

Role of iron biofortification to improve growth, yield and chemical composition of maize

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Abstract

Mineral nutrient deficiencies are a worldwide problem that is directly correlated with food insecurity. The most common of these is iron (Fe) deficiency as more than one-third of the world's population suffers from iron deficiency-induced anemia mainly in developing countries. This study was planned to overcome iron deficiency through its biofortification in maize plants. For this purpose, a pot experiment was conducted to monitor efficiency of iron biofortification on maize and soil health. Fe was applied through seed, soil and foliar mode. The experiment had 07 treatments with 04 replicates; $T_1 = Control$, $T_2 = Control$, $T_$ Seed priming with 1.0% FeSO₄ solution, T_3 = Seed priming with 2.0% solution of FeSO₄, T_4 = Addition of 10 kg ha⁻¹ FeSO₄ to soil, $T_5 =$ Addition of 20 kg ha⁻¹ FeSO₄ to soil, $T_6 =$ Addition of 1% FeSO₄ solution to leaves as spray and $T_7 =$ Addition of 2% FeSO₄ solution to leaves as spray. Seed priming with Fe solution was done before sowing. Soil treatments were applied one week after sowing. Foliar application of iron was accomplished at 2 stages viz. 30 and 60 days after germination of maize plants. Plant samples were obtained at maturity and analyzed for iron concentration. Soil samples were collected from all the pots and subsequently analyzed for iron concentration. Results depicted that highest N (0.95%), P (0.20%), K (2.06%), Ca (0.2%), Na (0.27%) and Fe (190 ppm) concentration in maize plant was achieved when Fe was applied as foliar application of FeSO₄ @ 2 % solution (T₇) compared to other treatments. However, soil organic matter (0.90 %), P (8.375 ppm) and K (182.25 ppm) was recorded by the addition of 20 kg ha⁻¹ FeSO₄ to soil (T₅). Thus, the mode of foliar application of Fe had beneficial effects on nutritional composition in maize crop and soil than soil application and seed priming. © 2022 Department of Agricultural Sciences, AIOU

Keywords: Biofortification, Growth, Iron, Maize, Yield

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Introduction

Maize (Zea mays L.) is cultivated in every part of the world. It has the ability to survive in a variety of climates including temperate, subtropical and tropical regions. It is a versatile crop which provides foodstuff for humans and livestock (Abebe & Ronda, 2015). A grain of maize consisted of the essential vitamins like A and B, protein 10%, starch 72%, sugar oil 8.5%, fiber 5.81% and ash 1%. In Asia the maize occupies 2nd position after rice, while in Pakistan 3rd most important crop after wheat and cotton. The maize is grown in Pakistan on about 1168.5 thousand hectares area while the total grain production is 4944.2 thousand tones. Average yield of maize in Pakistan is 4.23 t ha⁻¹ which is two to three times lesser than the average vield of developed countries (Awan & Aslam, 2015). Maize is usually refined to make a few different varieties of products like cereal, glucose, flour and breakfast wheat. The corn flour is used for making chapatis or breadcrumbs which are consumed in some northern states of India (Mehta & Dias, 1999).

Iron deficiency is a widespread nutritional disorder in the world. According to the World Health Organization

(WHO) the constraints of Fe lead to serious consequences on human body and economic growth of the countries (Becher et al., 2017). Most people are affected by Fe deficiency all over the world which may be five hundred million to two billion. Fe deficiency is significantly high in under developed countries (Ahmed et al., 2012; Ishfaq et al., 2021). Fe is a necessary micronutrient for the production of plants and its deficiency results in decreased photosynthesis causing leaves of plants colorless (Malakoti & Tehrani, 1999). Fe exists in soil in the form of ferrous Fe $^{2+}$ and ferric Fe $^{3+}$. The ferric compounds have the least solubility in soil solution, but the availability is depending upon the soil aeration and pH. The pH ranges from 7.4-8.5 will decrease the Fe concentration in alkaline calcareous soil. The capacity of plants to respond to iron availability in soil eventually influences human's nutrition either through reduced crop production or Fe content of nutritive tissue (Morrissey & Guerinot, 2009).

Iron is the greatest plentiful micronutrient in the crust present in the form of ferromagnesian silicate (López-Millán et al., 2013). The existence of iron in soil is approximately 1-5% contained in the plough layer. The iron occurred in soil in the

form soil silicate minerals like iron oxide, and hydroxide which are responsible for the reddish and yellowish color of the soil (Givnish et al., 2014).

Maize based diet is commonly lacking the compulsory micro element like Fe, zinc (Zn), vitamin A and its consumers get stunted growth, prone to anemia, less recreation and infant mortality (Saltzman et al., 2013). In the developed world the majority of residents depend on a cereal-based diet having insufficient Fe bioavailability (Cakmak et al., 2010). Fe biofortification is a sustainable and cost-effective agricultural strategy to reduce malnutrition (White & Broadley, 2009). Hemoglobin is the non-heme enzyme that secures iron in a non-toxic shape and hemoglobin iron is bioavailable in human beings as iron sulfate (Lonnerdal, 2007).

Good nutritious foods depend upon the adequate nutrient intakes and other compounds concentration. Developing countries have a problem of less food quantity with inferior quality and nutrition (Gani et al., 2018). Different strategies could be used to decrease the malnutrition (Hussain et al., 2017; Grujcic et al., 2021), but biofortification is an excellent, convenient, doable and economical practice (Adeyeye et al., 2019; Nishanth et al., 2021; Kiran et al., 2022). Insufficiency of micronutrients ultimately leads to hidden hunger as intake of such food can cause exposure to diseases due to malnutrition (FAO, 2021). The idea of biofortification is attractive and feasible to improve the problematic soil and ultimately enhance the growing conditions for crops. Biofortified foods have great potential to combat nutritional disorders in countries with poor diet resources (Jeong & Guerinot, 2008; Koç & Karayiğit, 2021). Therefore, assessing the bio-availability of iron is necessary in order to manage the iron nutrition in crops. Krishnaraj et al. (2020) reported positive effects of Fe and Zn foliar application on maize plant growth and production of maize crop. Foliar application of Fe and Zn formulation 17:17 at 0.2 % increased the production of the maize crop by 21%. Over all the results depicted that foliar application significantly increased crop growth and yield performance compared to fertilizer applications. Similarly, Qureshi et al. (2020) informed that biofortification of Fe and Zn improves the plant average yield of different maize varieties.

Keeping in view the low average yield of maize and predominating iron deficiency in the human beings, this research work was carried out to overcome the iron deficiency through its biofortification in maize plants and to study the impact of iron biofortification on the soil properties.

Materials and Methods

A pot trail was carried out in the research area of Department of Soil and Environmental Sciences University of Sargodha, Punjab, Pakistan during the winter season, 2020 to investigate the effect of iron on growth and yield of maize crop and differentiation in different methods of application. Soil samples were taken from the research area of the college of agriculture. Seven treatments were applied with four replications. Five seeds of maize (Hybrid R1) were sown in twenty-eight pots and three healthy plants were maintained in each pot after germination. Recommended dose of NPK was applied to each pot. Canal water was applied to all pots. All the agronomic practices were done as per necessity. Iron application was done as per treatment plan. After harvesting the yield and yield parameters were noted. Plant samples were collected from each pot for nitrogen, phosphorus, potassium, calcium and sodium determinations in the laboratory. At the end, soil samples were also collected from all the pots and shifted to the laboratory for chemical analysis. According to the design, every pot was filled with 07 kg of soil for seed sowing. Hybrid R1 variety was used in this experiment. Following treatments were used $T_1 = Control$, $T_2 =$ Seed priming with 1.0 % FeSO₄ solution, T_3 = Seed priming with 2.0 % solution of FeSO₄, T_4 = Addition of 10 kg/ha FeSO₄ to soil, T_5 = Addition of 20 kg/ha FeSO₄ to soil, T_6 = Addition of 1.0 % FeSO₄ solution to leaves as spray, and $T_7 =$ Addition of 2.0 % FeSO₄ solution to leaves as spray.

Fertilizers of N, P, K were added to every pot at different levels, individually. Different levels of iron were used: 1.0 % solution, 2.0 % solution, 10 kg/ha, 20 kg/ha, 1.0 % solution of foliar application and 2.0 % solution of foliar application. The NPK sources used for fertilizer were urea for nitrogen 2 g/pot, single super phosphate (SSP) for phosphorus 3 g/pot, and muriate of potash (KCl) for potassium 1 g/pot. In every pot, 5-6 seeds were sown. Plants were removed after 15-20 days of seed germination and three plants per pot were left for best growth. Pots were irrigated to maintain moisture at field capacity.

Plant sampling and analysis

Plants were sampled from each pot. Wet digestion of plant samples was adopted for the determination of N, P, K, Ca, Na and Fe concentrations using nitric acid and perchloric acid combination. P was determined with a colorimetric method with a spectrophotometer. K and Na concentrations were determined by flame photometer while Ca and Fe contents were determined by atomic absorption spectrophotometer.

Soil sample collections and analysis

Soil samples were taken from each pot for physical and chemical analyses. Preparation of soil samples was done for further analysis. Analytical methods of United States Department of Agriculture [USDA], (1969) or otherwise mentioned were adopted for laboratory analysis. Soil extract was obtained and then Fe was determined using a method described by Lindsay & Norvell (1978). Walky & Black (1934) method was adopted for organic matter investigation. Determination of phosphorus was done using а spectrophotometer following the protocol given by Olsen et al. (1954). Flame photometer was hired for K analysis using a method suggested by Affinnih et al. (2014). Statistical analysis of data by using software statistics 8.1 were carried out (Steel et al., 1997).

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Table 1 Soil physico-chemical analysis

Parameters	Units	Values
pH	-	8.2
EC	dSm^{-1}	1.85
Carbonates	m mol _c L ⁻¹	-
Bicarbonates	m mol _c L ⁻¹	6.6
Chlorides	m mol _c L^{-1}	6.34
Sulphates	m mol _c L^{-1}	5.56
Calcium + Magnesium	m mol _c L^{-1}	7.0
Sodium	m mol _c L ⁻¹	10.25
Potassium	ppm	1.25
Sodium adsorption ratio	-	5.48
Soil organic matter	%	0.76
Phosphorus	ppm	6.2

Results and Discussion

Nitrogen (N) concentration (%) in maize plants

The minimum N concentration (0.7%) was recorded for control treatment having zero dose of Fe. Foliar application of Fe @ 2% solution (T₇) remained better to all other treatments. The observed N concentrations of maize plants of these two treatments (T₇ and T₃) were 0.95 and 0.93 % respectively (Fig. 1). Foliar application of Fe proved more efficient than soil application in maize N concentration. Although, seed priming and soil application also enhanced the N concentration significantly when compared with control. Results of Davies et al. (2020) reported that biofortification of Fe at vegetative and productive stages of maize enhanced N concentration in maize crop.

Phosphorus (P) concentration (%) in maize plants

The minimum P concentration (0.12%) was recorded for control treatment having zero dose of iron. Foliar application of Fe @ 2 % solution (T₇) remained better to all other treatments. Foliar application of Fe proved more efficient than soil application in maize P concentration (Fig. 2). Seed priming and soil application also enhanced the P concentration significantly when compared with control. Results of Parent et al. (2020) favored these findings. Hussain et al. (2017) also reported that P was more at flowering and reproductive stage of maize crop. (NH₄)₂HPO₄ was the mode of when P coupled with Fe.



Fig. 1 Effect of Iron biofortification on N concentration (%) of maize crop



Fig. 2 Effect of Iron biofortification on P concentration (%) in plant of maize crop

Potassium (K) concentration (%) in maize plants

The minimum K concentration (1.94%) was recorded for control treatment having zero dose of iron (Fig. 3). Foliar application of Fe @ 2 % solution (T₇) remained better to all other treatments. Foliar application of Fe proved more efficient than soil application in maize K concentration. Seed priming and soil application also enhanced the K concentration significantly when compared with control. Results of Adnan (2020) were also in the same direction. Izsáki (2017) reported that K was more at the flowering and reproductive stage of the maize crop. K₂SO₄ was in the mode of K when coupled with iron.



Fig. 3 Effect of Iron biofortification on K concentration (%) in plant of maize crop

Calcium (Ca) concentration (%) in maize plant

The minimum Ca concentration (0.11%) was recorded for control treatment having zero dose of iron that was reached to maximum concentration of 0.2 % in treatment T₇ (Fig. 4). Foliar application of Fe @ 2 % solution (T₇) remained better to all other treatments. Foliar application of Fe proved more efficient than soil application in maize Ca concentration. Seed priming and soil application also enhanced the Ca concentration significantly when compared with control. Results of Abbas et al. (2021) favored these findings. Similarly, Gaj et al. (2018) reported that Ca was more at flowering and reproductive stage of maize crop when supplement with Fe.



Fig. 4 Effect of Iron biofortification on Ca concentration (%) in plant of maize crop

Sodium (Na) concentration (%) in maize plant

Data of this study suggested that Na concentration of maize crop was increasing substantially due to Fe fertilization in addition to recommended dose of NPK. Application of Fe (soil application at different rates, seed treatment and foliar application) produced more Na concentration of maize. All treatments were different significantly when compared with each other statistically (Fig. 5). The minimum Na concentration (0.2%) was recorded for control treatment having zero dose of Fe. Foliar application of Fe @ 2 % solution (T_7) remained better to all other treatments. Foliar application of Fe proved more efficient than soil application in maize Na concentration. Results of Saroj et al. (2018) also favored these findings. Likewise, Butcher et al. (2018) reported that Na was more at flowering and reproductive stage of maize crop.



Fig. 5 Effect of Iron biofortification on Na concentration (%) in plant of maize crop

Iron (Fe) concentration (mg kg⁻¹) in maize plant

Data of this study suggested that Fe concentration of maize crop was increasing substantially due to Fe fertilization in addition to recommended dose of NPK. Application of Fe (soil application at different rates, seed treatment and foliar application) produced more Fe concentration of maize. All treatments were different significantly when compared with each other statistically (Fig. 6). The minimum Fe concentration (150 mg kg⁻¹) was recorded for control treatment having zero dose of iron. Foliar application of Fe @ 2% solution (T_7) remained better to all other treatments. Results of Stewart et al. (2020) favored these findings. Results of Kumar & Salakinkop (2018) proved that foliar application of Fe enhanced the iron concentration in plants of maize crop. In another research, Rout & Sahoo (2015) reported that plant metabolism such as internal respiration and chemosynthesis of maize crop significantly increased by applying the foliar application of Fe on maize crop.



Fig. 6 Effect of Iron biofortification on Fe concentration (ppm) in plant of maize crop

Organic matter (%) of soil

Soil organic matter (SOM) was significantly affected by the use of Fe sulphate. Among all over the treatments, T_5 (soil application of 20 kg ha⁻¹ FeSO₄) produced the maximum SOM (0.91%) (Fig. 7). Minimum SOM (0.76 %) was noted in T_1 (control) which was followed by T_6 (foliar application of 1.0% FeSO₄ solution) and T_7 (foliar application of 2.0% FeSO₄ solution) that recorded 0.77% and 0.8% soil organic matter respectively. However, results suggested by Kandil (2016) implied that soil organic matter decreased with the use of iron sulphate in soil. Results of Björnerås (2019) also suggested that soil properties like soil organic matter, cation exchange capacity, soil texture, structure, porosity, soil chemistry, electrical conductivity and soil color reduced significantly due to Fe sulphate.



Fig. 7 Effect of Iron biofortification on organic matter (%) of soil

Phosphorus (P) of soil (ppm)

P content was significantly affected by the use of Fe sulphate. Among all the treatments, the T_5 (soil application of 20 kg ha⁻¹ FeSO₄) proved the maximum responsive regarding soil P content (8.37 ppm) followed by the treatments T_4 (soil application of 10 kg ha⁻¹ FeSO₄) with value of 8.05 ppm (Fig. 8). Minimum P was (7.08 ppm) in T_1 (control). The work of the previous scientists also supported these results that applied Fe sulphate to improve the soil properties. Similar results were suggested by Prasad and Chakraborty (2019) who reported that P decreased with the use of Fe sulphate in soil.



Fig. 8 Effect of Iron biofortification on Phosphorus (ppm) of soil

Potassium (K) of soil (ppm)

K application in maize crop showed significant results to FeSO₄. Data about K (ppm) in maize crop displayed that iron sulphate has significant effects on K (ppm) in maize. Among all the treatments, the T_3 (seed priming with 2.0 % FeSO₄ solution) produced the maximum K (184.3 ppm) followed by the treatments T_5 (soil application of 20 kg ha⁻¹ FeSO₄) with a value of 182.25 ppm (Fig. 9). Contrary, minimum K value (133.1 ppm) was found in T_1 (control). The work of the previous scientists also supported these results who applied FeSO₄ to the soil. Similar results were suggested by Awad-Allah & Elsokkary (2020) who reported that K decreased with the use of FeSO₄ in soil.



Fig. 9 Effect of Iron biofortification on potassium (ppm) of soil

Conclusion

Findings of present research implied that application of Fe as micronutrient improved crop growth and Fe content of maize plants. Biofortification of Fe significantly improved the Fe content of maize and soil. Although application of Fe by any method gave positive results. But the mode of foliar application of Fe had the highest beneficial effects on nutritional composition in maize crop and soil than soil application and seed priming.

References

- Abbas, M., Abdel-Lattif, H., & Shahba, M. (2021). Ameliorative effects of calcium sprays on yield and grain nutritional composition of maize (*Zea mays L.*) cultivars under drought stress. *Agriculture*, 11(4), 285-298.
- Abebe, W., & Ronda, F. (2015). Flowability, moisture sorption and thermal properties of tef [Eragrostis tef (Zucc.) Trotter] grain flours. *Journal of Cereal Science*, 63, 14-20.
- Adeyeye, S., Ayofemi, O., & Idowu-Adebayo, F. (2019). Genetically modified and biofortified crops and food security in developing countries. *Nutrition & Food Science*, 49, 978-986.

- Adnan, M. (2020). Role of potassium in maize production: A review. Open Access Journal of Biogeneric Science and Research, 3(5), 1-4.
- Affinnih, K. O., Salawu, I. S., & Isah, A. S. (2014). Methods of available potassium assessment in selected soils of Kwara State, Nigeria. Agrosearch, 14(1), 76-87.
- Ahmed, M., Xu, J., & Xu, P. X. (2012). EYA1 and SIX1 drive the neuronal developmental program in cooperation with the SWI/SNF chromatin-remodeling complex and SOX2 in the mammalian inner ear. *Development*, 139(11), 1965-1977.
- Awad-Allah, E. F., & Elsokkary, I. H. (2020). Influence of potassium nutrition and exogenous organic acids on iron uptake by monocot and dicot plants. *Open Journal of Soil Science*, 10(10), 486-500.
- Awan, A. G., & Aslam, A. (2015). Impact of agriculture productivity on economic growth: A case study of Pakistan. *Global Journal of Management and Social Sciences*, 1(1), 57-71.
- Becher, B., Spath, S., & Goverman, J. 2017. Cytokine networks in neuroinflammation. *Nature Reviews Immunology*, 17(1), 49-59.
- Bjorneras, C., Škerlep, M., Floudas, D., Persson, P., & Kritzberg, E. S. (2019). High sulfate concentration enhances iron mobilization from organic soil to water. *Biogeochemistry*, 144(3), 245-259.
- Butcher, K., Wick, A. F., DeSutter, T., Chatterjee, A., & Harmon, J. (2018). Corn and soybean yield response to salinity influenced by soil texture. *Agronomy Journal*, 110 (4), 1243-1253.
- Cakmak, I., Pfeiffer, W. H. & McClafferty, B. (2010). Biofortification of durum wheat with zinc and iron. *Cereal Chemistry*, 87(1), 10-20.
- Davies, B., Coulter, J. A., & Pagliari, P. H. (2020). Timing and rate of nitrogen fertilization influence maize yield and nitrogen use efficiency. *PLOS ONE*, 15(5), e0233674. https://doi.org/10.1371/journal. pone.0233674
- FAO. The State of Food Security and Nutrition in the World 2021. In Building Climate Resilience for Food Security and Nutrition; Food and Agriculture Org.: Rome, Italy, 2021.
- Gaj, R., Budka, A., Górski, D., Borowiak, K., Wolna-Maruwka, A., & Bak, K. (2018). Magnesium and calcium distribution in maize under differentiated doses of mineral fertilization with phosphorus and potassium. *Journal of Elementology*, 23(1), 137-150.
- Gani, G., Gulsar, B., Bashir, O., Bhat, T. A., Naseer, B., Qadri, T., & Jan, N. (2018). Hidden hunger and its prevention by food processing: A review. *International Journal of Unani and Integrative Medicine*, 2, 1-10.
- Givnish, T. J., Barfuss, M. H., Van Ee, B., Riina, R., Schulte, K., Horres, R., & Sytsma, K. J. (2014). Adaptive radiation, correlated and contingent evolution, and net species diversification in

Bromeliaceae. *Molecular Phylogenetics and Evolution*, 71, 55-78.

- Grujcic, D., Yazici, A. M., Tutus, Y., Cakmak, I., & Singh, B. R. (2021). Biofortification of silage maize with zinc, iron and selenium as affected by nitrogen fertilization. *Plants*, *10*, 391-400.
- Hussain, S., Hussain, M. B., Gulzar, A., Zafar-ul-Hye, M., Aon, M., Qaswar, M., & Yaseen, R. (2017). Right time of phosphorus and zinc application to maize depends on nutrient–nutrient and nutrient–inoculum interactions. *Soil Science and Plant Nutrition*, 63(4), 351-356.
- Ishfaq, M., Wakeel, A., Shahzad, M. N., Kiran, A., & Li, X. (2021). Severity of zinc and iron malnutrition linked to low intake through a staple crop: A case study in eastcentral Pakistan. *Environmental Geochemistry & Health*, 43, 4219-4233.
- Izsáki, Z. (2017). Effect of potassium supplies on the nutritional status of maize (*Zea mays* L.). Communications in Soil Science and Plant Analysis, 48(19), 2347-2358.
- Jeong, J., & Guerinot, M. L. (2008). Biofortified and bioavailable: the gold standard for plant-based diets. *Proceedings of the National Academy of Sciences*, 105(6), 1777-1778.
- Kandil, H. (2016). Effect of ferrous sulphate with and without organic matter on growth, yield and nutrients content of Chickpea (*Cicer arietinum* L.). *International Journal of Pharm Tech Research*, 9(12), 133-138.
- Kiran, A., Wakeel, A., Mahmood, K. Mubaraka, R. Hafsa, & Haefele, S.M. (2022). Biofortification of staple crops to alleviate human malnutrition: Contributions and potential in developing countries. *Agronomy*, *12*, 452. https:// doi.org/10.3390/agronomy12020452
- Koç, E., & Karayiğit, B. (2021). Assessment of biofortification approaches used to improve micronutrient-dense plants that are a sustainable solution to combat hidden hunger. *Journal of Soil Science and Plant Nutrition*, 22(1), 475-500.
- Krishnaraj, M., Senthil, K., Shanmugasundaram, R., Prabhaharan, J., & Subramanian, E. (2020). Effect of chelated iron and zinc application on growth and productivity of maize (*Zea mays L.*) in subtropical climate. *Journal of Pharmacognosy and Phytochemistry*, 9(6), 1212-1216.
- Kumar, N., & Salakinkop, S. R. (2018). Agronomic Biofortification of Maize with Zinc and Iron Micronutrients. *Modern Concepts and Development in Agronomy*, (4), 87-90.
- Lindsay, W. L., & Norvell, W. A. (1978). Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Science Society of America Journal*, 42(3), 421-428.
- Lonnerdal, B. (2007). Trace element transport in the mammary gland. *Annual Review of Nutrition*, 27, 165-177.
- López-Millán, A. F., Grusak, M. A., Abadía, A., & Abadía, J. (2013). Iron deficiency in plants: An insight from proteomic approaches. *Frontiers in Plant Science*, 4, https://doi.org/10.3389/fpls.2013.00254

- Malakoti, M. J., & Tehrani, M. M. (1999). Effects of micronutrients on the yield and quality of agricultural products. *Tarbiat Modarres University Publications*, 22, 292-294.
- Mehta, D. C., & Dias, F. F. (1999). Maize: Perspectives and applications in India. *Starch*, *51*(2-3), 52-57.
- Morrissey, J., & Guerinot, M. L. (2009). Iron uptake and transport in plants: the good, the bad, and the ionome. *Chemical Reviews*, *109* (10), 4553-4567.
- Nishanth, S., Prasanna, R., Hossain, F., Muthusamy, V., Shivay, Y. S., & Nain, L. (2021). Interactions of microbial inoculants with soil and plant attributes for enhancing Fe and Zn biofortification in maize genotypes. *Rhizosphere*, 19, 100421. https://doi.org/10.1016/j.rhisph.2021.100421
- Olsen, S. R., Cole, C. V., Watanabe, F., & Dean, L. A. (1954). Estimation of available phosphorus in soils. Circular No. 939. United States Department of Agriculture.
- Parent, S. É., Dossou-Yovo, W., Ziadi, N., Leblanc, M., Tremblay, G., Pellerin, A., & Parent, L. E. (2020). Corn response to banded phosphorus fertilizers with or without manure application in Eastern Canada. Agronomy Journal, 112(3), 2176-2187.
- Prasad, R., & Chakraborty, D. (2019). Phosphorus Basics: Understanding Phosphorus Forms and Their Cycling in the Soil. *Alabama Cooperative Extension System*. 151, 292-315.
- Qureshi, M. T., Iqbal, N., Noorka, I. R., & Waheed, H. (2020). Bio-fortification of iron and zinc improves the biomass, uptake, distribution and yield of different maize varieties. *Journal of Plant Nutrition*, 44(1), 120-129.
- Rout, G. R., & Sahoo, S. (2015). Role of iron in plant growth and metabolism. *Reviews in Agricultural Science*, *3*, 1-24.

- Saltzman, A., Birol, E., Bouis, H. E., Boy, E., De Moura, F. F., Islam, Y., & Pfeiffer, W. H. (2013). Biofortification: progress toward a more nourishing future. *Global Food Security*, 2(1), 9-17.
- Saroj, S., Parihar, N. N., Vitnor, S. S., & Shukla, P. K. (2018). Effect of sodium nitroprusside on paddy and maize under different levels of salt stress. *Journal of Pharmacognosy* and Phytochemistry, 7, 266-270.
- Steel, R. G., Torrie, J. H., & Dickey, D. (1997). Principles and Procedures of Statistics, a Biometrical Approach. The McGraw-Hill Co., Inc. New York.
- Stewart, Z. P., Paparozzi, E. T., Djanaguiraman, M., & Shapiro, C. A. (2019). Lipid-based Fe-and Znnanoformulation is more effective in alleviating Fe-and Zn-deficiency in maize. *Journal of Plant Nutrition*, 42(14), 1693-1708.
- Stewart, Z. P., Paparozzi, E. T., Wortmann, C. S., Jha, P. K., & Shapiro, C. A. (2021). Effect of foliar micronutrients (B, Mn, Fe, Zn) on maize grain yield, micronutrient recovery, uptake, and partitioning. *Plants*, 10(3), 528; doi: 10.3390/plants10030528
- Stewart, Z. P., Paparozzi, E. T., Wortmann, C. S., Jha, P. K., & Shapiro, C. A. (2020). Foliar micronutrient application for high-yield maize. *Agronomy*, 10, 1946; doi:10.3390/agronomy10121946
- United States Department of Agriculture [USDA]. (1969). Diagnosis and improvement of saline and Alkali soils (USDA). Handbook. 60. US. Govt., Printing Office, Washington.
- Walky, A., & Black, I. A. (1934). An examination of the Degtiareff method for determining soil organic matter and proposed modification of the chromic acid titration method. *Soil Science*, 63, 29-38.
- White, P. J., & Broadley, M. R. (2009). Biofortification of crops with seven mineral elements often lacking in human diets–iron, zinc, copper, calcium, magnesium, selenium and iodine. *New Phytologist*, 182(1), 49-54.