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Iron toxicity effects on the growth and biochemical analysis of Lady's finger (*Abelmoschus esculentus*) plants

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Abstract
Heavy metals are environmental pollutants that pose serious risks to agricultural productivity vitally by affecting plant's health. Their accumulation in environment can lead to toxic effects, disrupting growth and metabolic processes. This study investigates the Iron toxicity effects on the growth and biochemical analysis of Lady's Finger (*Abelmoschus esculentus*) plants. The experimental plants were divided into 4 different groups. Group 1 plants, which served as the control, received no ferrous iron treatment, while groups 2, 3, and 4 were exposed to ferrous iron treatments of 100, 200, and 400 mg, respectively. Results indicate that more exposure to ferrous iron significantly reduced the growth parameters such as germination percentage, root & shoot length, fresh & dry weight and vigour index. Biochemical analysis illustrates that higher concentration of ferrous iron leads to a notable reduction in carbohydrates and proteins levels. Furthermore, the activities of enzymic antioxidants such as catalase & super oxide dismutase were reduced significantly under ferrous iron toxicity compromising the plant's defense mechanisms against oxidative stress. These findings highlight the harmful effects of ferrous iron in the health of lady's finger plants and underscore the importance of developing strategies to ameliorate the environmental pollution due to heavy metals in agriculture.

Keywords: Lady's finger, iron, heavy metals, toxicity, soil pollution, environment

Introduction
Globally, Heavy metal (HMs) pollution has become a widespread problem, affecting the environment and posing serious health issues to humans. The primary factor contributes to this problem are industrialization, rapid urbanization and alterations in land use, especially in developing countries like India with being more populated. The variety of environmental pollutants has increased dramatically due to advent of economic globalization and industrial revolution with numerous anthropogenic activities

In recent decades, HMs toxicity has emerged as a major abiotic stressor that causes various environmental pollution (Castro *et al*., 2011) [6]. Consequently, Soil crop systems exemplify the interactions between biotic and abiotic factors in the environment. It is well known that HMs make a major contribution to pollutions in the environment due to human activities such as electroplating, mining, smelting, intensive agriculture, sludge dumping, military operations, power transmission, energy and fuel production (Nedelkoska and Doran, 2000) [30]. Nevertheless, higher concentrations of both essential and non-essential HMs in the soil can induce growth inhibition and toxic symptoms in most of plant species (Li *et al*., 2010) [22].

Soil gets contaminated by HMs accumulation due to various sources which includes emissions from rapidly growing areas of industries, disposal of high metal wastes, leaded gasoline and paints, land application of fertilizers, mine tailings, spillage of petrochemicals, wastewater irrigation, sewage sludge, animal manures, pesticides, coal combustion residues and atmospheric deposition (Zhang *et al*., 2010) [41]. Moreover, HMs adversely affect soil biota by soil–microbe interactions and microbial processes (Gall *et al*., 2015) [12].

Uptake of HMs by plants from soils at elevated concentrations can pose greater health risks taking into consideration implications of food-chain. Consuming food crops contaminated with HMs is a primary food chain pathway for human exposure. Long term ingestion of these toxic HMs has detrimental consequences on human health and the linked harmful effects becoming perceptible only after several years of exposure (Khan *et al*., 2008) [19].

Iron (Fe) is one of the important elements for all plants which plays an important role in biological processes such as chlorophyll biosynthesis, photosynthesis and development of chloroplast (Olaleye *et al*., 2009) [31].

It is a key component of cellular redox systems like heme proteins which includes catalase (CAT), superoxide dismutase (SOD), peroxidase, cytochromes, Fe-S proteins like ferredoxin and aconitase (Marschner, 1995) [26]. Plant's excessive absorption of Fe leads to direct toxicity which damages the plant cells. Initial symptoms appear on younger leaves, where Fe accumulates in little brown dots, which is known as bronzing (Baruah and Nath, 1996) [5]. Once over bioaccumulation stage of Fe, leaves exhibit chlorosis and at progressive stages of toxicity necrosis can occur causing the leaves to gets dry and eventually die later. Indirect toxicity arises from limited absorption of various nutrients includes potassium, phosphorous, calcium, magnesium and Fe itself which occurs due to the precipitation of Fe on epidermis of rice roots. Symptoms of toxicity are typically linked with Fe deposition within the roots (Sahrawat, 2010) [37].

Abelmoschus esculentus, commonly known as Lady Finger or Okra is widely popular across the globe as a vegetable for its health benefits and nutritional values (Khatun *et al*., 2011) [20]. It exhibits various biological properties such as hepatoprotective (Honda *et al*., 2003) [17], hypolipidemic, anti-diabetic (Fan *et al*., 2014) [9], anti-fatigue, antioxidant (Lin *et al*., 2014) [23], gastroprotective (Ortac *et al*., 2018), neuroprotective (Mathew *et al*., 2014) [27], cardioprotective (Vindika *et al*., 2018) [40] and anticancer (Solomon *et al*., 2016) [39] effects. HMs presence in crops of agriculture pose a major risk, especially in vegetables like lady's finger, which is essential in many diets across the world. Bioaccumulation of HMs in lady's finger results in threatening health effects for consumers. As the plant absorbs these HMs from contaminated water and soil, toxic substances can enter the food chain leading to risks of neurological disorders, chronic illnesses and developmental problems. Research illustrates that HMs contamination can adversely affect the lady's finger's growth and yield.

Effective restoration and protection of soil ecosystems contaminated by HMs need their remediation strategy and its characterization. Understanding the interactions between lady's finger and HMs is important for developing strategies to alleviate the contamination risks. Implementing sustainable practices in agriculture, monitoring the water and soil qualities and promoting awareness among consumers and farmers are the most important steps in addressing this problem. So, our study was performed to investigate the Iron Toxicity effects on the growth and biochemical analysis of Lady's Finger (*Abelmoschus esculentus*) plants.

Materials and Methods

The experimental protocol deduced in order to fulfill the objectives were carried out with standard procedures. Lady's finger seeds were obtained from agricultural shop, Puducherry. Ferrous sulphate was used to induce Iron toxicity.

Seed Sterilization

Uniform sized seeds were selected. All the seeds were surface sterilized with 0.1% mercuric chloride for 2-3 minutes to avoid fungal infection. These seeds were taken out immediately and washed several times with distilled water.

Polyethylene bag experiment

Polyethylene bag culture experiments were conducted to study the effect of the HMs toxicity in Lady's Finger plants. The growth medium in the polyethylene bags consist of artificially polluted soil at level of 100, 200 and 400 mg of ferrous sulphate. 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 initial phase, the Lady's finger plants were divided into four different groups. Group 1 bag with soil served as control, that did not receive any Fe treatment. In contrast, groups 2, 3 and 4 were exposed to Fe treatments of 100, 200 and 400 mg, respectively. The plants were cultivated under conditions of relative humidity, natural photoperiod and average temperature.

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 (in cm)

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

Shoot Length (in cm)

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

Fresh Weight (in gm)

The fresh weight of the whole plant is determined using electronic balance.

Dry Weight (in gm)

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

Vigour Index

For Vigour index data were 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 [1].

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

Biochemical Estimations

Estimation of Carbohydrates

The carbohydrate content was estimated by the method of Hedge and Hofreiter (1962) [16]. For sample preparation, 1 g of fresh leaves was ground with 50 pml of potassium hydroxide then centrifuged for 15 min and the residue was discarded. The supernatant was made upto 100 ml for sample. The optical density (OD) was recorded at 640 nm against blank.

Estimation of proteins

The protein content was estimated by Lowry's method (1951) [25]. For sample preparation, 1 g of fresh leaves was ground with 10 ml trichloroacetic acid, then centrifuged for 15 min

and the supernatant was discarded. The pellet was centrifuged with 5 ml of sodium hydroxide and the pellet was discarded. The supernatant was made upto 100 ml for sample. The optical density was recorded at 660 nm against blank.

Enzyme assays and analysis

Estimation of Catalase

Leaves were homogenized in 100 mM phosphate buffer (pH 7). The activity of catalase (CAT: EC 1.11.1.6) was assayed by the method of Sinha (1972) [38].

Estimation of Superoxide dismutase

Leaves were homogenized in 100 mM sodium pyro phosphate buffer (pH 8.3). Superoxide dismutase (SOD: EC 1.15.1.1) was assayed by the method of Kakkar *et al*., 1984 [18].

Statistical analysis

Results were expressed as means ± standard deviation of 6 plants per group. Data were analyzed 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).

Results and Discussion

Human exposure to HMs is a complex problem, arising from different routes which includes contaminated water drinking, contaminated air inhalation and food intake that is already contaminated with toxic metals. Effectively, these metals often get accumulated in soil and water, especially in regions with elevated contamination levels. This bioaccumulation primarily occurs via two major routes such as water-root-crop and soil-root-crop route, which results in HMs incorporation into the food chain, affecting both human and animals (Rai *et al*., 2019) [36]. Considering the risks, the Food and Agriculture Organization (FAO) and World Health Organization (WHO) have established safety guidelines for concentration of HMs in water, vegetables and fruits and other different food products (FAO/WHO, 2011). HMs consumption exceeding these recommended maximum allowable concentrations (MAC) through intake of diets can pose major threats to human health (Nakhaee *et al*., 2019) [29].

Germination percentage, Root length and Shoot length

Table 1 shows the effect of Fe stress in Lady's finger plants on germination percentage (%), root length (in cm) and shoot length (in cm) of different experimental groups. To assess the seed quality, it is widely recognized that germination percentage is the most important indicator (Liu *et al*., 2011) [24]. Germination percentage and vigour index can reflect the overall germination status. In our study, germination percentage and vigour index were selected to investigate the effect of Fe on seed germination. Results showed a significant reduction in germination rate, root length and shoot length of *Abelmoschus esculentus* seedling under different doses of Fe as compared to control plants. This toxicity appeared as inhibition in the plant growth (Dubey *et al*., 1987) [7]. Additionally, reduction in germination percentage correlates with increasing concentrations of HMs showed that elevated HMs concentrations inhibit the process of germination which can be attributed to toxic effects of ions involved (Kiran and Munzuroglu, 2004) [21]. A low germination rate in plants leads to inadequate seedling establishment and weak vegetative growth, ultimately leading to a significant decrease in potential yield.

Table 1: Effect of ferrous iron stress in Lady's finger plants 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	15.65±1.35	28.98±2.21
Fe – 100 mg	80	13.29±1.02	24.86±1.76
Fe – 200 mg	50	8.56±0.63	16.97±1.30
Fe – 400 mg	30	5.60±0.46	9.19±0.89

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).

Fresh weight, dry weight and vigour index

Table 2 shows the effect of Fe toxicity on fresh weight, dry weight and vigour index on different groups of lady's finger plant. These observations were recorded at 30th day after sowing. A significant reduction of fresh weight, dry weight and vigour index were observed under Fe toxicity in lady's finger plant as compared to control plant which is consistent with previous studies indicating that elevated concentrations of Fe resulted in reduced plant weight, grain yield and overall growth retardation (Olaleye *et al*., 2001) p32<. The inhibitory consequences of higher concentrations of Fe in root & shoot length and leaf area may be attributed to HMs toxic effects on photosynthesis, reduction in cell division, protein synthesis and respiration. These factors obviously contribute to the retardation of plant's normal growth (Oncel *et al*., 2000) [33]. Moreover, there was a clear direct relationship between concentration of HMs and vigour index reduction, as HMs concentration levels increased, the vigor index also correspondingly decreased.

Table 2: Effect of ferrous iron stress on fresh weight, dry weight and vigour index on different experimental groups of lady's finger plants.

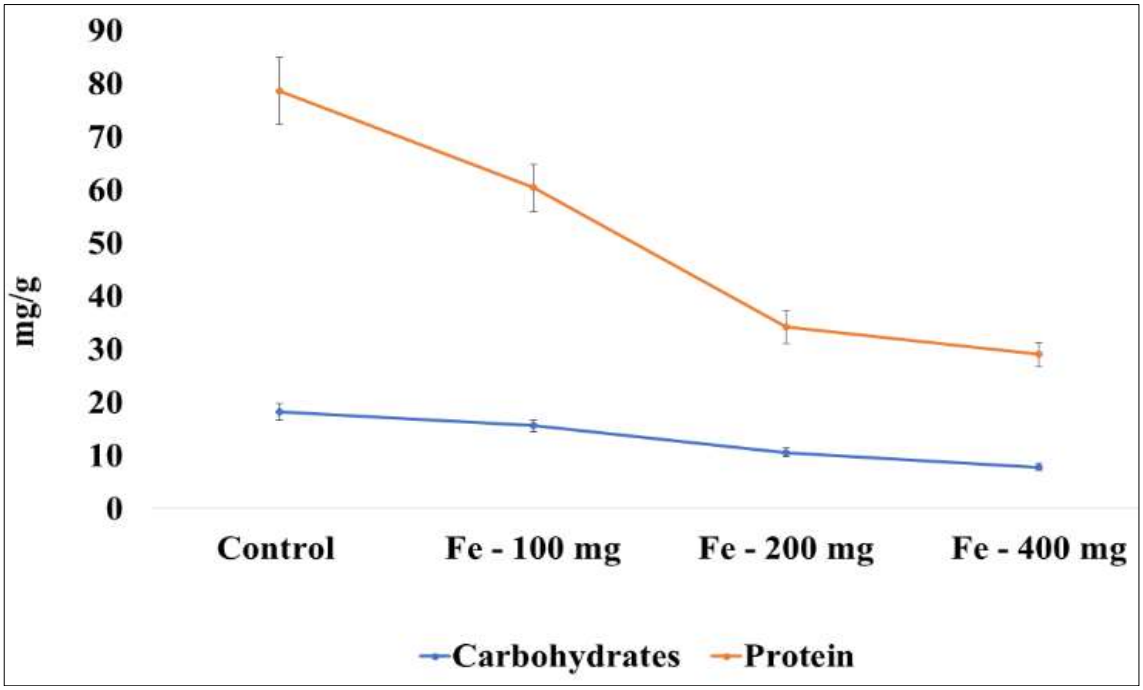
Groups	Fresh weight (g)	Dry weight (g)	Vigour index
Control (C)	6.76±0.57	2.89±0.24	4016.7±210.56
Fe – 100 mg	6.25±0.38	2.32±0.18	3052±98.54
Fe – 200 mg	4.64±0.39	1.64±0.14	1276.5±65.29
Fe – 400 mg	3.06±0.25	1.26±0.10	443.7±59.91

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).

Carbohydrates and protein contents

Figure 1 depicts the effect of Fe on total carbohydrate and protein on three different groups of tested plants. These observations were recorded at 30th day after sowing the seeds. Regarding carbohydrate contents in lady's finger plants with respect to Fe toxicity, the present study revealed that various concentrations of Fe induce significant reduction in carbohydrate contents as compared to control plants. This observed reduction in carbohydrate levels in Fe treated plants may be attributed to various interconnected biochemical and physiological factors. At maximum Fe concentration, most significant reduction of carbohydrate contents was observed. This correlates with the previous study by Abraham *et al*., 2015 [2] who reported that HMs adversely reduced the levels of carbohydrates possibly due to decreased phytochemical activities and alterations in pigment composition, both of which are necessary for carbon dioxide assimilation and synthesis of carbohydrates.

Protein contents in lady's finger under the effect of various concentrations of Fe, our results indicated that under lower concentrations of ferrous iron shows slight reduction in protein contents, while increased Fe concentrations, resulted in a more pronounced reduction. Protein contents was too much sensitive to toxicity of Fe as compared to carbohydrates. This significant decrease in protein contents under higher HMs concentrations in case of Fe may be due to toxic action of these HMs on enzymic reactions responsible for protein biosynthesis (Osman *et al.*, 2004) [35]. Furthermore, the overall plant health is adversely affected under toxicity conditions, resulting in reduced growth and lower biomass accumulation. This reduced biomass may also reflect reduced protein contents, as concentration of protein typically correlates with plant growth.



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).

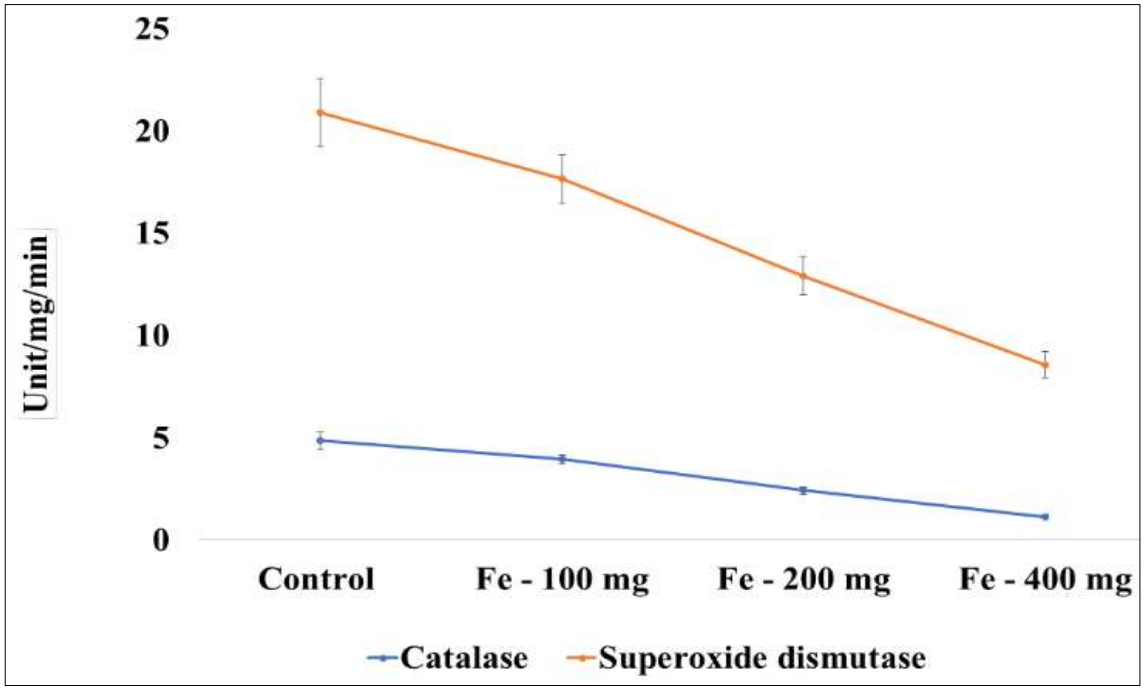
Fig 1: Effect of ferrous iron stress on carbohydrate and protein contents on different experimental groups of lady's finger plants.

Enzymic antioxidants (Catalase and Super oxide dismutase)

Excess of HMs can cause phytotoxic consequences through different mechanism, one of these being the reactive oxygen species (ROS) overproduction, which interrupts the cellular redox environment leading to oxidative stress (Erdei *et al.*, 2002) [8], while it is well known that ROS is a significant factor which contributes to HMs toxicity (Gratao *et al.*, 2005). There is also limited information on their relative efficacy in causing oxidative damage and consequences on the antioxidant system. Furthermore, excess concentration of Fe accelerates the ROS synthesis which are highly toxic, ultimately can cause significant damage to carbohydrates, proteins, DNA and lipids leading to necrosis (Arora *et al*, 2002) [4].

Figure 2 illustrates the effect of Fe stress on enzymic antioxidants on different experimental groups of lady's finger plants. Our present study reveals that decreased catalase (CAT) activity in Fe tested lady's finger plants with respective to various increased concentrations of HMs treatment when compared to normal control plants. This significant reduction in CAT may be attributed to its inactivation through reactions with superoxide ions, can impair the detoxification of H_2O_2 leading to increased oxidative stress. Similar kind of experimental results were observed in previous reports by Hameed *et al.* 2011 [15] showed that HMs suppress the activity of CAT in lady's finger, which is seemingly attributed to inhibited enzymatic synthesis.

Generally, when plants exposure to environmental stresses can result in oxidative damage, as the balance between the synthesis of ROS and their detoxification by the antioxidant system is disrupted (Gomez *et al.*, 1999) [13]. Increased capacity for scavenging or detoxifying activated oxygen species is associated with the ability to tolerate environmental stresses (Foyer *et al.*, 1994) [11]. Superoxide dismutase (SOD), an important metalloprotein, catalyzes the dismutation of super oxide to hydrogen peroxide (H_2O_2) and molecular oxygen (Allen, 1995) [3]. SOD is also considered as a key enzyme in regulating intracellular ROS concentrations. Consequently, increased activity of SOD within plant cells revealed that it plays a beneficial role in managing cellular ROS levels and repairing oxidative damage (Miller *et al.*, 2008). In our study we observed significant decrease in the activity of SOD in response to Fe stress in lady's finger plants, with respective to increased concentrations of Fe when compared to normal control plants. This decreased antioxidant status is likely to increase oxidative stress in lady's finger plants.



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).

Fig 2: Effect of ferrous iron stress on Catalase and Superoxide dismutase on different experimental groups of lady's finger plants.

Conclusion

The present findings affirm that Fe exerts significant toxic effects in lady's finger plants, as evidenced by a marked significant reduction in growth parameters which includes germination percentage, root & shoot lengths, fresh & dry weights and vigour index as compared to control plants. Additionally, the carbohydrate and protein contents were notably decreased in plants exposed to Fe stress, underscore the adverse effect on the quality of nutrition. Furthermore, the activity of important enzymic antioxidants such as CAT and SOD, was diminished, revealing compromised oxidative stress response processes in these plants. These results collectively underline the detrimental effects of Fe toxicity on plant growth, development and biochemical mechanisms, suggesting the necessity for further investigation into ameliorating strategies to alleviate HMs stress in agriculture field.

References

1. Abdul-Baki AA, Anderson JD. Vigor determination in soybean seed by multiple criteria. Crop Sci. 1973;13:630-633.
2. Abraham K, Ramesh P, Damodharan T. Effect of cadmium chloride ($CdCl_2$) on biochemical contents of *Arachis hypogaea* L. Int J Adv Res Eng Appl Sci. 2015;4(11):52-69.
3. Allen RD. Dissection of oxidative stress tolerance using transgenic plants. Plant Physiol. 1995;107:1049-1054.
4. Arora A, Sairam RK, Srivastava GC. Oxidative stress and antioxidative system in plants. Curr Sci. 2002;82:1227-1238.
5. Baruah KK, Nath BC. Changes in growth, ion uptake, and metabolism of rice (*Oryza sativa* L.) seedlings at excess iron in growth medium. Indian J Plant Physiol. 1996;2:122-125.
6. Castro R, Caetano L, Ferreira G, Padilha P, Saeki M. Banana peel applied to the solid-phase extraction of copper and lead from river water. Chem Res. 2011;50:3446-3451.
7. Dubey RC, Dwivedi RS. Effect of heavy metals on seed germination and seedling growth of soybean. Proc Natl Acad Sci Lett. 1987;10:121-123.
8. Erdei L, Tari I, Csiszar JI, Pecsvaradi A. Osmotic stress responses of wheat species and cultivars differing in drought tolerance: some interesting genes (Advices for gene hunting). Acta Biol Szeged. 2002;46(3):63-65.
9. Fan S, Zhang Y, Sun Q. Extract of okra lowers blood glucose and serum lipids in high fat diet-induced obese C57BL/6 mice. J Nutr Biochem. 2014;125:702-709.
10. FAO/WHO. Joint FAO/WHO food standards programme codex committee on contaminants in foods, food CF/5 INF/1. Fifth session. The Hague, the Netherlands: FAO/WHO; c2011.
11. Foyer CH, Lelandais M, Kunert KJ. Photooxidative stress in plants. Physiol Plant. 1994;92(4):696-717.
12. Gall JE, Boyd RS, Rajakaruna N. Transfer of heavy metals through terrestrial food webs: A review. Environ Monit Assess. 2015;187(4):201.
13. Gomez JM, Hernandez JA, Jimenez A, del Rio LA, Sevilla F. Differential response of antioxidative enzymes of chloroplasts and mitochondria to long-term NaCl stress of pea plants. Free Radic Res. 1999;31:11-18.
14. Gratao PL, Polle A, Lea PJ, Azevedo RA. Making the life of heavy metal stressed plants a little easier. Funct Plant Biol. 2005;32(6):481-494.
15. Hameed A, Qadri NT, Mahmooduzzafar T. Differential activation of the enzymatic antioxidant system of *Abelmoschus esculentus* L. under $CdCl_2$ and $HgCl_2$ exposure. Braz J Plant Physiol. 2011;23:46-54.
16. Hedge JE, Hofreiter BT. In: Whistler RL, Be Miller JN, editors. Carbohydrate chemistry. 17th ed. New York: Academic Press; c1962.
17. Honda AH, Nakagawa S, Ashida H, Kanazawa K. Simultaneous determination of all polyphenols in vegetables, fruits, and teas. J Agric Food Chem. 2003;51:571-581.

18. Kakkar PS, Das B, Viswanathan PN. A modified spectrophotometric assay for superoxide dismutase. Indian J Biochem Biophys. 1984;21:130-132.
19. Khan S, Cao Q, Zheng YM, Huang YZ, Zhu YG. Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. Environ Pollut. 2008;152:686-692.
20. Khatun H, Rahman A, Biswas M, Islam AU. Water soluble fraction of *Abelmoschus esculentus* L. interacts with glucose and metformin hydrochloride and alters their absorption kinetics after co-administration in rats. ISRN Pharm. 2011;260:537.
21. Kiran Y, Munzuroglu O. The effects of lead on the seed germination and seedling growth of lens (*Lens culinaris* Medic.). Firat Univ J Sci Eng. 2004;16(1):1-9.
22. Li Q, Cai S, Mo C, Chu B, Peng L. Toxic effects of heavy metals and their accumulation in vegetables grown in saline soil. Ecotoxicol Environ Saf. 2010;73:84-88.
23. Lin Y, Liu HL, Fang J, Yu CH, Xiong YK, Yuan K. Anti-fatigue and vaso-protective effects of quercetin-3-O-gentiobiose on oxidative stress and vascular endothelial dysfunction induced by endurance swimming in rats. Food Chem Toxicol. 2014;68:290-296.
24. Liu TT, Wu P, Wang LH, Zhou Q. Response of soybean seed germination to cadmium and acid rain. Biol Trace Elem Res; c2011.
25. Lowry OH, Roseborough NJ, Farr AL, Randall RL. Protein measurement with Folin-phenol reagent. J Biol Chem. 1951;193:265-275.
26. Marschner H. Functions of mineral nutrients: micronutrients. In: Marschner H, editor. Mineral Nutrition of Higher Plants. 2nd ed. London: Academic Press; c1995. p. 313-404.
27. Mathew M, Subramanian S. *In vitro* screening for anticholinesterase and antioxidant activity of methanolic extracts of Ayurvedic medicinal plants used for cognitive disorders. PLoS One. 2014, 9.
28. Miller G, Shulaev V, Mitter R. Reactive oxygen signaling and abiotic stress. Physiol Plant. 2008;133:481-489.
29. Nakhaee S, Amirabadizadeh A, Brent J, Mehrpour O. Impact of chronic lead exposure on liver and kidney function and hematologic parameters. Basic Clin Pharmacol Toxicol. 2019;124(5):621-628.
30. Nedelkoska TV, Doran PM. Characteristics of heavy metal uptake by plant species with potential for phytoremediation and phytomining. Miner Eng. 2000;13:549-561.
31. Olaleye AO, Ogunkunle AO, Singh BN, Akinbola GE, Tabi FO, Fayinminu OM, *et al*. Ratios of nutrients in lowland rice grown on two iron-toxic soils in Nigeria. J Plant Nutr. 2009;32:1-17.
32. Olaleye AO, Tabi FO, Ogunkule AO, Singh BN, Sahrawat KL. Effect of toxic iron concentration on the growth of lowland rice. J Plant Nutr. 2001;24:441-457.
33. Oncel I, Keles Y, Ustun AS. Interactive effects of temperature and heavy metal stress on the growth and some biochemical compounds in wheat seedlings. Environ Pollut. 2000;107(3):315-320.
34. Ortaç D, Cemek M, Karaca T, Buyukokuroglu ME, Ozdemir ZO, Kocaman AT, *et al*. *In vivo* anti-ulcerogenic effect of okra (*Abelmoschus esculentus*) on ethanol-induced acute gastric mucosal lesions. Pharm Biol. 2018;56:165-175.
35. Osman OMEH, El-Naggar AH, El-Sheekh MM, El-Mazally E. Differential effects of Co^{2+} and Ni^{2+} on protein metabolism in Scenedesmus obliquus and Nitzschia perminuta. Environ Toxicol Pharmacol. 2004;16:169-178.
36. Rai PK, Lee SS, Zhang M, Tsang YF, Kim KH. Heavy metals in food crops: health risks, fate, mechanisms, and management. Environ Int. 2019;125:365-385.
37. Sahrawat KL. Reducing iron toxicity in lowland rice with tolerant genotypes and plant nutrition. Plant Stress. 2010.
38. Sinha KA. Colorimetric assay of catalase. Anal Biochem. 1972;47:389-394.
39. Solomon S, Muruganantham N, Senthamilselvi MM. Anticancer activity of *Abelmoschus esculentus* (flowers) against human liver cancer. Int. J Pharm Biol Sci. 2016;6:154-157.
40. Vindika S, Kuruwitaarachchige D, Inoka U, Sirimal P, Jayantha W. Cardio protective activity of *Abelmoschus esculentus* (Okra). Int. J Food Sci Nutr. 2018;3:39-43.
41. Zhang MK, Liu ZY, Wang H. Use of single extraction methods to predict bioavailability of heavy metals in polluted soils to rice. Commun Soil Sci. Plant Anal. 2010;41(7):820-831.