Foliar Nutrient Supplementation with Micronutrient-Embedded Fertilizer Increases Biofortification in Eggplant Fruit and Soil Biological Activity While Enhancing Plant Productivity

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Abstract

Micronutrient malnutrition or hidden hunger remains a major global challenge for human health and wellness. The problem results from soil micro- and macro-nutrient deficiencies combined with imbalanced fertilizer use. Micronutrient-embedded NPK (MNENPK) complex fertilizers have been developed to overcome the macro- and micro-element deficiencies to enhance the yield and nutritive value of key crop products. We investigated the effect of foliar applications of an MNENPK fertilizer containing N, P, K, Fe, Zn & B in combination with traditional basal NPK fertilizers in terms of eggplant yield, fruit nutritive quality and on soil biological properties. Applying a multi-element foliar fertilizer improved the nutritional quality of eggplant fruit, with a significant increases in the concentration of Fe (+26%), Zn (+34%), K (+6%), Cu (+24%), and Mn (+27%), all of which are essential for human health. Increasing supply of essential micronutrients during the plant reproductive stages increased fruit yield, as a result of improved yield parameters. The positive effect of foliar fertilizing with MNENPK on soil biological parameters (soil microbial biomass carbon, dehydrogenase, alkaline phosphatase) also demonstrated its capacity to enhance soil fertility. This study suggests that foliar fertilizing with a multi-nutrient product such as MNENPK at eggplant flowering and fruiting stages, combined with the recommended-doses of NPK fertilizers is the optimal strategy to improve the nutritional quality of eggplant fruits and increase crop yields, both of which will contribute to reducing micronutrient malnutrition and hunger globally.

Introduction

Eggplant (*Solanum melongena L.*) or brinjal is the fifth most important vegetable crop globally[1–2], grown on approximately 1.86 million hectares of land, with annual global production of around 54.1 million tonnes worth over USD 10 billion[2]. While eggplant is produced across Asia and Europe and in many African nations[3], of the total global eggplant production China and India are the major producers, generating 61 % and 23 %, respectively, of the total annual yield[2]. The average eggplant-fruit yield in South and East Asia is far below the potential productivity, primarily as a result of abiotic and biotic plant stressors and poor awareness of the correct nutrition and other management practices among farmers[4]. Eggplant is a high nutrient-exhausting crop, as a result of its high biomass production and long-growing season. A 60 t ha^{-1} eggplant crop removes from the soil approximately 190 kg ha^{-1} nitrogen (N), 10.9 kg ha^{-1} phosphorus (P), and 128 kg ha^{-1} potassium (K), as well as significant amounts of micronutrients[5]. Most eggplant farmers apply only primary-nutrient fertilizers which leads to low and variable crop yields and sub-optimal nutrient concentrations in the fruit. The ongoing use of primary-nutrient fertilizers combined with limited use of organic or micronutrient fertilizers has led to multi-nutrient deficiencies in the majority of soils in the Indian subcontinent [6–7]. Across India, approximately 89, 80 and 50 % of the arable soils are deficient in N, P and K, respectively. Further, zinc (Zn), boron (B), iron (Fe), manganese (Mn), molybdenum (Mo), and copper (Cu) deficiencies have been reported in around 40, 33, 12, 5, 11 and 3 % of Indian soils, respectively[7–9]. Similarly, up to 51 % of the arable soils in China are deficient in Zn, with deficiencies in Mo, N, Mn, Cu and Fe in 47, 34.5, 21, 7 and 5 % of farmland soils, respectively, with large macronutrient (i.e. N, P, K) deficiencies also[10]. Considering the widespread multi-nutrient deficiencies in soils on which eggplants are grown, efficient nutrient management strategies to overcome these deficiencies are vitally important[7].

Modern hybrid, high-yielding eggplant varieties are more responsive to applied fertilizers than traditional varieties. Such varieties have high production potential, they require regular fertilization during both vegetative and reproductive stages[11]. Water-soluble multi-nutrient fertilizers could provide sufficient nutrients to the plant throughout its growing season and could reduce flower and fruit drop, thus improving crop yield and quality[12].

Additionally, crop cultivation in nutrient-deficient soils results in foods with low nutrient concentrations, particularly of micronutrients, which contributes to malnutrition and hidden hunger in many emerging-economy countries[13–14]. Globally, micronutrient malnutrition, arising either from inadequate consumption of fruits and vegetables or from consumption of foods which are low or deficient in essential micronutrients, results in approximately 1.7 million deaths annually[7,15,16]. Eating foods which have been biofortified to increase their micronutrient content is a useful pathway to overcome malnutrition for many[7,13].

In order to reduce malnutrition it is imperative to supply crops with micronutrients in addition to the macronutrients required for plant growth. The efficiency of inorganic micronutrients applied into soils is low as they become easily fixed to soil particles[17]. Applying a foliar spray of micronutrient fertilizers is an effective option to enhance plant nutrient-use efficiency (NUE)[18–12]. The recent innovation of micronutrient-embedded NPK fertilizers (MNENPK) enables growers to cater to the specific multi-micronutrient demands of individual crops at specific plant growth stages. Until now, only single- or two-nutrient foliar fertilizers have been tested for their efficacy in eggplant biofortification and yield improvement. As well, there have been no systematic field trials conducted on foliar applications of MNENPK fertilizer in eggplant crop and the subsequent effects on soil health and enzyme activity, and there is little information available on partitioning of key macro- and micro-nutrients when fertilized with MNENPK in different plant parts. The current investigation was conducted to (1) test the effectiveness of MNENPK fertilizers on eggplant growth and yield, (2) quantify the nutrient biofortification of different eggplant parts (including fruit) under MNENPK fertilizers, and (3) document soil biological activity under diverse fertility scenarios, in a south Asian semi-arid agro-ecology.

Results

Plant and fruit growth parameters and fruit yield

Of the main-plot NPK fertilizer treatments, RDF3 produced the tallest plants (54.8 cm) with the highest number of branches per plant (8.8), LAI (6.3), number of flowers per cluster (5.8), fruit length (16.7 cm), number of fruits per plant (9) and fruit yield (29.0 t/ha). All parameters were significantly higher under RDF3 treatments than under RDF2 and RDF1 treatments (Fig. 1a, 1b and 1c). Plant growth and fruit yield were lowest under RDF1, with plant height of 43.5

cm, number of branches per plant at 5.9, LAI at 4.6, number of flowers per cluster at 3.7, fruit length at 13.7 cm, number of fruits per plant at 4.46 and fruit yield at 11.81 t/ha.

The treatment MNEPK1 had the lowest values for all plant and fruit growth parameters, and fruit yield (Fig. 1a, 1b and 1c).

There were no statistical differences ($p>0.05$) between treatments MNEPK1 and MNEPK2 in terms of plant height (48.3 and 50.4 cm, respectively), number of branches per plant (7.22 and 7.52, respectively) and number of fruits per plant (6.17 and 6.91, respectively). These later two treatments were significantly ($p<0.05$) different in terms of LAI (5.31 and 5.58, respectively), number of flowers per cluster (4.55 and 4.89, respectively), fruit length (14.43 and 14.91 cm, respectively) and fruit yield (18.21 and 20.40 t/ha, respectively). Treatment MNENPK4 had the greatest plant height (51.7 cm), number of branches per plant (8.22), LAI (5.85), number of flowers per cluster (5.24), fruit length (15.83 cm), number of fruits per plant (7.83) and fruit yield (24.19 t/ha), however treatment MNENPK4 did not differ statistically ($p>0.05$) from treatment MNENPK3 in terms of all growth parameters except LAI and fruit length.

Soil Fertility Status

Plant-available NPK in the RDF1 treatment were 141.3, 12.1 and 206.3 kg ha^{-1}, respectively; this increased in the RDF2 (171.1, 13.3 and 237.5 kg ha^{-1}, respectively), and the RDF3 (182.2, 13.9 and 259.2 kg ha^{-1}, respectively) treatments. There was no significant difference between the MNENPK foliar-supplementation treatments in terms of plant-available N or K, however the $MNENPK_3$ (14.2 kg ha^{-1}) and $MNENPK_4$ (14.6 kg ha^{-1}) treatments were higher in plant-available P than the $MNENPK_1$ (12.9 kg ha^{-1}) treatment (Table 1).

Table 1
Effect of diverse fertility scenarios and MNENPK treatments on soil after two eggplant crops

Treatment	Available N (kg ha^{-1})	Available P (kg ha^{-1})	Available K (kg ha^{-1})
Diverse fertility scenario			
RDF_1	141.3_c	12.1_c	202.3_c
RDF_2	175.1_b	13.8_b	238.8_b
RDF_3	182.2_a	15.5_c	263.2_a
CD (p=0.05)	9.88	1.21	21.6
Micronutrient embedded NPK (MNENPK) fertilizer			
$MNENPK_1$	164.6_a	12.9_c	224.1_a
$MNENPK_2$	159.6_a	13.6_{bc}	233.7_a
$MNENPK_3$	170.1_a	14.2_{ab}	239.3_a
$MNENPK_4$	167.0_a	14.6_a	242.2_a
LSD (p=0.05)	NS	0.97	NS
Interaction RDF×MNENPK	NS	NS	NS

*Means followed by a similar lowercase letter within a column are not significantly different at $p < 0.05$ according to Tukey's HSD test; NS = non-significant; data are pooled means of 2017-18 and 2018-19 cropping seasons

Soil Microbial Parameters

Soil alkaline phosphatase, acid phosphatase activity and soil microbial biomass carbon (SMBC) varied significantly ($p<0.05$) under different NPK treatments (Table 2). There was no significant difference ($p>0.05$) of different NPK fertilizer treatments on urease activity. Under the RDF3 treatment, soil alkaline phosphatase (14.31 µg PNP g $soil^{-1}$ h^{-1}), acid phosphatase activity (4.60 µg PNP g $soil^{-1}$ h^{-1}) and SMBC (524 µg g $soil^{-1}$) were highest, with lower levels of soil alkaline phosphatase (13.74 and 13.44 µg PNP g $soil^{-1}$ h^{-1}), acid phosphatase activity (4.54 and 4.14 µg PNP g $soil^{-1}$ h^{-1}) and SMBC (509.8 and 465.9 µg g $soil^{-1}$) in the RDF2 and RDF1 treatments, respectively. The lowest enzyme activity and SMBC occurred in the RDF1 treatment.

The effect of the MNENPK foliar-supplementation treatments was significant ($p<0.05$) in terms of SMBC and all enzyme activity examined. Highest levels of soil dehydrogenase (3.41 µg TPF g $soil^{-1}$ d^{-1}), alkaline phosphatase (14.87 µg PNP g $soil^{-1}$ h^{-1}), acid phosphatase (4.85 µg PNP g $soil^{-1}$ h^{-1}) and SMBC (543.8 µg g $soil^{-1}$) were recorded under the MNENPK4 treatment. Urease activity was significantly higher (18.4 µmoles ammonia g^{-1} h^{-1}) in the MNENPK2 and MNENPK3 treatments compared to the MNENPK1 treatment (17.2 µmoles ammonia g^{-1} h^{-1}). The lowest rates of dehydrogenase (2.79 µg TPF g^{-1} soil

d^{-1}), alkaline phosphatase (12.69µg PNP g $soil^{-1}$ h^{-1}), acid phosphatase (3.92 µg PNP g $soil^{-1}$ h^{-1}), urease (17.2 µmoles ammonia g^{-1} h^{-1}) and SMBC (454.3 µg g $soil^{-1}$) were observed in treatment MNENPK1, which were significantly lower than in all other MNENPK treatments.

Table 2
Effect of diverse fertility scenarios and multi-micronutrient foliar fertilization on soil microbial activities of eggplant

Treatment	Dehydrogenase (µg TPF g $soil^{-1}$ d^{-1})	Alkaline phosphatase (µg PNP g $soil^{-1}$ h^{-1})	Acid phosphatase (µg PNP g $soil^{-1}$ h^{-1})	Urease (µmole ammonia g^{-1} h^{-1})	SMBC (µg g $soil^{-1}$)
Diverse fertility scenario					
RDF_1	3.09_c	13.44_c	4.14_c	17.50_a	465.9_b
RDF_2	3.19_b	13.74_b	4.54_b	18.7_a	509.8_b
RDF_3	3.25_a	14.31_a	4.60_a	17.8_a	524.0_a
LSD (p=0.05)	0.08	0.61	0.28	NS	29.48
Micronutrient embedded NPK (MNENPK) fertilizer					
$MNENPK_1$	2.79_d	12.69_{cd}	3.92_d	17.2_c	454.3_d
$MNENPK_2$	3.16_c	13.29_{bcd}	4.28_{bc}	18.4_{abc}	485.3_c
$MNENPK_3$	3.36_b	14.47_{abc}	4.64_{abc}	18.4_{ab}	516.2_b
$MNENPK_4$	3.41_a	14.87_{ab}	4.85_{ab}	17.9_{ab}	543.8_a
LSD (p=0.05)	0.07	0.48	0.24	1.28	18.1

*Means followed by a similar lowercase letter within a column are not significantly different at $p < 0.05$ according to Tukey's HSD test; data are pooled means of 2017-18 and 2018-19 cropping seasons. SMBC=soil microbial biomass carbon

Micronutrient Concentrations In Fruit, Shoots And Leaves

Micronutrient concentrations in eggplant fruit, shoots and leaves increased with greater applications of NPK fertilizer and of MNENPK foliar-supplementation (Table 3). The concentration of Cu in the RDF1 treatment was 0.44, 0.53 and 0.47 mg kg^{-1} in the fruit, shoots and leaves, respectively. This increased to 0.59, 0.75 and 0.68 mg kg^{-1}, respectively, in the RDF3 treatment. Similarly, concentrations of Fe, Zn and Mn in the fruit, shoots and leaves increased with increasing fertilizer concentration from the RDF1 treatment to the RDF3 treatment (Table 3).

The MNENPK foliar-supplementation significantly improved the concentration of micronutrients in the plant parts. The lowest concentrations of Cu (0.46, 0.54 and 0.48 mg kg^{-1}), Fe (2.76, 3.41 and 2.76 mg kg^{-1}), Zn (1.16, 1.48 and 1.19 mg kg^{-1}) and Mn (2.80, 3.43 and 2.97 mg kg^{-1}) were observed in the fruits, shoots and leaves, respectively, of plants in the MNENPK1 treatment. The highest concentrations of Cu (0.57, 0.73 and 0.67 mg kg^{-1}), Fe (3.49, 4.11 and 3.49 mg kg^{-1}), Zn (1.55, 2.01. and 1.63 mg kg^{-1}) and Mn (3.55, 4.12 and 3.75 mg kg^{-1}) were observed in the fruit, shoots and leaves, respectively, of plants in the MNENPK4 treatment. There were no statistical differences in micronutrient concentrations between treatments MNENPK3 and MNENPK4.

A significant ($p>0.05$) interaction effect between NPK and MNENPK treatments was observed between different micronutrients (Fig. 2a &2b). The highest concentrations in eggplant fruit of Cu (0.673 mg kg^{-1}), Fe (4.25 mg kg^{-1}), Zn (1.83 mg kg^{-1}) and Mn (4.21 mg kg^{-1}) were observed in the RDF3-MNENPK4 treatment; although there was no statistical difference between this and the RDF3-MNENPK3 treatment. Increasing the application rate of the multi-nutrient fertilizer from the MNENPK1 treatment (0 kg MNENPK ha^{-1}) to the MNENPK4 treatment (0.75 kg MNENPK ha^{-1}), while retaining the NPK fertilizer application rate at RDF1 (0 kg NPK ha^{-1}) did not result in an increase in micronutrient concentration in eggplant fruit. However, applying the MNENPK fertilizer under RDF2 (75% of RDF) and RDF3 (100% of RDF) did result in increased micronutrient concentrations in eggplant fruit.

K Concentration In Fruit, Shoots And Leaves

The concentration of K in eggplant fruit, shoots and leaves increased significantly with increasing K under higher fertilizer applications (Table 3). The highest K content was observed in fruit (0.26%), shoots (0.28%) and leave (0.26%) under the RDF3 treatment, although there was no statistical difference between the K concentrations in shoots and leaves in the RDF2 and RDF3 treatments. The lowest K contents were observed under the RDF1 treatment (0.21, 0.22 and 0.21% for fruit, shoots and leaves, respectively), significantly lower than in the RDF2 and RDF3 treatments.

K concentration in fruit, shoots and leaves increased significantly ($p>0.05$) with increasing applications of the MNENPK fertilizer, with maximum concentrations observed in the MNENPK4 treatment (Table 3).

Table 3
Effect of diverse fertility scenarios and multi-micronutrient foliar fertilization on micronutrient concentrations in eggplant fruit, shoots and leaves

Treatment	Cu (mg kg^{-1})			Fe (mg kg^{-1})			Zn (mg kg^{-1})			Mn (mg kg^{-1})			K (%)		
	Fruit	Shoot	Leaf	Fruit	Shoot	Leaf	Fruit	Shoot	Leaf	Fruit	Shoot	Leaf	Fruit	Shoot	Leaf
Diverse fertility scenario															
RDF_1	0.44_c	0.53_c	0.47_c	2.45_b	3.17_b	2.55_c	1.13_c	1.45_c	1.19_b	2.57_c	3.20_c	2.74_c	0.209_c	0.215_b	0.213_b
RDF_2	0.52_b	0.65_b	0.60_b	3.27_b	3.88_b	3.37_b	1.46_b	1.83_b	1.56_a	3.38_b	3.89_b	3.57_b	0.242_b	0.252_a	0.251_a
RDF_3	0.59_a	0.75_a	0.68_a	3.78_a	4.33_a	3.93_a	1.64_a	2.03_a	1.61_a	3.77_a	4.33_a	3.97_a	0.264_a	0.275_a	0.262_a
CD (*p=0.05*)	0.05	0.06	0.06	0.28	0.44	0.35	0.12	0.17	0.14	0.29	0.39	0.28	0.021	0.025	0.022
Micronutrient embedded NPK (MNENPK) fertilizer															
$MNENPK_1$	0.46_c	0.54_c	0.48_c	2.76_c	3.41_c	2.76_c	1.16_c	1.48_d	1.19_c	2.80_c	3.43_c	2.97_c	0.231_a	0.237_a	0.234_a
$MNENPK_2$	0.50_b	0.62_b	0.56_b	3.11_b	3.71_b	3.11_b	1.39_b	1.71_c	1.43_b	3.17_b	3.73_b	3.36_b	0.246_a	0.245_a	0.238_a
$MNENPK_3$	0.55_a	0.68_a	0.63_a	3.31_{ab}	3.94_{ab}	3.31_{ab}	1.52_{ab}	1.88_b	1.57_a	3.44_a	3.95_{ab}	3.64_a	0.241_a	0.251_a	0.244_a
$MNENPK_4$	0.57_a	0.73_a	0.67_a	3.49_a	4.11_a	3.49_a	1.55_a	2.01_a	1.63_a	3.55_a	4.12_a	3.75_a	0.245_a	0.257_a	0.248_a
CD (*p=0.05*)	0.03	0.05	0.04	0.21	0.25	0.21	0.09	0.11	0.09	0.21	0.24	0.21	NS	NS	NS

*Means followed by a similar lowercase letter within a column are not significantly different at $p < 0.05$ according to Tukey's HSD test; data are pooled means of 2017-18 and 2018-19 cropping season

GGE Biplot Analysis

GGE bioplot analysis was undertaken on concentrations of the micronutrients Zn, Mn, Cu and Fe and the macronutrient K in eggplant fruit, shoots and leaves. In the GGE analysis 12 treatment-combination effects and five test environments (i.e. the four micronutrients and K) were examined under the different experimental treatments. A GGE analysis of eggplant growth traits in terms of their performance under the experimental treatments was also undertaken (Fig. 3).

GGE biplot for nutrient concentration in eggplant

In the GGE biplot analysis of micronutrients in eggplant fruit, the first two principal components (PC) explained 98.2% and 1.8% of variation, respectively. Similarly, in the GGE biplots of nutrient concentrations in eggplant shoots and leaves the first two PCs explained 97.9% and 95.8%, and 2.1% and 3.3% of variation, respectively. The 'which won where/what' polygon shows the two-dimensional view of multiple environments, exhibiting the best treatment across the environments and also assists in identifying the interaction pattern between treatments, years and traits (Fig. 4a). Rays divide the polygon into sectors[23]. The polygon shows that T12 (RDF_3-$MNENPK_4$) has the highest concentrations in eggplant fruit of Cu (0.67 mg kg^{-1}), Fe (4.25 mg kg^{-1}), Zn (1.83 mg kg^{-1}) and Mn (4.21 mg kg^{-1}), closely followed by T11 (RDF_3-$MNENPK_3$).

Treatments T10, T11, and T12 are positioned in the same mega environment and are relatively close to each other, indicating that the RDF_3-$MNENPK_3$ and RDF_3-$MNENPK_4$ fertilizer applications led to the highest Cu concentration in eggplant fruits. Similar trends in nutrient concentrations were observed in shoots (Fig. 5a) and leaves (Fig. 6a).

The mean vs. stability

GGE biplots assist in identifying the treatment with the highest micronutrient concentration in fruit and the best stability. The ideal nutrient concentration and greater treatment stability are determined by a biplot's average environment coordinate (AEC). The normal lines to the AEC which pass via through origin of the biplot are the "AEC ordinate" (Fig. 4b). In both directions of the AEC ordinate, points which are further away from the origin have lower stability and higher treatment by environment (T × E) interactions. The instability of any treatment is directly proportional to the absolute length of the projection on the AEC[24]. Treatments T10, T11, and T12 were observed to be ideal treatments in terms of nutrient concentration and stability.

Ranking experimental treatments

Ranking treatments within GGE biplots are used to determine the order of efficiency of the treatments (Fig. 4c). The most efficient treatment combination is one which results in the highest nutrient fortification and greatest stability across the test environments; this is placed at the centre of the concentric circles. Treatments closer to the concentric circles of the 'ideal treatment' have higher mean nutrient biofortifications and higher stability for nutrient accumulation. Of the various treatment combinations, the best-ranking treatments were T12 > T11 in the inner orbit, followed by T10 > T8 in the second orbit, > T7 > T9 >T6. Treatments T1 to T4 were within the two outermost circles, indicating their relatively lower performance in terms of nutrient accumulation in eggplant fruit. Fig. 4d, 5b, 6b rank the nutrient concentrations relative to the 'model environment' depicted by the smallest circle on the AEC axis. A nutrient closer to the intersection of the straight lines has a superior ranking, whereas those farther from the intersection have lower rankings. Nutrient concentrations in eggplant fruit were ranked from Cu ≈ Fe >Mn> Zn > K, while in eggplant shoots concentrations were Mn=Fe > Cu >Zn> K (Fig. 4b). In Figure 4b, Mn and Fe are located closer to the circles while other nutrients are further away. Nutrient concentrations in eggplant leaves were Mn> Fe>Cu >Zn>K. Excepting Mn, the remaining nutrients lay outside and away from the concentric circles (Fig. 5b).

GGE biplot for growth and yield parameters

For GGE biplot analysis of micronutrients in eggplant fruit, the first two principal components (PC) explained 99.5% and 0.5% of the variation, respectively. The 'which won where/what' polygon indicated that the treatments T12 (RDF3-MNENPK4) and T11 (RDF3-MNENPK3) had the highest growth and yield parameters (Fig. 3a). The presence of all growth parameters and crop yield in the same mega-environment indicates that all these parameters follow a uniform pattern of performance in these treatments. Treatments T10, T9, T8 and T7 were the second-best group of treatments in terms of crop yield and plant growth. The mean vs. stability biplots indicated that treatments T8 and T9, followed by treatments T10, T12 and T11 had the highest stability in terms of eggplant growth and yield performance. Similarly, fruit yield was the most stable trait under different treatment combinations, followed by flowers per cluster and fruits per plant (Fig. 3b). Fruit length and number of branches plant were the least stable traits. The ranking-treatments graph shows the performance order of various experimental treatments. The order for growth and yield was T12>T11>T10>T8>T9>T7; all these treatments were in the same convex hull (Fig. 3c). Treatments T1 to T5 performed relatively poorly in terms of growth and yield. The ranking environments biplot (Fig. 3d) indicated that fruit yield was the most stable trait. The number of fruits per plant, number of branches per plant and the number of flowers per cluster were in the same orbit and this group was the second-most-stable trait group. The LAI was lying furthest from the concentric circle and was the least stable trait.

Discussion

The supply of essential plant nutrients in optimum proportions at different growth stages is vital to increase crop yield and nutrient-use efficiency[25–27]. Optimal nutrient supply is particularly important in crops like eggplant with a heavy nutrient demand[28]. In this experiment, the balanced supply of essential NPK macronutrients supplemented by micronutrients applied as foliar spray at critical growth stages significantly improved eggplant yield and growth parameters[29–30].

Foliar application of nutrients, especially micronutrients at later crop stages is of prime importance in enhancing the crop yields and increasing the use efficiency of micronutrients[31]. Moreover, absorption of foliar-applied nutrients is much faster than those applied into the soil[18–32]. During reproductive stages, roots are less efficient and nutrients are transported from leaves to grain or fruit[33]. Therefore, foliar feeding at fruit-development stages will supplement the amount of nutrients required to be extracted from plant leaves.

Supply of macro-nutrients like N, P and K in optimal proportion is required for proper plant growth, to reduce flower and fruit drop, and for the development of effective rooting systems which will facilitate absorption of soil nutrients[34–35]. In this research, soil applications of the RDF combined with later stage foliar fertilizing with multi-nutrient fertilizer increased the nutrient concentrations in fruits, shoots and leaves: this improved uptake of micronutrients, facilitated by well-grown plants which were a consequence of well-developed root systems resulting from ensured macronutrient supply.

Increases in concentrations of Cu and Mn in various plant parts may be a result of the development of improved source-sink channels arising from increased microbial activity in the rhizosphere (Table 2), and improved root development[34] leading to vigorous plant growth (Fig. 1), and enhanced accumulation of these micronutrients.

Significant improvement in microbial enzyme activity and soil microbial biomass carbon (SMBC) was recorded with increasing fertilizer application. Microbes require nutrients for their growth, development and metabolism[36–37]. Nutrient application enhances both above ground and below ground growth of plants, thereby increasing the rhizosphere area[38] and facilitating greater microbial activity[39]. This may contribute to the increased enzyme activity and SMBC with increasing nutrient supply observed in this experiment. Improvement in soil enzyme activity was also observed by Bana et al.[37] and Chen et al.[40] as a result of improved crop nutrition, resulting in rhizo-deposition of nutrient-rich decayed roots and root exudates as a microbial substrate. Hartman and Richardson[41]; Pal et al.[42] and Aeron et al.[43]also highlighted the importance of N and P availability for microbial activity.

Plants absorb nutrients from the soil for their growth and development, which can lead to the depletion of essential plant nutrients in agricultural soils[44]. Adding essential plant nutrients via exogenous sources like fertilizers or organic manures is necessary to sustain crop yields[6]. A regular supply of nutrients at optimum levels can also improve the health and nutrient status of soils[45]. In our study, a significant enhancement in soil nutrient status was observed with the application of fertilizers. The enhancement in soil nutrient levels was observed after crop harvest due to the balanced essential nutrient supplies in plant-available forms, which also led to high rhizospheric biomass production, increasing soil organic matter and microbial activity, and ultimately improving soil fertility[46–48].

There was no effect of the multi-nutrient fertilizer on plant-available N and K in the soil, as it was applied to plant leaves. Furthermore, the amount of the multi-nutrient fertilizer applied to the crop was too low to affect soil nutrient status. Enhanced microbial activity, specifically alkaline phosphatase, in the soil as a result of foliar nutrition may have increased plant-available P in the soil. An increase in plant-available soil P due to improved plant nutrition was also reported by Meena et al.,[38] and Pal et al.[42] in similar soils and agro-ecologies.

Conclusion

This research has demonstrated that foliar application of novel micronutrient-embedded NPK (MNENPK) fertilizers assists in biofortification of essential micronutrients (Fe and Zn) in eggplant fruits, which are crucial for healthy human nutrition. Application of the MNENPK fertilizer also increased the concentration and plant uptake of other micronutrients (Cu and Mn) through positive interactions, thus further improving the nutritional profile of eggplant fruit. Combined with the RDF of NPK, foliar supplementation with MNENPK is a cost-effective, sustainable strategy, which is readily accessible to farmers and will increase the yield and micronutrient concentration in eggplant, while improving soil fertility. Therefore, foliar sprays of MNENPK complex fertilizers combined with other modern crop management practices should be recommended to eggplant farmers in South Asia and other similar agro-ecologies. Further investigation into the biofortification potential of foliar fertilizers in other important vegetable crops should be a major research priority.

Method And Materials

Experimental site

A two-year (2017-2019) field experiment was conducted at the Division of Agronomy, ICAR–Indian Agricultural Research Institute, New Delhi (28˚4'N, 77˚12'E, 228.6 m altitude), on a sandy loam Inceptisol soil. Composite soil samples were taken at 0-150 mm depth before sowing, using a core sampler. Soil samples were analyzed for soil physical and chemical properties. The experimental soil had low organic carbon and plant-available N, moderate levels of plant-available P and plant-available K, and was slightly alkaline (Table 4). The plant-extractable Zn, Fe, Mn, Cu within the composite soil samples was 0.58, 4.82, 5.2 and 1.71 mg kg^{-1}, respectively, before the experiment commenced.

Table 4
Physical and chemical properties of soil of the experimental field

Particulars	Content	Method of analysis
A. Soil particle size analysis		
Sand (%)	61.6	Modified hydrometer method (Bouyoucos, 1962)
Silt (%)	12.6	
Clay (%)	25.8	
Soil texture class	Sandy loam	
B. Other soil physical analysis		
1. Field capacity (%)	18.81	Pressure plate apparatus (Richards,1954)
2. Permanent wilting point (%)	6.47	Pressure membrane apparatus (Richards, 1954)
3. Bulk density (Mg m^{-3})	1.56	Core method (Piper, 1966)
4. Infiltration rate (cm hr^{-1})	1.06	Double ring infiltrometer
C. Soil chemical analysis		
1. Organic carbon (%)	0.45	Walkley and Black method, (Jackson, 1973)
2. Available N (kg ha^{-1})	162.5	Modified Kjeldahl's method, (Jackson,1958)
3. Available P (kg ha^{-1})	13.9	Olsen's method, (Olsen'1954)
4. Available K (kg ha^{-1})	231.2	Flame photometer method, (Jackson, 1958)
5. pH (1:2.5 soil: water)	7.7	Blackman's Xeromatic pH meter, (Jackson, 1958)
6. EC (dS m^{-1} at 25°C)	0.35	Jackson, 1958

Treatment Details

The experiment was conducted in a split-plot design replicated thrice with gross plot size of 14.6 m^2. The recommended dose of NPK-fertilizer (RDF) for eggplant is 150 kg N ha^{-1}, 26.2 kg P ha^{-1}, and 49.6 kg K ha^{-1}. There were three recommended dose of NPK-fertilizers (RDF) main-plot treatments *viz*., control in which no fertilizer was applied (RDF1), 75% recommended dose of NPK-fertilizers (RDF2) and 100% recommended dose of NPK-fertilizers (RDF3). The

sub-plot treatments were applications of micronutrient-embedded NPK complex fertilizer (MNENPK) at rates of 0, 0.25, 0.5, and 0.75 kg MNENPK ha^{-1} (Table 5). The MNENPK product used in the present study contains 2.5% N, 3.91% P, 15.65% K, 0.1% Fe, 0.15% Zn, and 0.1% B, with 100% solubility in water.

Management of crop

Eggplant seedlings of the 'Pusa Shyamla' variety were grown in raised beds (7.0×1.5×0.15 m) in the second week of July in both experimental years. The seed rate was 250 g ha^{-1}. In the main experimental plots, 50% of fertilizer N and 100% of fertilizer P and K were applied before the eggplant seedlings were transplanted. The remainder of the N fertilizer was applied in two equal splits, at flowering and fruit development. Seedlings were transplanted into the main field at four weeks of age, at a spacing of 0.65×0.65 m. A light irrigation of 45 mm depth was applied after transplanting. For weed control, pendimethalin was applied at 0.75 kg active ingredient (a.i.) ha^{-1} as pre-emergence, followed by manual weeding at 25 and 45 days after transplanting (DAT). MNENPK was applied as a foliar spray at the flowering and fruiting stages, as per the experimental treatment plan, using a battery-powered knapsack sprayer (Table 5). For protection from fruit and shoot borer infestations, emamectin benzoate at 200 g ha^{-1} was applied during flowering and fruit formation stages. Eggplant fruits were harvested at regular intervals at horticultural maturity. The fruits used for nutrient analysis were harvested at peak fruiting stage from randomly selected plants within each experimental plot.

Plant growth, yield and yield-attributing parameters

Key eggplant growth parameters, plant height, the number of branches per plant and the leaf area index, were recorded at the time of the third fruit harvest, from the inner rows of plots, leaving a border row on all plot sides. The yield-attributing traits (i.e. number of branches per plant, number of flowers per cluster, fruit length, and number fruits per plant) and the fruit yield were measured at horticultural maturity.

Soil sampling and analyses of chemical status and enzymatic activity

Soil samples from 0-150 mm depth were collected using a core sampler to examine the effect of the treatments on soil health. Samples were taken at eggplant flowering to determine soil microbial activity and at harvest to determine soil fertility status[49].

Plant-available soil N was estimated using the modified Kjeldahl method[50]. Plant-available P was determined using the Olsen method[51], and plant-available K by the flame photometer method[52]. Quantification of plant-extractable Zn, Mn, Fe and Cu was done by DTPA before the commencement of the experiment[52]. To estimate topsoil microbial enzyme activity, samples were analyzed for soil microbial biomass carbon (SMBC)[54], dehydrogenase[55], alkaline phosphatases[56], acid phosphatases[57] and urease activities[58].

Table 5
Details of treatments applied to eggplant

Treatment		Treatment combination	Treatment description
Fertility scenario 1	T1	RDF1-MNENPK1	Control (no fertilizer application) + Control [no foliar application of MNENPK)]
	T2	RDF1-MNENPK2	Control (no fertilizer application) + Foliar application of MNENPK @0.25 kg ha^{-1} at flowering and fruiting stages
	T3	RDF1-MNENPK3	Control (no fertilizer application) + Foliar application of MNENPK @0.50 kg ha^{-1} at flowering and fruiting stages
	T4	RDF1-MNENPK4	Control (no fertilizer application) + Foliar application of MNENPK @0.75 kg ha^{-1} at flowering and fruiting stages
Fertility scenario 2	T5	RDF2-MNENPK1	Application of 75% of recommended dose of fertilizer (RDF) + Control [no foliar application of MNENPK)]
	T6	RDF2-MNENPK2	Application of 75% RDF + Foliar application of MNENPK @0.25 kg ha^{-1} at flowering and fruiting stages
	T7	RDF2-MNENPK3	Application of 75% RDF + Foliar application of MNENPK @0.50 kg ha^{-1} at flowering and fruiting stages
	T8	RDF2-MNENPK4	Application 75% RDF + Foliar application of MNENPK @0.75 kg ha^{-1} at flowering and fruiting stages
Fertility scenario 3	T9	RDF3-MNENPK1	Application of 100% RDF + Control [no foliar application of MNENPK)]
	T10	RDF3-MNENPK2	Application of 100% RDF + Foliar application of MNENPK @0.25 kg ha^{-1} at flowering and fruiting stages
	T11	RDF3-MNENPK3	Application of 100% RDF + Foliar application of MNENPK @0.50 kg ha^{-1} at flowering and fruiting stages
	T12	RDF3-MNENPK4	Application of 100% RDF + Foliar application of MNENPK @0.75 kg ha^{-1} at flowering and fruiting stages

Estimating Nutrient Concentrations In Plant Parts

Eggplant leaves, shoots and fruits were dried, ground and digested to determine the concentrations K and of four key micronutrients, Zn, Fe, Mn, and Cu. The K concentrations were determined using a flame photometer and compared with standards ranging from 0–100 parts per million (ppm) of potassium chloride. Zn, Fe, Mn and Cu concentrations were estimated using an atomic absorption spectrophotometer[49]. The most sensitive wavelengths for Zn, Fe, Mn and Cu were 213.7 nm, 248.7 nm, 279.5 nm and 324.6 nm, respectively.

Data Analyses

Means from two years and three replications in each treatment were compared using the least significant difference (LSD) test at a 95% confidence interval (Table 6). Analyses of variance (ANOVA) were conducted using SAS software, version 9.4.

A genotype main effect plus genotype by environment interactions (GGE) biplot analysis was conducted using R to determine the effects of treatments (T) and the interaction effects of treatments × environments (T × E) of the main-plot treatments and MNENPK sub-treatments, following the approach of Yan *et al.*[59]and Yan and Kang[60]. The GGE biplot analysis was performed to determine the "which won where/what" polygon which enables the identification of the best-performing and the least variable treatments. Similarly, other polygons comparing mean *vs.* stability, or ranking treatments and environments graphically were used to determine the most stable treatment combinations, to rank treatments on various indices and to quantify treatments in different mega-environments or sub-groups[23].

The following statistical GGE biplot model was used for data analyses:

$$\mathrm{Y_{ij}} - \mathrm{B_j} = \sum_{\mathrm{k=1}}^{\mathrm{t}} \lambda_{\mathrm{k}} \alpha_{\mathrm{ik}} \delta_{\mathrm{jk}} + \epsilon_{\mathrm{ij}}$$

Where Y_{ij} is the nutrient fortification in the fruit/leaf/shoot with treatment effect i (i = 1,....., n) in environment j (j = 1, …., p), and B_j is the mean of nutrient fortification in the j^{th} environment. The Y_{ij} data matrix was decomposed into *k* principal components (PC) (1 to t with $t \leq \min(p, n-1)$. The λ (1,…., t) are the singular values for the respective PC with $\lambda 1 \geq \lambda 2 \ldots \geq \lambda t \geq 0$; α_{ik} (k = 1,…, t) are the eigenvectors for PC_1, PC_2, …, PC_t, respectively, for each entry *i*; δ_{jk} are the eigenvectors for PC_1, PC_2,…, PC_t, respectively, for each tester *j*, and ε_{ij} is the residual of the model. The first two PC generated from subjecting the singular-value decomposition to the data were used to construct two-dimensional GGE biplots. The data were centred on the applied NPK fertilizer (i.e. main-plot treatments) while comparing between MNENPK treatments, and centred on the applied MNENPK fertilizer (i.e. sub-plot treatments) when comparing between NPK fertilizer treatments. Symmetric scaling (f=0.5) was used for the "which won where/what" pattern. The angles between environmental vectors defined the correlations[61–62].

Policy and plant use guidelines

The authors confirm that the eggplant variety (*Pusa Shymla*) used in the present study was a released variety which is under wide cultivation and was in accordance to international, national, and/or institutional guidelines.

Statement of permission to use specimens of Endangered Species

The authors confirms that no any collection of plant or seed specimens was practiced in the present study. The present research does not involve any species at risk of extinction and the convention on the trade in endangered species of wild fauna and flora.

Table 6
Analysis of variance of growth, yield attributes, yield and nutrient concentration in eggplant

Source	DF	BPP	FL	FY	FPP	LAI	NFC	PH	Micronutrient content			
									Cu	Fe	Mn	Zn
		MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS
Year	1	2.56	0.76	41.79	69.70	0.02	0.02	40.50	0.00095	0.00001	0.00001	0.00031
Rep (year)	4	0.07	4.59	3.63	2.40	0.47	0.56	3.47	0.00012	0.0051	0.0052	0.00089
A	2	67.79	45.42	1439.04	131.03	21.45	24.08	649.76	0.1727	12.3678	8.9126	2.1032
Year*A	2	2.83	2.61	0.40	21.42	0.14	0.07	2.93	0.0018	0.06	0.00001	0.0327
A*rep (year)	8	0.09	1.25	4.25	1.26	0.04	0.06	2.30	0.0015	0.0606	0.0609	0.0115
B	3	3.06	2.09	60.86	8.04	1.40	1.61	39.15	0.0474	1.7822	2.0062	0.6297
Year*B	3	0.15	0.62	2.07	2.34	0.01	0.02	0.20	0.00001	0.00001	0.00001	0.0025
B*rep (year)	12	0.17	0.58	5.02	0.57	0.04	0.02	4.98	0.0006	0.0248	0.0247	0.0047
A*B	6	0.19	0.20	2.00	0.76	0.06	0.14	0.91	0.007	0.3020	0.2451	0.0699
Year*A*B	6	0.07	0.26	0.41	0.68	0.02	0.01	1.33	0.00004	0.00001	0.00001	0.0027
Error	24	0.09	0.82	2.49	0.62	0.01	0.02	6.72	0.0014	0.0536	0.0557	0.0105

*Source = Source of variation, Rep = Replication, A = Main plot (NPK treatment), B = Sub-plot (MNENPK treatment), MS = Mean square, DF = Degree of freedom, BPP = Branches per plant, FL= Fruit length, FY = Fruit yield, FPP = Fruits per plant, LAI = Leaf area index, NFC = Number of fruits per cluster, PH= Plant height

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Figures

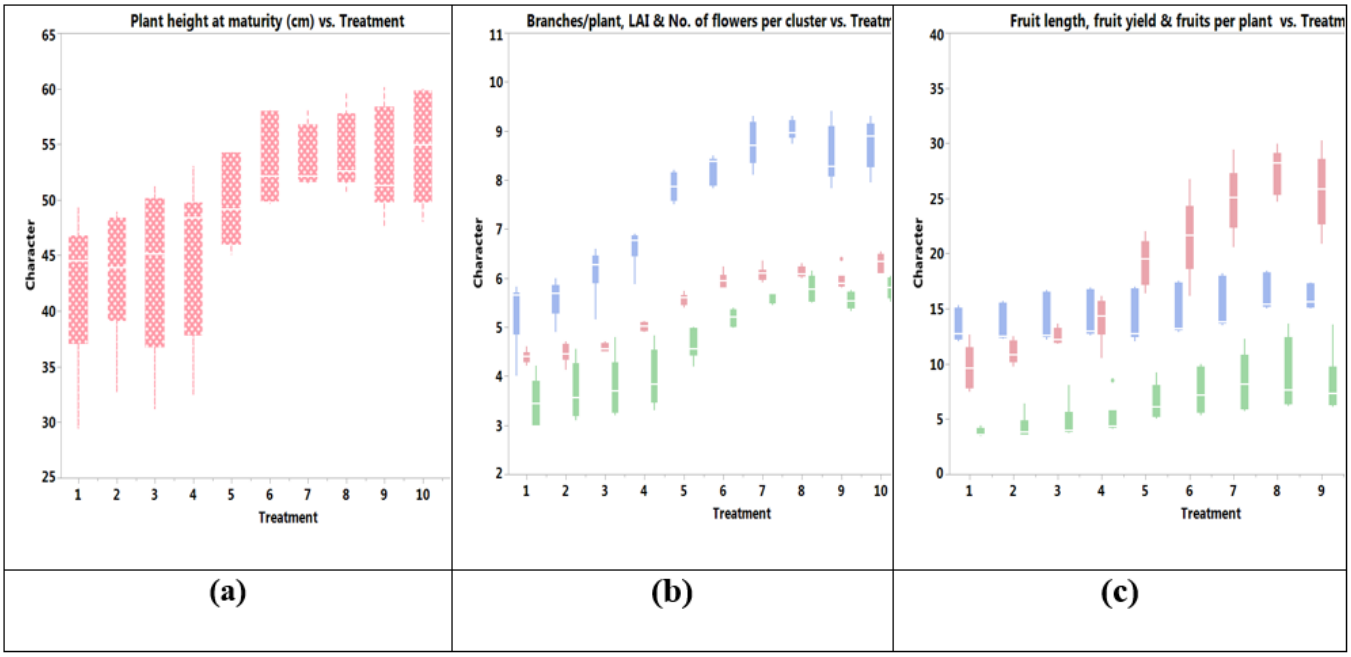

Figure 1

Effect of diverse fertility scenarios and MNENPK fertilizer treatments on (a) plant height, (b) growth parameters and (c) yield attributes and yield of eggplant (pooled data of 2017-18 and 2018-19 cropping seasons). #1= RDF1-MNENPK1; 2= RDF1-MNENPK2; 3= RDF1-MNENPK3; 4= RDF1-MNENPK4; 5= RDF2-MNENPK1; 6= RDF2-MNENPK2; 7= RDF2-MNENPK3; 8= RDF2-MNENPK4; 9= RDF3-MNENPK1; 10= RDF3-MNENPK2; 11= RDF3-MNENPK3; 12= RDF3-MNENPK4

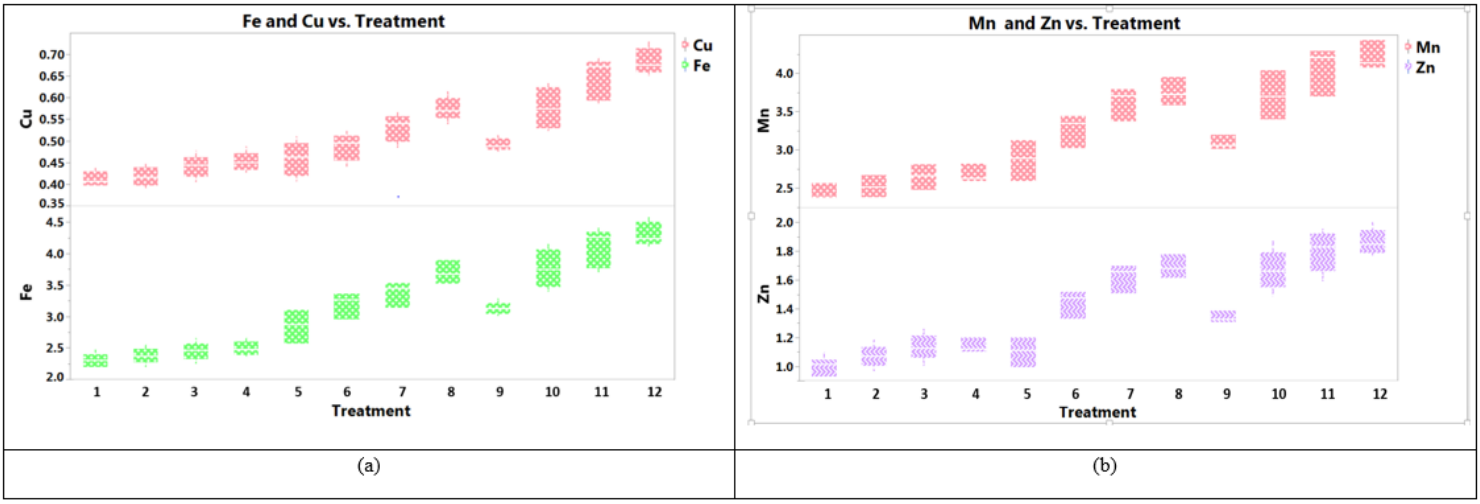

Figure 2

Interaction between NPK and MNENPK fertilizer treatments on eggplant fruits in terms of (a) Cu & Fe, (b) Mn & Zn content (pooled data of 2017-18 and 2018-19 cropping seasons).#1= RDF1-MNENPK1; 2= RDF1-MNENPK2; 3= RDF1-MNENPK3; 4= RDF1-MNENPK4; 5= RDF2-MNENPK1; 6= RDF2-MNENPK2; 7= RDF2-MNENPK3; 8= RDF2-MNENPK4; 9= RDF3-MNENPK1; 10= RDF3-MNENPK2; 11= RDF3-MNENPK3; 12= RDF3-MNENPK4

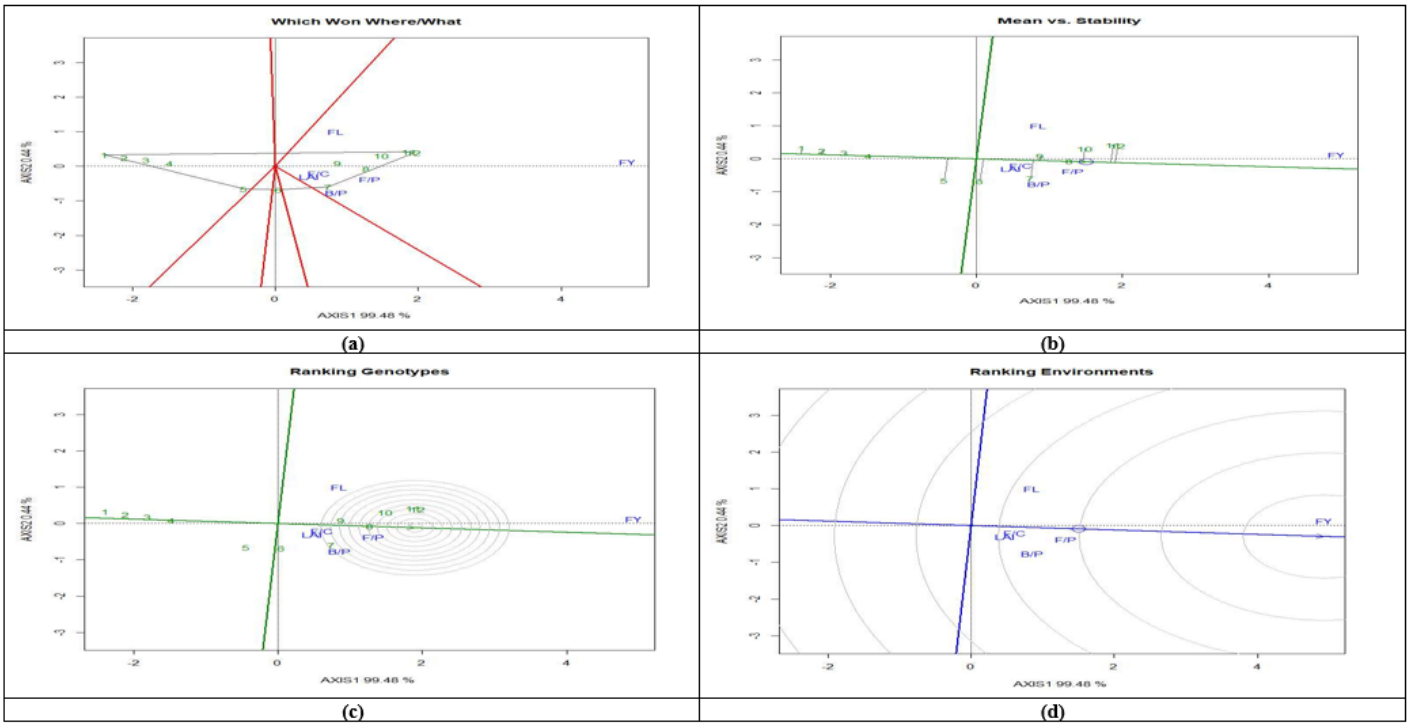

Figure 3

GGE biplot analysis of eggplant yield parameters under experimental treatments. (a) polygon view (Which Won Where/What), (b) Mean vs. stability, (c) Ranking Genotypes (mean ranking treatments), (d) Ranking Environments. AXIS 1 Principal Component 1, AXIS 2 Principal Component 2. #1= RDF1-MNENPK1; 2= RDF1-MNENPK2; 3= RDF1-MNENPK3; 4= RDF1-MNENPK4; 5= RDF2-MNENPK1; 6= RDF2-MNENPK2; 7= RDF2-MNENPK3; 8= RDF2-MNENPK4; 9= RDF3-MNENPK1; 10= RDF3-MNENPK2; 11= RDF3-MNENPK3; 12= RDF3-MNENPK4

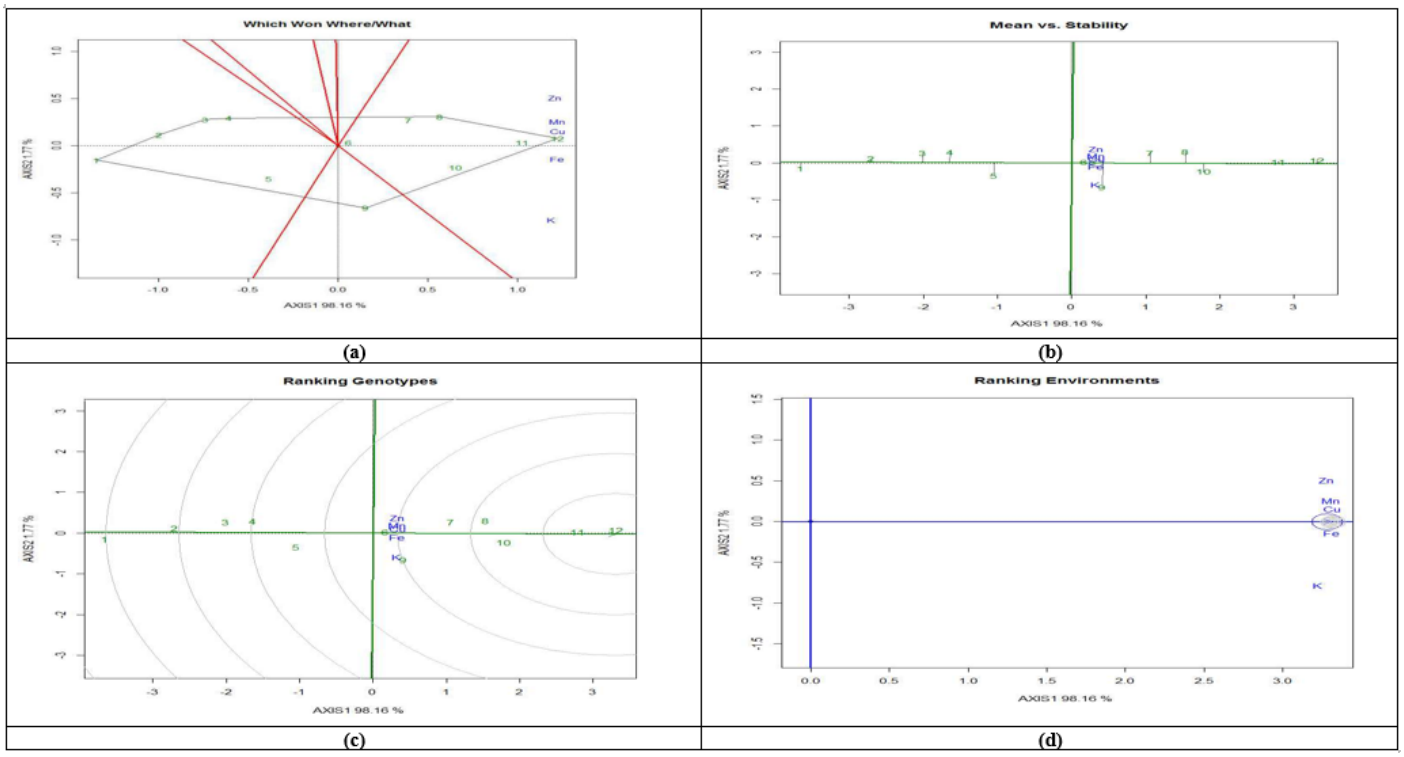

Figure 4

GGE biplot analysis of nutrient concentration in eggplant fruits under experimental treatments. (a) polygon view (Which Won Where/ What), (b) Mean vs. stability, (c) Ranking Genotypes (means ranking treatments), (d) Ranking Environments. AXIS 1 Principal Component 1, AXIS 2 Principal Component 2. #1= RDF1-MNENPK1; 2= RDF1-MNENPK2; 3= RDF1-MNENPK3; 4= RDF1-MNENPK4; 5= RDF2-MNENPK1; 6= RDF2-MNENPK2; 7= RDF2-MNENPK3; 8= RDF2-MNENPK4; 9= RDF3-MNENPK1; 10= RDF3-MNENPK2; 11= RDF3-MNENPK3; 12= RDF3-MNENPK4

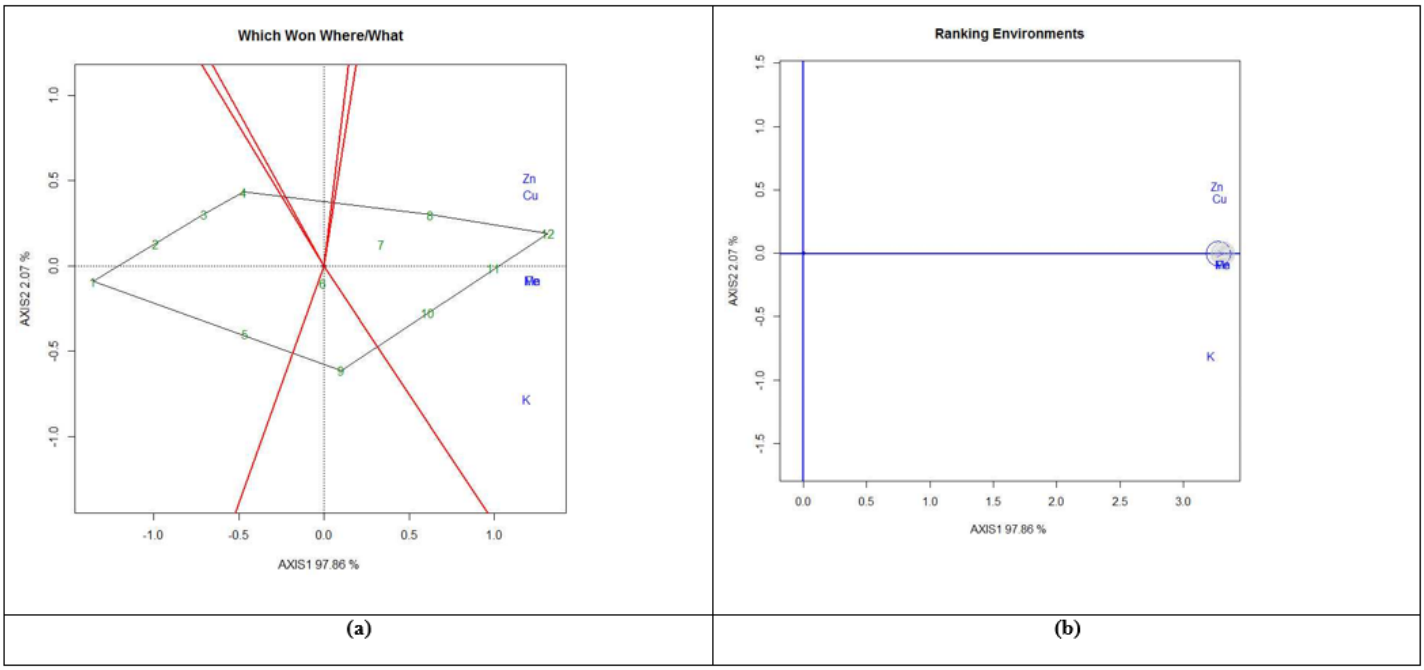

Figure 5

GGE biplot analysis of nutrient accumulation in eggplant shoot under experimental treatments. (a) polygon view (Which Won Where/ What), (b) Ranking Environments. AXIS 1 Principal Component 1, AXIS 2 Principal Component 2. #1= RDF1-MNENPK1; 2= RDF1-MNENPK2; 3= RDF1-MNENPK3; 4= RDF1-MNENPK4; 5= RDF2-MNENPK1; 6= RDF2-MNENPK2; 7= RDF2-MNENPK3; 8= RDF2-MNENPK4; 9= RDF3-MNENPK1; 10= RDF3-MNENPK2; 11= RDF3-MNENPK3; 12= RDF3-MNENPK4

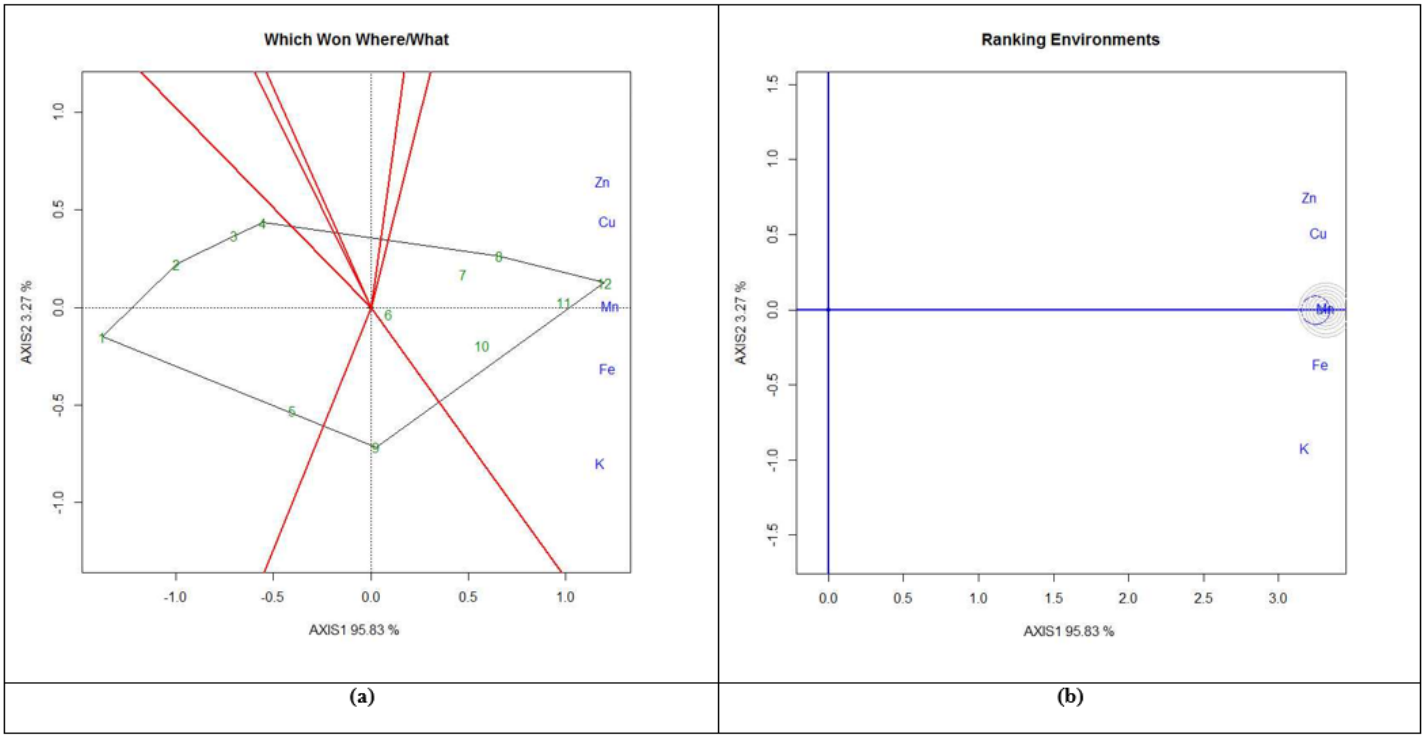

Figure 6

GGE biplot analysis of nutrient accumulation of eggplant leaf under experimental treatments. (a) polygon view (Which Won Where/ What), (b) Ranking Environments. AXIS 1 Principal Component 1, AXIS 2 Principal Component 2. #1= RDF1-MNENPK1; 2= RDF1-MNENPK2; 3= RDF1-MNENPK3; 4= RDF1-MNENPK4; 5= RDF2-MNENPK1; 6= RDF2-MNENPK2; 7= RDF2-MNENPK3; 8= RDF2-MNENPK4; 9= RDF3-MNENPK1; 10= RDF3-MNENPK2; 11= RDF3-MNENPK3; 12= RDF3-MNENPK4