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Enhancing Nutritional Value of Urdbean (*Vigna mungo* L. hepper) Through Agronomic Biofortification with Zinc and Iron

Anand Dinesh Jejal [a++*], Milind Dattatraya Giri [a#], Shirish Ganpat Nagoshe [a++], Yogesh Achyutrao Shaniware [b†] and Sujal Sanjay Bachhav [c‡]

[a] *Department of Agronomy, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, India.*
[b] *Department of Agricultural Botany, College of Agriculture, Dhule, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, Pin: 424004, India.*
[c] *Division of Soil Science, College of Agriculture, Pune, India.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Zn & Fe deficiencies are prevalent nutritional concerns globally, particularly in developing countries, leading to various health complications such as stunted growth, anaemia, and compromised immune function. Traditional methods of addressing these deficiencies, such as dietary supplementation, are often limited in effectiveness, especially in regions with resource constraints.

[++] *M.Sc. Scholar;*
[#] *Associate Professor;*
[†] *M.Sc. (Agriculture) Genetics and Plant Breeding;*
[‡] *M. Sc Soil Science;*
**Corresponding author: E-mail: anandjejal@gmail.com;*

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Biofortification emerges as a promising strategy to address iron and zinc deficiencies in populations with limited access to diverse diets. In this study, we investigate the efficacy of foliar applications of zinc sulphate ($ZnSO_4$) and ferrous sulphate ($FeSO_4$) at different growth stages of Urdbean (*Vigna mungo*) to enhance the concentration of these essential minerals in the grains. By utilizing agronomic practices, we aim to increase the nutritional value of urdbean grains, thereby contributing to the alleviation of malnutrition.
The study was conducted at the Research Farm of the Department of Agronomy, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. The experimental design comprised a randomized block design with three replications. Ten treatments were evaluated, including various combinations of foliar applications of $ZnSO_4$ and $FeSO_4$ at flower and pod initiation stage, along with control treatments. Foliar sprays were applied plot-wise as per the designated treatments.
Among the treatments, T7, which involved the foliar application of both $ZnSO_4$ and $FeSO_4$ at flower and pod initiation stages, exhibited the highest increase in Zn & Fe concentration in urdbean seeds. Conversely, treatment T10, which received 100% RDF showed lesser improvements in nutrient concentration compared to other treatments.
Foliar applications of $ZnSO_4$ and $FeSO_4$ at specific growth stages effectively increased the concentration of Zn & Fe in urdbean grains along with yield. Further optimization of nutrient application rates and timing may be necessary to balance nutritional enhancement with yield considerations. Biofortification through agronomic practices holds promise as a sustainable approach to addressing nutritional deficiencies and promoting food security in resource-constrained regions.

Keywords: Vigna mungo; foliar application; nutritional malnutrition; agronomic biofortification; zinc; iron; PDKV micro grade X.

1. INTRODUCTION

Micronutrient deficiencies affecting over 2 billion people globally [1], primarily stem from consuming monotonous diets low in nutrient-rich foods [2]. They are particularly common in developing nations where diets rely heavily on staple crops [3]. 43.8% of children in five Indian states have a zinc deficiency. The states with the highest prevalence of zinc deficiency are Orissa (51.3%), Uttar Pradesh (48.1%), and Gujarat (44.2%) while iron also have same concern [4]. Zn & Fe deficiencies can result in stunted physical growth, cognitive impairments, weakened immunity, metabolic issues, and increased prenatal health risks [5].

Addressing micronutrients deficiencies involves various programs aimed at enhancing vitamin and mineral intake or reducing nutrient loss from the body. Strategies encompass dietary diversification, food fortification, and supplementation. Moreover, public health initiatives such as deworming, vaccination, improved sanitation, hygiene, healthcare, and nutrition education play crucial roles in micronutrients deficiencies prevention [6,7]. Each strategy has its merits and drawbacks and should be tailored to local contexts. For instance, dietary diversification stands out as the most sustainable approach, tackling the root cause of micronutrients deficiencies. However, access and affordability of diversified foods pose challenges in resource-constrained settings [8-10]. Food biofortification offers broad impact and cost-effectiveness compared to supplementation but is limited to centrally processed foods, presenting difficulties for societies reliant on local food sources. Supplementation is preferable in severe cases for rapid improvement, despite logistical challenges [11,12-15].

Agronomic biofortification involves enhancing micronutrient content in food crops' edible parts through mineral fertilizer application (Foliar or soil applied) [16]. While this method can benefit resource-poor rural populations, ensuring access to fertilizers, In Soil, excessive use may lead to nutrient imbalance, soil and water contamination, necessitating environmental monitoring [17]. Effective agronomic biofortification requires targeting locally adapted food crops and varieties.

Urdbean (*Vigna mungo L. Hepper*) exhibits agronomic advantages, such as moisture stress tolerance, soil basicity tolerance, disease resistance urdbean has high nutritive value and consist proteins, vitamins and minerals. 100 g of urdbean contains about 24 per cent protein, 62 per cent carbohydrate, 1.7 per cent fats and is the richest among the various pulses in phosphoric acid, along with 7.2 mg Iron, 3 mg Zinc, 360 mg phosphorous & 1240 mg Potassium & being 5 to 10 time richer than others (Food Data Central) Indigenous to Asia, Urdbean ranks as the fourth most significant Pulse crop after Arhar *(Cajanas cajan*), Soybean (*Glycine max*), Mungbean (*Vigna radiata (L.) Wilczek)*. In India, the area of production of Kharif Urdbean is 32.13 m ha, with 15.07 million tons production & productivity of 469 Kg ha^{-1} (Anonymous, 2023). Hence, this study seeks to evaluate the potential impact of agronomic biofortification on Urdbean grain Zn and Fe concentrations, alongside investigating varietal and environmental factors influencing Zn and Fe biofortification responses.

2. MATERIALS AND METHODS

2.1 Site Specification and Characteristics

The study was conducted at a farm located at the Department of Agronomy, Dr. Panjabrao Deshmukh Krishi Vidyapeeth (PDKV), Akola. The coordinates of the location are approximately 20.70, 77.03, and an elevation of 286 meters above mean sea level. This area is situated in the Central Maharashtra Plateau Zone, experiencing the Kharif season from June to October. The soil under investigation had a pH value of 8.3, electrical conductivity (EC) of 0.29 dS m^{-1}, and organic carbon (OC) content of 0.59 %. Initial macronutrient concentrations in the soil were measured as 198 available Nitrogen Kg ha^{-1}, 19.60 Kg ha^{-1} Phosphorous and 396 Kg ha $^{-1}$ potassium, micronutrient concentrations in the soil were measured as 0.9 mg kg^{-1} for Zn, 4.86 mg kg^{-1} for Fe. The region has a subtropical climate characterized by hot, rainy summers and dry winters. Annual rainfall typically ranges from 700 to 900 mm, with the majority occurring between July and September, accounting for about 70% of the total precipitation. Foliar application as per treatment is applied on crop at flower and / or pod initiation stage, while RDF of 20;40;20 NPK per ha. Is applied on each crop plot where septa hydrate form of Zn & Fe is used ($ZnSO_4.7H_2O$ and $FeSO_4.7H_2O$) for spraying and Micro Grade X is also used (as shown in Table 1)

2.2 Sample Collection

Matured and dried urdbean pods were taken from each plot and the crop samples were hand threshed to produce approximately 1 kg of grain representing a sample and whole-grain samples were packed in sample envelop [18].

2.3 Sample Analysis

When the plants reached physiological maturity, they underwent manual harvesting, with samples of both grain and haulm collected for analysis. To determine the dry weight of various plant components, the samples were air-dried initially, followed by oven drying at 65 °C for 48 hours. Subsequently, a mechanical grinder was employed to finely powder the oven-dried plant samples. For digestion, 1.0 g of haulm and 0.5 g of grain samples were taken and subjected to a mixture of di-acids (HNO_3 and $HClO_4$) in a 3:1 ratio on an electric hot plate. The digested extracts were then analysed for micronutrient content, including Fe & Zn using an atomic absorption spectrophotometer (Model AAS 240 FS, Company Varian, Germany) [19]. For protein First Nitrogen is extracted by using Kjeldahl method from seeds and the Nitrogen (%) is multiple by 6.25 for protein.

3. RESULTS

3.1 Impact of Foliar Application of Zn and Fe on Seed and Haulm Yield of Urdbean

The study evaluated ten different treatments (T1 to T10) to assess their impact on seed and haulm yields in a crop. Among these treatments, T7 showed superior results with the highest seed yield with a seed yield of 1462 Kg ha^{-1} and haulm yield of 2875 Kg ha^{-1} which is at par with T6 with seed yield of 1459 Kg ha^{-1} and haulm yield of 2873 Kg ha^{-1}, followed closely by T5 with a seed yield of 1436 Kg ha^{-1} and haulm yield of 2838 Kg ha^{-1}. Conversely, T9, which reduced the fertilizer dose to 75% of RDF while combining $ZnSO_4$ and $FeSO_4$ at both flowering initiation (FI) and pod initiation (PI) stages, showed inferior results with a seed yield of 1310 Kg ha^{-1} and a haulm yield of 2295 Kg ha^{-1}. These findings demonstrate the varying efficacy of different treatments in influencing seed and haulm yields in the crop, providing valuable insights for agricultural practices.

3.2 Impact of Foliar Application of Zn and Fe on Concentration in Grain and Haulm of Urdbean

In this study, the impact of ten different treatments (T1 to T10) on iron (Fe) and zinc (Zn) concentrations in seeds and haulms, along with the protein content in seeds, was investigated.

Among the treatments, T7 exhibited the most significant enhancement in nutrient

Table 1. Treatments details

Symbol	Details
T1	RDF + Foliar application of 0.50% $ZnSO_4$ at flower initiation
T2	RDF + Foliar application of 0.50% $FeSO_4$ at flower initiation
T3	RDF + Foliar application of 0.50% $ZnSO_4$ at pod initiation
T4	RDF + Foliar application of 0.50% $FeSO_4$ at pod initiation
T5	RDF + Foliar application of 0.50% $ZnSO_4$ + 0. 50% $FeSO_4$ at flower initiation
T6	RDF + Foliar application of 0.50% $ZnSO_4$ + 0. 50% $FeSO_4$ at pod initiation
T7	RDF + Foliar application of 0.50% $ZnSO_4$ + 0.50% $FeSO_4$ at flower and pod initiation
T8	RDF + Foliar application of "PDKV Liquid Micro Grade X" at flower and pod initiation
T9	75 % RDF + Foliar application of 0.50% $ZnSO_4$ + 0.50% $FeSO_4$ at flower and pod initiation
T10	RDF (Control)

Table 2. Effect of different foliar application of yield of Urdbean

Symbol	Treatment Details	Seed Yield (Kg ha^{-1})	Haulm Yield (Kg ha^{-1})
T1	RDF + FA of 0.50% $ZnSO_4$ at FI	2854	4285
T2	RDF + FA of 0.50% $FeSO_4$ at FI	2839	4254
T3	RDF + FA of 0.50% $ZnSO_4$ at PI	2853	4276
T4	RDF + FA of 0.50% $FeSO_4$ at PI	2844	4265
T5	RDF + FA of 0.50% $ZnSO_4$ + 0. 50% $FeSO_4$ at FI	2868	4304
T6	RDF + FA of 0.50% $ZnSO_4$ + 0. 50% $FeSO_4$ at PI	2873	4333
T7	RDF + FA of 0.50% $ZnSO_4$ + 0.50% $FeSO_4$ at FI & PI	2875	4337
T8	RDF + FA of "PDKV Liquid Micro Grade X" at FI & PI	2837	4222
T9	75 % RDF + FA of 0.50% $ZnSO_4$ + 0.50% $FeSO_4$ at FI and PI	2295	3575
T10	RDF (Control)	2760	4085
	S.E. (m)+	166	216
	CD (P=0.05)	NS	NS
	CV (%)	10.34	8.95
	GM	2790	4194

Table 3. Effect of different foliar application on nutritional value of urdbean crop

Symbol	Treatment Details	Zn Concentration in Seed (mg Kg^{-1})	Zn concentration in haulm (mg Kg^{-1})	Fe Concentration in Seed (mg Kg^{-1})	Fe concentration in haulm (mg Kg^{-1})	Protein content in seed (%)
T1	RDF + FA of 0.50% $ZnSO_4$ at FI	27.97	33.47	153.67	230.50	23.12
T2	RDF + FA of 0.50% $FeSO_4$ at FI	27.71	33.21	158.67	238.00	22.20
T3	RDF + FA of 0.50% $ZnSO_4$ at PI	27.80	33.30	147.00	220.50	22.75
T4	RDF + FA of 0.50% $FeSO_4$ at PI	25.84	31.34	155.67	233.50	22.62
T5	RDF + FA of 0.50% $ZnSO_4$ + 0. 50% $FeSO_4$ at FI	33.58	39.08	166.00	249.00	23.37
T6	RDF + FA of 0.50% $ZnSO_4$ + 0. 50% $FeSO_4$ at PI	34.80	40.30	168.00	252.00	23.67
T7	RDF + FA of 0.50% $ZnSO_4$ + 0.50% $FeSO_4$ at FI & PI	35.66	41.16	169.67	254.50	23.88
T8	RDF + FA of "PDKV Liquid Micro Grade X" at FI & PI	25.71	31.21	145.18	217.76	21.91
T9	75 % RDF + FA of 0.50% $ZnSO_4$ + 0.50% $FeSO_4$ at FI and PI	24.98	30.48	144.77	217.15	19.58
T10	RDF (Control)	23.79	29.29	143.33	215.00	20.58
	S.E. (m)+	0.42	0.42	2.28	3.42	0.31
	CD (P=0.05)	1.24	1.24	6.78	10.17	0.91
	CV (%)	2.51	2.11	2.55	2.55	2.36
	GM	28.78	34.28	155.59	232.79	22.37

Where, FA: Foliar Application, FI: Flower Initiation, PI: Pod Initiation, RDF: Recommended dose of fertilizer concentrations, with Fe concentrations of 169.50 mg/kg in seeds and 254.61 mg/kg in haulms, and Zn concentrations of 35.12 mg/kg in seeds and 41.40 mg/kg in haulms. Additionally, T7 showed the highest protein content in seeds at 23.25%. Following closely, T6 also demonstrated notable improvements, particularly in Fe and Zn concentrations in both seeds and haulms, with protein content in seeds reaching 22.62%.

Conversely, T9, which reduced the fertilizer dose to 75% of the Recommended Dose of Fertilizer (RDF) while combining ZnSO4 and FeSO4 at both flowering initiation (FI) and pod initiation (PI) stages, displayed inferior results, exhibiting decreased Fe and Zn concentrations in seeds and haulms, along with a reduced protein content in seeds. These findings highlight the efficacy of different treatments in enhancing nutrient concentrations and protein content in seeds, providing valuable insights for agricultural practices aimed at improving crop quality and nutritional value.

4. DISCUSSION

The study findings underscored the effectiveness of biofortification in augmenting micronutrient concentrations via foliar application of Zn & Fe in urdbean. Detailed discussions on the outcomes of various parameters are presented in subsequent sections.

4.1 Grain and Haulm Yield with Zn and Fe Application

The foliar application of $ZnSO_4$. $7H_2O$ (0.5%) + $FeSO_4$. $7H_2O$ (0.5%) demonstrated efficacy in improving both grain and straw yield in urdbean, potentially due to synergistic interactions among zinc (Zn), and iron (Fe). [20] Zn foliar application may have contributed to increased yield by supporting photosynthesis, cell division, protein synthesis, membrane structure retention, and resistance against pathogens. Moreover, the role of Zn in carbohydrate, lipid, protein, and nucleic acid synthesis, as well as chlorophyll formation, likely contributed to improved crop performance [21,22,23,24]. Additionally, foliar application of Fe contributed to increased grain and straw yield by enhancing carbohydrate and protein synthesis, photosynthesis rate, growth promoter synthesis, seed maturation, nucleic acid metabolism, and chlorophyll synthesis. The translocation of photosynthates in reproductive structures facilitated by Fe foliar spray further led to increased effective branching, test weight, and ultimately grain and straw yield in urdbean [25,26].

Furthermore, the application of double micronutrients exhibited superior grain and straw yield compared to single micronutrient applications, likely due to synergistic interactions among Zn, and Fe. This finding is supported by treatment T7, which utilized all two micronutrients at both stage (Flower and pod initiation) and showed the highest grain and straw yield compared to treatments involving only one micronutrient (T1, T2, T3 and T4) (Ismal, 2017 and Ismal and Alam, 2016). Consistent with these results, previous studies have indicated that joint application of Zn and Fe has a greater impact on urdbean yield compared to their individual application. Additionally, research by Ali *et al.* highlighted that combining Zn results in higher seed yield compared to sole applications of Fe or Zn in rice crop.

4.2 Zn & Fe Concentration in Urdbean

The sole and combined application of Zn and Fe led to the increase in micronutrient concentration in urdbean grain and haulm as compared to the control which might be due to the immediate absorption of available micronutrients by plant leaves [27]. Foliar application of Zn enhanced grain and haulm Zn concentrations which is an outstanding method to produce grains with an adequate quantity of Zn. This approach would surely help in reducing malnutrition owing to Zn deficiency. A study demonstrated the potential of Zn in enhancing its concentration in the grain of Mungbean [28]. Similar results were observed for the concentration of Fe in grain and haulm of Mungbean [29]. Increased Fe concentration in haulm in comparison to grain might be associated with the presence of Fe storage proteins and non-heme proteins, which possess a good binding capacity for Fe. So, combined Zn and Fe application in the present study exhibited a positive influence on Zn and Fe content of urdbean grain and haulm thus it can be inferred that Zn and Fe possess a similar mechanism for translocation to grains [30]. The enhancement in nutrient content might be due to an increased absorption as well as assimilation of the micronutrients that resulted in balanced nutritional value in the crop for higher growth and thereby higher nutrient content [31].

5. CONCLUSIONS

Zn & Fe are vital micronutrients crucial for human health. Urdbean, as a key short-duration legume crop, holds the potential to enhance both productivity and nutrient quality through biofortification methods. This study elucidated the impact of supplementing Zn and Fe using ZnSO4.7H2O and FeSO4.7H2O on the yield and quality of urdbean. The combined foliar application of ZnSO4.7H2O (0.5%) + FeSO4.7H2O (0.5%) notably increased micronutrient concentration and uptake in urdbean. Among individual micronutrient applications, FeSO4.7H2O (0.5%) treatment exhibited superior outcomes compared to treatments involving ZnSO4.7H2O and Micro Grade X alone. These findings underscore the effectiveness of biofortification through the combined application of ZnSO4.7H2O (0.5%) + FeSO4.7H2O (0.5%) in enhancing the nutritional quality and economic returns of urdbean.

6. RECCOMENDATIONS & IMPLICATIONS

The fortification of seeds with agronomic biofortification techniques has yielded promising results in enhancing the Zn & Fe content in both seed and straw of urdbean. These findings suggest that implementing such strategies in farmer fields could significantly improve the nutritional quality of urdbean seeds and straw, consequently benefiting both human and animal health. Further studies are recommended to thoroughly investigate the effects of these interventions on human and animal health outcomes.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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