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Comparative Analysis of Iron Content in Drumstick Leaves of Different Maturity Collected from Different Regions of Jaipur, Rajasthan, India

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Abstract

This study presents a comparative assessment of Iron (Fe) content in *Moringa oleifera* leaves of different sizes collected from 5 different regions of Jaipur, Rajasthan, India. The leaves were classified as small, medium and large based on their dimensions and their Fe content was estimated using Atomic Absorption Spectroscopy (AAS) The leaf size ranged from 4 cm-18 cm and maximum size was recorded for those collected from Vidyadhar nagar. Maximum Fe content was noted in small 0.83 ± 0.01 mg/g, medium 0.91 ± 0.07 mg/g and large 0.97 ± 0.06 mg/g sized leaves collected from Barodia Scheme. The relative order of Fe concentration according to the regions was obtained as follows: Barodia Scheme > Sahakar Nagar > Ashok Nagar > Vidyadhar Nagar > Himmat Nagar The variation in Fe content of leaves of different trees can be due to the variable Fe uptake which in turn can be attributed to the variability in soil composition, Fe availability in soil and habitat conditions. This study provides preliminary findings based on which a detailed study can be conducted to further identify the optimum soil, environment and harvesting conditions for maximum yield in terms of Fe content. Further, findings of the present study strongly support that *M. oleifera* leaves can serve as an excellent Fe supplement to cure anemic and other Fe deficient conditions.

Keywords

Moringa oleifera, AAS, Fe supplement, leaf size

Introduction

Minerals are essential for functioning of a living system due to their involvement in various cellular, metabolic and physiological pathways. Based on their function(s), elements including Ca, P, K, Na, Cl, Mg, Fe, Zn, I, Cr, Cu, F, Mo, Mn, and Se have been rendered as "essential minerals" for human body. These essential elements prominently function as activators or cofactors for various enzymes/hormones and organic molecules that mediate/regulate different biological processes such as synthesis of DNA/RNA/protein and other biomolecules, respiration, energy generation through ATP, and many more [1, 2]. Fleet, Replogle [3] evaluated the functions of different essential minerals and reported that "in addition to being intracellular messengers (P and Ca) and metalloprotein and enzyme cofactors (Fe, Cu, and Zn), they are also necessary for bone health (P, Mg, and Ca), osmoregulation (P, Na, and K), redox and acid-base balance (K, Na, Se, and Ca).

Among these essential minerals, Fe has been rendered as a fundamental element for various biological processes such as oxygen transport, mitochondrial function maintenance, DNA synthesis, respiration, ATP generation, antioxidant protein function, DNA-damage repair in hypoxic cancer cells and many more [4, 5]. Fe is also an important/essential constituent (prosthetic group or cofactor) of various enzymes and is thus involved in activation of various metabolic pathways [6]. In addition, Fe bound proteins (also known as hemoprotein) and non-heme enzymes mediate redox reactions and electron transfer [7]. It is majorly present in erythrocytes as a constituent of heme moiety of hemoglobin, and its deficiency is one of the major causes for anemia [8]. It is also present in storage compounds (ferritin and hemosiderin) and in myoglobin (muscle cells). Thus, maintenance of appropriate Fe levels is critical for normal functioning of human body and other living beings.

Various currently available Fe fortified foods and synthetic/chemically derived Fe (oral) supplements have been reported to exhibit several harmful side effects including adverse sensory changes in food, gastrointestinal irritations and inflammation [9], which has generated the need for development of supplements that are less/non- toxic for human health. EAT-Lancet Commission on Food, Planet, Health stated that "a diet rich in plant-based foods and with fewer animal source foods confers both improved health and environmental benefit and assures sustainability of natural resources" [10].

Plants serve as storehouse of various types of essential minerals and elements along with wide variety of phytochemicals that confer the diverse medicinal and non-medicinal properties to a plant. Plant derived supplements have gained lot of interest in past few decades due to their non-toxic and naturally derived formulations. Studies based on assessment of mineral/elemental composition of different plants & their bioavailability upon consumption have been conducted [2].

M. oleifera Lam., commonly known as drumstick tree is one such mineral rich medicinally important multi-purpose tree. It is often referred as the "miracle tree" due to its potential to serve as remedy for more than 300 diseases and disorders [11]. The plant is well known for its edible aerial parts and is used as key ingredient in various food preparations. Almost all parts of the plant are edible and are consumed in various forms such as soups and sauces (flowers and leaves), food garnish (leaves), herbal tea (leaves), porridge (leaves), spices (leaves), pickles (seeds), etc. [11]. Its leaves are also consumed raw as a snack, and its roots are used as a substitute for horseradish (a condiment) [11].

The tree as a whole serves as a reservoir of the essential vitamins B6, B2, and C, minerals Fe, Mg, and Ca and digestible proteins [12, 13]. In fact, its leaves are reported to possess "higher amounts of Ca (4x), protein (2x), ascorbic acid (7x), vitamin A (4x), Fe (3x) and K (3x) than that in milk, oranges, carrots, spinach and banana", whilst its flowers, immature pods and young shoots serve as a rich source of methionine, P, Ca, and Fe [14, 15]. This nutritional rich composition of the plant makes it an excellent source of food for remediation of malnutrition and nutrient deficiencies.

Owing to the rich nutrient profile of the drumstick leaves, majority of the commercially available nutritional supplements are leaf based formulations [11]. High Fe content and wide applications in food makes its leaves as an excellent source of Fe supplement. However, lack of proper information about the optimum harvesting conditions to obtain maximum nutrient value is one of the major associated limitations. Therefore, in this study leaves of different dimensions were collected from different regions of Jaipur, Rajasthan, India to determine the effect of leaf dimension on Fe content.

Material and Methods

Plant samples

Leaves of different dimensions were collected from drumstick tree located in different regions (Himmat nagar, HN; Ashok vihar, AV; Vidyadhar nagar, VN; Barodia scheme, BS and Sahakar Marg, SM) of Jaipur, Rajasthan, India. Based on their dimensions, leaves were classified into small (< 8 cm), medium (8 – 10 cm) and large (> 10 cm). Samples were collected in triplicates for each dimension and were processed for further analysis as mentioned in subsequent sections.

Preparation of samples

All collected leaf samples were washed with distilled water and were dried in hot air oven at 120 °C until constant dry weight was obtained. The dried leaves were then crushed into fine powder using mortar pestle and used for Fe content estimation.

Wet digestion method

Each of the homogenized leaf sample was treated with conc. HNO_3 (5 ml) as per method given by Kazi, Sahtio [16]. The mixture was then covered with a watch glass followed by heating on a hot plate for 1 h at 80 - 100 °C. The mixture was then treated with HNO_3 (5 ml) and 30% H_2O_2 (2 ml) by heating for 1 h. The watch glass was removed, and the mixture was heated until it was semi-dried. Further, 2 N HNO_3 (5 ml) was added to the semi-dried mixture, which was then heated for 2 min, cooled, diluted with 2 N HNO_3 and filtered using Whatman Filter Paper No. 42. The filtrate was collected in a volumetric flask and its volume was made up to 25 ml with deionized water.

Determination of Fe content using AAS

The absorbance of digested leaf samples was recorded using 'Model A' Analyst 300 AAS (Perkin Elmer) as per standard operating procedure. The concentration of Fe was determined using calibration curve obtained by measurement of absorbance of series of standard solutions of known Fe concentration (prepared from its 1000 ppm standard stock solution) HNO_3 was used as blank and all the readings were taken in triplicates.

Results

Determination of leaf size

Variations in size of small, medium, and large leaves collected from trees of different locations were recorded and has been summarized in Table 1.

Determination of Fe content

The Fe content was quite higher in medium and large sized leaves than that of small sized irrespective of the location of the sample collection (Table 2). However, the content was comparable in medium and large sized leaves and no major differences were recorded in their content (Table 2).

Significant differences were recorded in Fe content of leaves collected from different locations, such that highest content was recorded in small (0.74 ± 0.03 mg/g), medium (0.91 ± 0.07 mg/g), and large (0.97 ± 0.06 mg/g) sized leaves collected from SM and BS respectively. The Fe contents of leaves collected from trees located in AV, HN and VN were nearly similar and were lower than that recorded in leaves of SM & BS (Figure 1).

Discussion

In present study, Fe content in *M. oleifera* leaves of different sizes collected from five different regions of Jaipur, Rajasthan, India was determined. The results showed relatively higher Fe content in all sizes of leaves collected from BS.

Additionally, the size of leaves also varied according to the cultivation regions in this study. The maximum leaf size was observed 18.5 cm and 10.2 cm, respectively from large leaves and medium leaves of VN area. However, highest leaf size i.e., 7.2 cm recognized from small leaves of AV area. The leaf width and length have significant role in the studies of plant species therefore morphological analysis of *M. oleifera* leaves is necessary to recognize dynamics of area and dimensions [17]. In the study of Macário, Ferraz [18], the range of leaf size was determined 0.695-2.831 cm with 29.717% coefficient of variation and the average length of leaves was observed 1.595 cm. Commonly the leaf length and width gave acceptable knowledge about the leaf area of plant species. A study conducted by Souza et al., 2019 in which they assessed about the size of bean seeds and revealed that the measurement of seeds size is crucial to choose seeds for higher yield. Maximum size of leaf is helpful to optimize radiation capture by plants under 15% less intensity in luminosity that is effective to increase the absorption of radiation by the mesophyll cells. As a consequence, it is indirectly augment the photosynthetic rate. It also diminishes the harmful effects on plant metabolism, which is governed by luminosity [19].

Table 1: Classification of leaves collected from different regions of Jaipur, Rajasthan (India).

Location of Sample Collection	Average leaf size ± SD (cm)		
Small	Medium		Large
HN	5.5 ± 0.461	9.2 ± 0.567	15.5 ± 0.414
AV	7.2 ± 0.234	9.7 ± 0.671	16.5 ± 0.574
VN	5.8 ± 0.515	10.2 ± 0.410	18.5 ± 0.673
BS	4.7 ± 0.138	8.2 ± 0.229	16.7 ± 0.455
SM	4.5 ± 0.112	9 ± 0.342	10.6 ± 0.387

HN Himmat Nagar, AV Ashok Vihar, VN Vidyadhar Nagar, BS Barodia Scheme, SM Sahakar Marg; n = 3.

Table 2: Fe content in *M. oleifera* leaves of different sizes collected from different regions of Jaipur, Rajasthan (India).

Location of Sample Collection	Average Fe content ± SD (mg/g)		
Small Leaf	Medium Leaf		Large Leaf
HN	0.231 ± 0.016	0.441 ±0.0104	0.618 ± 0.028
AV	0.322 ± 0.0087	0.564 ± 0.020	0.763 ± 0.016
VN	0.287 ± 0.039	0.459 ± 0.051	0.629 ± 0.013
BS	0.826 ± 0.013	0.911 ± 0.071	0.968 ± 0.059
SM	0.745 ± 0.029	0.799 ± 0.041	0.834 ± 0.047

HN Himmat Nagar, AV Ashok Vihar, VN Vidyadhar Nagar, BS Barodia Scheme, SM Sahakar Marg; n = 3.

Leaves from SN had the second highest Fe level (0.745 mg/g SL, 0.799 mg/g ML, and 834 mg/g LL) with respect to other three area (HN, AV, and VN). Furthermore, based on leaf sizes, large size of *M. oleifera* leaves had shown maximum Fe content as compared to medium and small leaf sizes. Consequently, it was observed that the Fe content in *M. oleifera* leaves depends on environment conditions as well as leaf sizes. Same species of plant can show variation in Fe content according to the area in which they are growing. Therefore, climatic conditions play a vital role to define the plant properties.

Likewise, in the study of Aslam, Anwar [2] the contents of different minerals in the leaves of *M. oleifera* were significantly varied from region to region in which the concentration of Fe in *M. oleifera* was found to be 205, 397 and 573 mg/kg from Bahawalnager, Sadiqabad and Chenabnager in Punjab. In 2014, Liyanage, Jayathilake [20] analyzed minerals in *M. oleifera* leaves from different regions of Sri Lanka in which

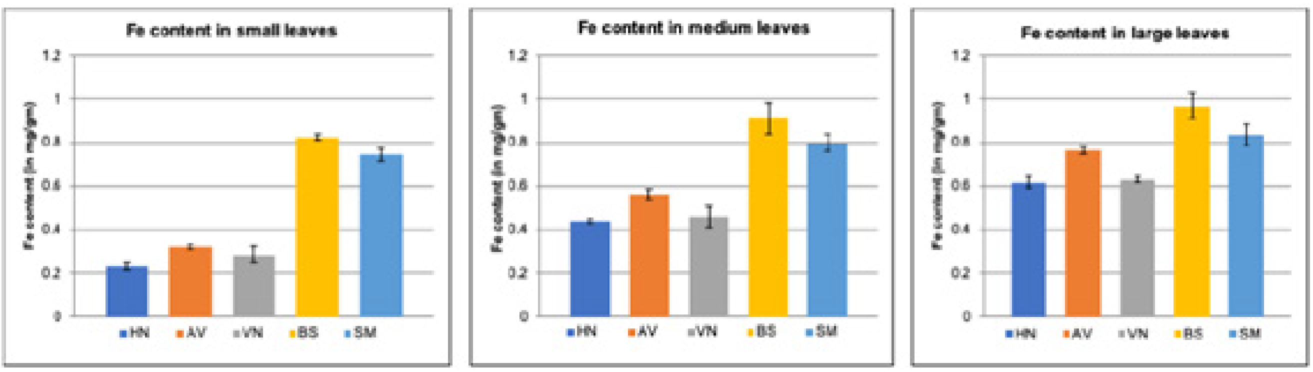

Figure 1: Fe content in (A) small (B) medium (C) large sized leaves of *M. oleifera* collected from different regions of Jaipur, Rajasthan, India.

maximum Fe level of 26.99 ± 12.0 mg/100 g was obtained from Polonnaruwa district followed by 13.09 ± 6.93 mg/100 g Fe from Kurunegala district which was significantly higher ($p<0.05$) compared to other districts such as Anuradhapura, Galle, Hambantota, Jaffna, Kandy, and Matara. According to the William, Iddrisu [21] research conducted in two zone of Ghana, the Fe concentration was obtained 26.83 mg/kg in the semi-deciduous forest zones and 25.04 mg/kg in Guinea Savanna of Ghana. The iron concentration in *M. oleifera* leaf samples from the semi-deciduous forest zone was found slightly higher than Guinea savanna. Jongrungruangchok, Bunrathep [22] reported a data on mineral components in *M. oleifera* leaves from eleven different agro-climatic regions of Thailand. The K, Ca and Fe level of *M. oleifera* leaves were found in the range of 1504.23-2054.05 mg/100gm, 1504.23 - 2054.05 mg/100 gm, 1510.41 - 2951.13 mg/100 gm and 20.31 - 37.60 mg/100 gm from different regions of Thailand, respectively. Abundance of minerals particularly P, K, Mg and Fe has been confirmed in the leaves of *M. oleifera* [23]. Aditama, Heri [24] reported 152.89 ppm Fe content in fresh *M. oleifera* leaves powder and 143.85 ppm in steam blanched *M. oleifera* leaves powder. Therefore, *M. oleifera* leaves has more mineral components specially Fe content hence it can be used as iron supplement.

Conclusions

The results of the present analysis demonstrated variations in Fe content with respect to both leaf size and location of the tree. The variation in Fe contents of leaves of different trees can be due to the variable Fe uptake which in turn can be attributed to the variability in soil composition, Fe availability in soil and habitat conditions. The findings of present study revealed that *M. oleifera* leaves of size > 10 cm contain maximum Fe content and thus could serve as an optimum source of Fe supplementation. This study provides preliminary findings based on which a detailed study can be conducted to further identify the optimum soil, environment conditions and harvesting conditions for maximum yield in terms of Fe content.

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