengebawy et al., 2021). Once absorbed by plants, HMs have the capacity to transfer through the food chain, thereby accumulating in the bodies of humans and animals, ultimately poses potential health risks (Selvi et al., 2019). Soil contamination caused by Hg is a serious concern, as it is released into the environment via various industrial processes. This leads to major threat to human health and ecosystem (Moreno et al., 2005).

Germination percentage, Root length and Shoot length

HMs can create high toxicity to all organisms, which includes plants and animals. Metals and chemicals at higher concentrations inhibits plant germination, growth and productivity, thereby affects the biochemical, physiological and genetic elements of plant systems. Effects of mercury on fenugreek seedlings explained in a concentration depend manner, which exhibits reduced germination percentage, root and shoot length in Hg tested plants, when its concentration is increasing more pronounced significant reduction were

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observed (Wang et al., 2003; Ling et al., 2010; Behera et al., 2018) which correlates with our present study which shows that significant reduction in seed germination, root and shoot length was observed in mercury treated plants. In fenugreek, the percentage of seed germination was significantly affected through impaired enzymatic activities, thus retarding germination of seeds and growth of fenugreek plants. Due to higher accumulation of metallic salts within the plant biomass, the suppression of seed germination and responses of plant growth have been attributed to the occurring of higher toxic effect syndrome (Singh and Singh, 1981). Hg is rapidly absorbed by the plant roots results in vast significant difference in root and shoot length as compared to control fenugreek plants. Ultimately, exposure of Hg in fenugreek plants causes reduction in rate of growth, enzyme activity inhibition, upsets water balance and mineral nutrition, change in branching pattern, altering the hormonal status that affects membrane permeability and its structure.

Fresh weight, dry weight and vigour index

For overall growth and physiological status, the fresh weight, dry weight and vigour index of plants are regarded as important indicators. For immediate biomass of the plant fresh weight is measured, while dry weight offers valuable insights regarding nutrient accumulation and resource allocation of plants. The vigour index, which integrates the germination percentage and growth parameters of seedlings, provides an extensive evaluation of plant vitality. Understanding these consequences is vital for developing strategies aimed at alleviating heavy metal toxicity in agricultural environments. By evaluating fresh weight, dry weight, and vigour index, researchers can determine the degree of contamination and its consequences on plant health. Results showed that reduced fresh weight, dry weight and vigour index under Hg toxicity in fenugreek plants were observed significantly as compared to the control plants. More pronounced significant reduced effect was observed in fenugreek plants tested with higher concentrations of Hg, suggesting HMs exhibiting toxicity based on concentration dependent manner often indicating stress and toxicity, which aliens with previous study suggested that increased concentration of mercuric chloride, leads to gradual decrease in fresh weight and dry weight were observed in *Cajanus cajan* L plants (Patnaik and Mohanty, 2013).

Carbohydrates and protein contents

Carbohydrates are synthesized by green plants through photosynthesis process from carbon dioxide and water, that serve as primary energy sources like glucose and storage forms like starch in plants. Additionally, carbohydrates executing different ecological functions in plants which includes protection against wounds and infections as well as in the mechanism of detoxification of foreign substances (Satvir et al., 2000). Results reveals that carbohydrate levels were significantly reduced in Hg tested fenugreek plants as compared to control plants. This reduced levels of carbohydrate in Hg treated leaves may be corresponded with stimulation of respiration rate or inhibition of photosynthesis. These reduced levels of carbohydrate contents not only affecting the plant's energy reserves but also its capability to withstand both biotic and abiotic stress.

Proteins are essential macromolecules made up of amino acids which is involved in various processes such as catalyzing enzyme catalysis, structural integrity, transport across membranes, and energy synthesizing reactions involving electron transport. In plants, protein synthesis from mRNA is more important for growth and development, particularly under stress conditions. Abiotic stress may disrupt some protein synthesis mechanism and promote others (Ericson and Alfinito 1984) with a common trend of significant reduction in the overall contents which correlates with our study shows that reduced levels of protein contents under Hg stressed conditions. More pronounced HMs stress was seen

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in high concentration of 100 mg of Hg treated fenugreek plants. These decreased levels of protein contents in fenugreek plants may be attributed to the enhanced protein degradation and due to increased activity of protease enzyme which was found to increase in more HMs stressed conditions. Moreover, Hg exposure may induce fragmentation of proteins and lipid peroxidation due to toxic effects of reactive oxygen species (ROS), thereby leads to reduced levels of protein contents. These biochemical properties may determine the toxic effects on plants (Zhou et al., 2008).

Chlorophyll a, chlorophyll b, Total chlorophyll and Carotenoids

In plants, chlorophyll and carotenoids are most important pigments playing vital roles in the process of photosynthesis and plant health. Hg stress leads to narrowed leaves and exhibits the symptoms of necrosis and chlorosis. Chlorophyll and carotenoid contents were decreased with increased concentration of Hg. Previous literature evidence shown by Caspi et al. (1999) demonstrated that Hg inhibits the synthesis of chlorophyll and carotenoids and delays the incorporation of these photosynthetic pigments into the photosystem complexes of fenugreek leaves. On the other hand, the decrease in net photosynthesis process as a result of reduced absorption of necessary mineral nutrients may be attributed for chlorosis in plants (Aghaz and Bandehagh, 2013). This in accordance with our study which shows that chlorophyll and carotenoids contents were significantly reduced upon increased concentrations of Hg thereby affecting the efficiency of photosystems. Different abiotic stresses have been shown to decrease chlorophyll contents in plants (Ahmad et al. 2007). The observed decreased levels of chlorophyll contents in plants under Hg stress may be attributed to the inhibition of key enzymes such as protochlorophyllide reductase and δ-aminolevulinic acid dehydratase (ALA dehydratase) (Van Assche and Clijsters 1990) which is also related to the impairment in the supply of Fe^{2+} and Mg^{2+}required for chlorophyll synthesis. This impairment leads to reduced photosynthetic efficiency, further hampers plant growth and development.

Catalase and Super oxide dismutase

Antioxidant enzymes play a crucial role in plant protection against different stresses. The induction of enzymic antioxidants which includes superoxide dismutase (SOD) and catalase (CAT) is an important defense mechanism to alleviate oxidative damage in polluted environments (Zhang et al. 2007). SOD is a key enzyme and an important component of antioxidative defense system, as it undergoes the catalytic process such that it dismutates the two molecules of superoxide (O^{-2}) to hydrogen peroxide (H_2O_2) and oxygen (Cakmak and Horst 1991). SOD can eliminate O^{-2}, decreases lipid peroxidation and maintain the cell membrane stability. Our study shows that, SOD activity in Hg treated plants showed significantly decreased results when compared to control fenugreek plants. More decreased antioxidant effect was observed in plants treated with 100 mg of mercury. This reduced activity of SOD indicates that Hg toxicity induces oxidative stress in plants by enhancing the formation of ROS (Olmos et al., 2003). Hg might induce generation of ROS through mechanisms which includes induction of lipid peroxidation, disruption of electron transport chain or interaction with the antioxidant defense system. The decreased SOD activity under high Hg stress might be due to enzyme damage caused by excessive free radicals production and peroxides.

Catalase (CAT) is an important enzyme found in various kind of organisms, especially in plants, where it plays a vital role in protecting cells from oxidative damage. This tetrameric enzyme which contains heme, is a key component of antioxidant defense system in plants. CAT facilitates the breakdown of H_2O_2, a potentially detrimental ROS into water and oxygen, thereby reducing oxidative

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stress. Being an eco-protective enzyme, CAT enables plants to pose defensive responses against toxicants, which can be detrimental to their health. The CAT activity of fenugreek plants was decreased under all of the Hg treatments with different concentration of 25 mg, 50 mg and 100 mg in comparison with the control plants. Increasing the concentration of Hg, the accumulation of ROS surpassed the enzymic adjustment capacity in plants, thereby inhibited the activity of CAT enzyme. Moreover, duration of HMs stress in plants also have an impact on the activity of CAT. The mechanism involving the inhibition of cellular enzymatic processes by Hg also involves its binding with the hydroxyl radical in amino acids, which regards as a major part linked to immune and allergic reactive conditions (Patra et al., 2004).

6. CONCLUSION

To conclude, this study indicates that high concentrations of Hg in fenugreek plants exhibits significant reduction in growth parameters which includes germination percentage, root length, shoot length, fresh weight, dry weight and vigour index. Similarly, the carbohydrates and protein contents are reduced in Hg treated plants. The photosynthetic pigments such as chlorophyll a, chlorophyll b, total chlorophyll carotenoid levels also reduced due to Hg toxicity, which also aliens with reduced levels of antioxidant enzymes such as catalase and super oxide dismutase in fenugreek plants. These results ultimately reflecting the plant attempt to alleviate the consequences of Hg toxicity.

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