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RESEARCH ARTICLE

BIOFORTIFICATION: AN ALTERNATIVE FOR ZINC AND IRON DEFICIENCY IN CEREALS - A REVIEW.

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Abstract

Iron and zinc deficiencies in human nutrition are widespread in developing Asian and African countries where cereal grains are the staple food. Effects are therefore underway to develop cereal genotypes with grains denser in Fe and Zn by traditional plant breeding or using genetic engineering techniques. This approach requires a long period and adequate funds. However, the products of genetic engineering are not well accepted in many countries. Also, there is a trade-off between yield and grain biofortification. Agronomic biofortification offers to achieve this without sacrificing on yield and with no problem of product acceptance. From the viewpoint of biofortification, foliar application has been reported to be better than the soil application of Fe and Zn, and for this purpose, chelated Fe and Zn fertilizers are better. When soil applied, water soluble sources of Zn are better. Soil application of Fe is not recommended. Agronomic biofortification depends upon management practices (tillage, water management, nutrient interactions), soil factors (amounts present, pH, mechanisms of Zn fixation other than pH), and plant factors (root characteristics, excretion of phytosiderophores and organic acids by roots, Zn utilization at the cellular level, translocation within plant and mechanisms of Zn accumulation in grain). Genetic and agronomic biofortification are complementary to each other. Once the genotypes having denser grains are developed, they will have to be adequately fertilized with Fe and Zn.

Introduction:-

Cereals are staple food in most developing low-income countries of Asia and Africa, where they may contribute as much as 60% of the dietary energy (Prasad *et al.,* 2014). Dietary deficiency of essential micronutrients such as zinc (Zn) and iron (Fe) affects more than two billion people worldwide (White and Broadley, 2009), mostly pregnant women and children below the age of five year, who suffer from severe acute malnutrition. In many parts of the world, micronutrient deficiency is a more widespread problem than poor dietary quality and low energy intake (Stewart *et al.,* 2010). Rice, the main staple food of Asia, is inherently very low in Zn and its high consumption relative to other foods contributes to high incidence of Zn deficiency in human populations in Asia (Phattarakul *et al.,* 2012). Iron (Fe) deficiency alone affects > 47% of all pre-school aged children globally, often leading to impaired physical growth, mental development and learning capacity. Zinc (Zn) deficiency, like iron, is thought to affect billions of people, hampering growth and development and destroying immune systems (Cakmak *et al.,* 2010).

Mineral malnutrition can be addressed through dietary diversification, mineral supplementation, food fortification and/or increasing mineral concentrations in edible crops (biofortification) (Hussain *et al.,* 2010). Agronomic biofortification is of great importance in enriching seeds with Zn and Fe. Soil applications are reported to have, in general, small increases in grain Zn concentration, while foliar applications result in remarkable increases in grain

Zn concentration in wheat (Cakmak *et al.,* 2010). Hari Ram et al (2015) showed that application of foliar Zn at tillering and milk stages proved beneficial in increasing grain Zn content in both rice and wheat. Zinc fertilization improved grain yield and Zn content of maize hybrids compared to synthetic variety (Kanwal *et al.,* 2010).

Foliar application of $FeSO_4.7H_2O$ at 0.5 and 1% levels at different growth stages of rice crop significantly increased grain yield and Fe concentration in rice grains. Five rice cultivars showed differential response to yield, concentration and uptake of Fe with its foliar spray (Singh *et al.*, 2013). Aciksoz *et al.*, (2011) revealed that inclusion of urea in foliar Fe fertilizers had a positive impact on grain Fe concentration in wheat. The combined application of Zn as seed priming (2.0%) and foliar spray (2.0%) can improve the performance of maize hybrids (Mohsin *et al.,* 2014).

Effect of zinc deficiency:-

Prasad *et al.,* (2013) Data on global mortality in children under 5 years of age due to different micronutrient deficiencies in 2013 (Table 1). Zn deficiency was next only to vitamin A deficiency and was responsible for over 453 thousand deaths.

Table: 1. Global mortality in children under 5 years of age.

Deficiency	Deaths
Vitamin A	666,771
Zinc	453,207
Iron	20,854
Iodine	3,619

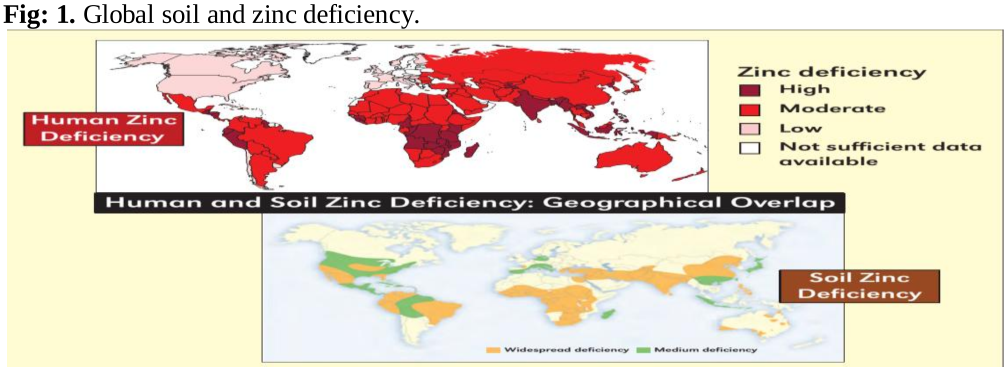
Cakmak *et al.,* (2008) According to a WHO report on the risk factors responsible for development of illnesses and diseases, Zn deficiency ranks 11^{th} among the 20 most important factors in the world and 5^{th} among the 10 most important factors in developing countries (Table 2).

Table: 2. Ten leading causes of illness and disease in low income countries.

Risk factors	Illness (%)
Underweight	14.9
Unsafe sex	10.2
Unsafe water	5.5
Indoor smoke	3.7
Zinc deficiency	3.2
Iron deficiency	3.1
Vitamin A deficiency	3.0
Blood pressure	2.5
Tobacco	2.0
Cholesterol	1.9

Alloway *et al.,* (2004) The regions with Zn-deficient soils are also the regions where Zn deficiency in human beings is widespread, for example in India, Pakistan, China, Iran and Turkey Figure 1 shows global distribution of the regions where Zn deficiency problem has been reported in crop plants. Possibly, there are many other regions or countries where Zn deficiency problem has not been reported or diagnosed.

Fig: 1. Global soil and zinc deficiency.

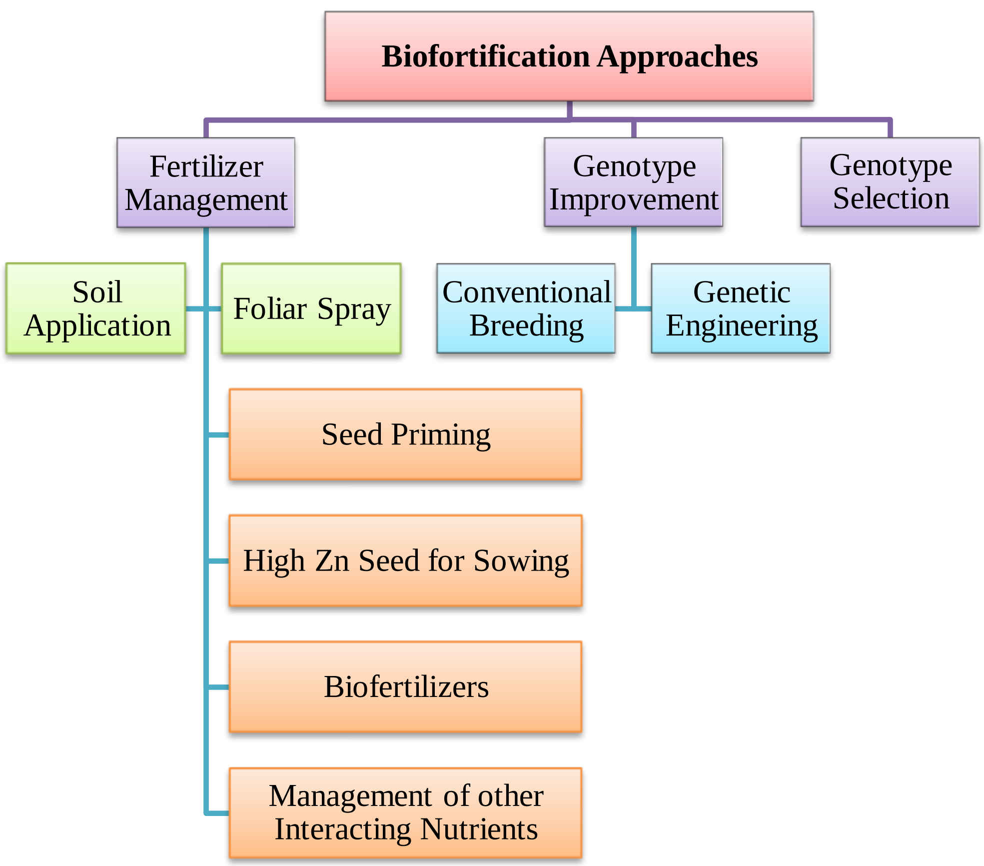


White and Broadley *et al*., (2005). Traditional interventions tackled mineral malnutrition by supplementation, food fortification and dietary diversification. None of these have been universally successful on sustainable basis because they require safe delivery systems, stable political policies, appropriate social infrastructures and continued investment for longer periods (Table 3). Fageria, (2008) Conventional crop breeding (especially hybrid development), molecular biology, genetic engineering and resource management can greatly help in enhancing the crops to feed the poor (Fig. 2).

Table: 3. Possible solution to mineral deficiency in human population.

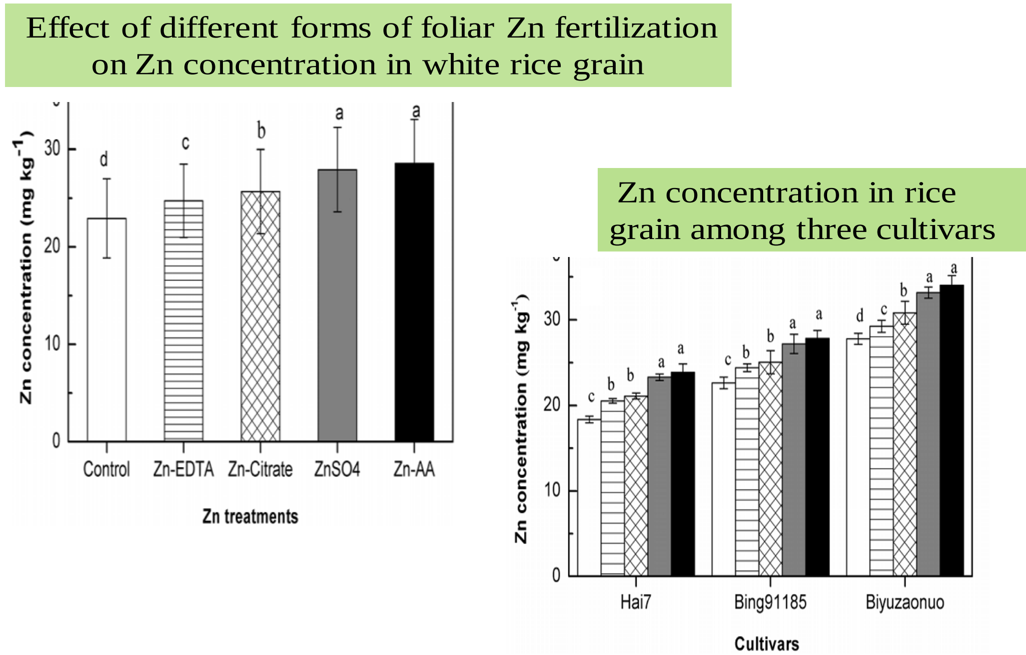
Intervention	Scope	Economics
Supplementation	It is generally recommended during pregnancy or in severe Zn deficiency for a shorter period.	It is costly and only recommended when a very quick response is required.
Fortification	It is effective but limited to urban areas.	It is very uneconomical if carried out for longer period of times.
Food Diversification/ modification	It is applicable only where alternative food products are available with high adoptability.	It is economically feasible and sustainable intervention.
Bio-fortification	It is targeted and reachable	It is cost effective and sustainable approach. It has added benefit of yield increase on Zn deficient soils and seems permanent solution.

Fig: 2. Approaches for zinc biofortification of staple food grains.



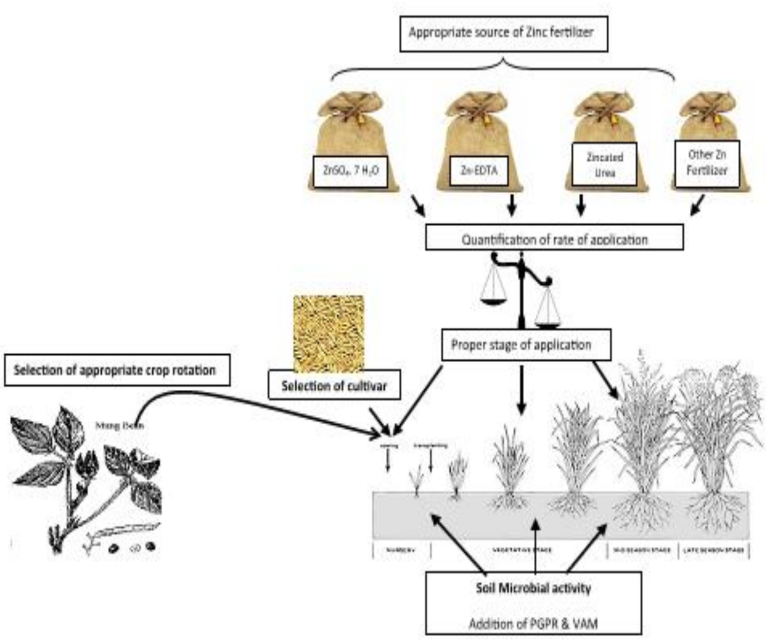
Wei *et al.,* (2012) found that regardless of cultivar, although polishing process decrease substantial amount Zn from mature grain, the polished rice obtained from foliar Zn applications was still contained 10.22–24.04% more Zn than those of control (Fig. 3) Similar trends were found in polished rice (Fig. 3), the cultivar Biyuzaonuo had the highest Zn concentration, while Hai7 had the lowest Zn concentration in all Zn treatments.

Fig: 3. Effect of different forms of foliar application on Zn concentration in white rice grain.



Singh and Prasad, (2014) agronomic biofortification is a win–win approach for developing countries which relies on exploitation of micronutrient dense cultivar applying zinc fertilizers to seeds, soil and/or foliar, at rates greater than those required for maximum yield, in order to increase the uptake of Zn into the plants and its translocation into seeds. This could be a more sustainable and cost effective strategy to improve Zn concentrations in rice grains. Application of soil microorganism and selection of suitable crop rotation has also been found very promising to increase zinc concentration in rice grain (Fig. 4).

Fig: 4. Major approach for agronomic bio-fortification .



Shivay *et al.*, (2015) studied significant increase in Zn concentration in grain over NPK fertilization was recorded with soil application of ZnSHH or Zn–EDTA, which were at par with two or three applications of 0.2% solutions of ZnSHH or Zn–EDTA or one or two applications of 0.5% solutions of ZnSHH or Zn–EDTA. A single application of 0.2% of ZSHH or Zn–EDTA was inferior to soil application of Zn and did not significantly increase Zn concentration in rice grain. In both the years of study Zn application either to soil or foliage significantly increased ZnMEI over no fertilizer control (Table 4).

Table: 4. Effect of sources, time and method of zinc application on Zn concentration in grain of basmati rice.

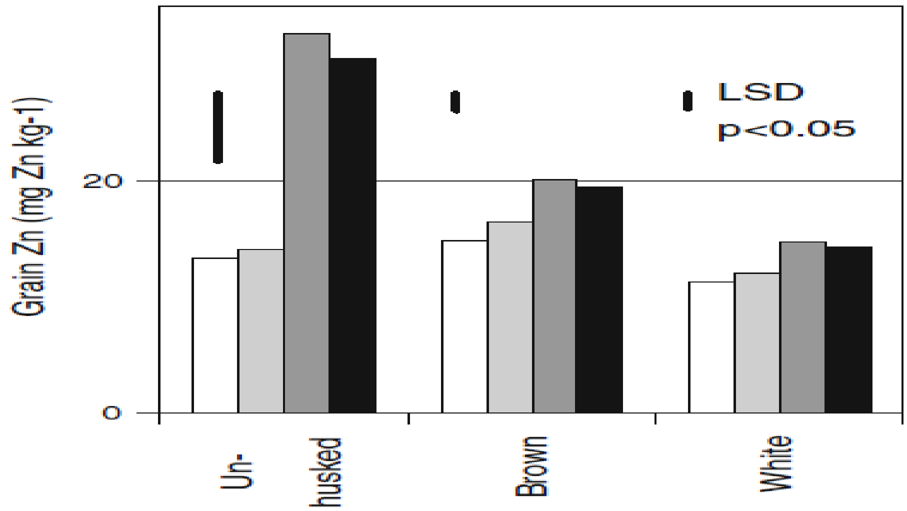
Treatment	Zn concentration in rice grain (mg kg^{-1})		Zn mobilization efficiency index (ZnMEI)	
	2010	2011	2010	2011
Absolute control	20.7	21.2	0.28	0.28
NPK (120 kg N+26.2 kg P+60 kg K)	23.1	23.6	0.29	0.29
NPK+5kg Zn ha^{-1}through ZnSHH as SA	26.4	26.9	0.30	0.30
NPK+ ZnSHH 0.2 % FSAT	24.8	25.3	0.29	0.29
NPK+ ZnSHH 0.2 % FSAT+B stages	26.3	26.8	0.30	0.30
NPK+ ZnSHH 0.2 % FSAT + B+ GF stages	26.8	27.3	0.30	0.30
NPK+ ZnSHH 0.5 % FSAT	25.4	25.9	0.30	0.30
NPK+ ZnSHH 0.5 % FSAT +B stages	26.6	27.1	0.29	0.30
NPK+ ZnSHH 0.5 % FSAT+ B+ GF stages	28.2	28.7	0.30	0.30
NPK+ 5kgZnha^{-1}through Zn–EDTA as SA	27.8	28.3	0.30	0.30
NPK+ Zn–EDTA 0.2 % FSAT	24.7	25.2	0.29	0.29
NPK + Zn–EDTA 0.2 % FSAT+B stages	26.6	27.1	0.30	0.30
NPK+ Zn–EDTA 0.2 % FSAT+ B+ GF stages	27.7	28.2	0.30	0.30
NPK+ Zn–EDTA 0.5 % FSAT	25.8	26.3	0.29	0.29
NPK+ Zn–EDTA 0.5 % FSAT+ B stages	28.2	28.7	0.30	0.30
NPK + Zn–EDTA 0.5 % FSAT +B +GF stages	29.8	30.3	0.31	0.31
CD (P=0.05)	2.1	1.7	0.01	0.02

Phattarakul *et al.*, (2012) revealed that Zn concentration in brown rice was increased by 25% and 32% by foliar and foliar + soil Zn applications, respectively, while there was only 2.4% increase with soil Zn application. In some locations in India and Thailand, foliar Zn application increased brown rice Zn by about 60% (Table 5). The effectiveness of foliar Zn application on grain Zn varied substantially between years for a given genotype, indicating important role of environmental conditions on seed deposition of foliar applied Zn. There were also some genotypes which showed better response to foliar Zn application such as IR68144 as compare to other varieties in Thailand. And also reported that the Zn concentration of un-husked, brown and white rice were all increased much more markedly by the foliar Zn applications made at milk stage while only minimal increases in grain Zn were found when Zn applications were made at tillering and booting stages (Fig. 5).

Table: 5. Zinc concentration of brown rice grown with different methods of Zn fertilizer treatments.

Country	Location	pH, OC (%) and DTPA-Zn (mg kg^{-1})	Year	Variety	Nil	Soil Zn	Foliar Zn	Soil + foliar Zn
					Zn in brown rice (mg Zn Kg^{-1})			
China	Anhui	4.8, 0.3 & 0.9	2008	GLY No.6	17.6a	20.4b	24.3c	26.2c
	Zhejiand	5.6, 0.1 & 4.3	2008	GLY No.6	23.3a	23.8a	26.6b	27.9b
India	Ludhiana	7.6, 0.4 & 6.5	2009	PR 120	23.6a	26.7a	28.9b	30.3b
			2010	PR 120	16.3a	20.3ab	23.6b	25.2b
	Langroya	8.8, 0.3 & 5.5	2009	PR 118	21.8a	25.1ab	27.0b	30.0b
	Kapurthala	7.6, 0.3 & 2.2	2010	PR 118	18.1a	23.0b	29.0c	24.9b
Thailand	Chiang Mai	6.2, 0.9, &2.1	2008	CNT 1	20.6a	20.6a	25.8b	26.6b
			2009	CNT 1	19.6a	20.4a	22.5b	24.3c
			2009	IR 68144	31.0a	31.5a	32.1a	35.7a
Mean					19.4	20.8	24.2	25.5
% increase in brown rice Zn over nil						2.4	24.7	31.9

Fig: 5. Zn concentration in un-husked, brown and white rice as affected by methods of foliar Zn application at different stages.



Prasad *et al*., (2014) showed that application of Zn as 2% zinc sulfate heptahydrate with coated urea, (5.2 kg Zn ha^{-1}) as soil applied significantly increased grain yield of rice as well as Zn concentration in rice grain and application of Zn as 0.5% $ZnSo_4$ $7H_2O$ (1.2 kg Zn ha^{-1}) as foliar application in case of biofortification recovery efficiency significantly better than other treatments (Table 6).

Table: 6. Effect of method, source and rate of Zn application on grain yield, Zn content and bio-fortification recovery efficiency of basmati rice.

Treatment	Grain yield (t ha^{-1})	Zn concentration in unhusked rice (mg kg^{-1})	Zn concentration in polished rice (mg kg^{-1})	BRE_{Zn} (%)
No Zn	3.92	30.4	26.1	-
25 kg $ZnSO_4.7H_2O$ ha^{-1} (5.3 kg Zn ha^{-1}) Soil application	5.20	47.5	40.3	1.42
0.2% $ZnSO_4.7H_2O$ foliar application (1.2 kg Zn ha^{-1})	4.99	52.6	28.8	2.42
Soil application of 1% ZnO-coated urea (2.6 kg Zn ha^{-1})	4.48	38.2	32.4	1.16
Soil application of 2% ZnO-coated urea (5.2 kg Zn ha^{-1})	5.13	44.7	37.9	1.24
Soil application of 1% $ZnSO_4.7H_2O$ coated urea (2.6 kg Zn ha^{-1})	4.69	40.3	34.1	1.55
Soil application of 2% $ZnSO_4.7H_2O$ coated urea (5.2 kg Zn ha^{-1})	5.27	49.7	42.1	1.61
CD (P=0.05)	0.45	4.5	-	-

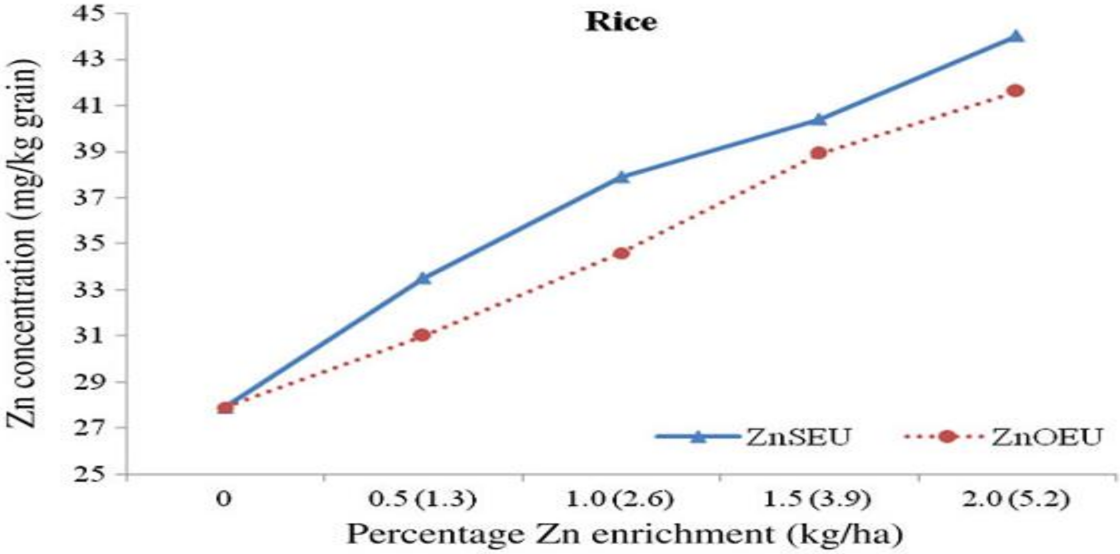
Purakayastha and Chhonkar (2001) examined that influence of the VAMF, Glomus etunicatum, was significant in enhancing grain yields of rice as compared to the un-inoculated control. The inoculation increased the content and uptake of Zn by rice (Table 7) and the highest content of Zn were observed in the NPK+ZnSO4 as compare to other treatment.

Table: 7. Grain yield and Zn content of rice without and with inoculation of roots with Glomus etunicatum.

	Grain yield (g pot^{-1})			Content of Zn (μg g^{-1})		
Fertilization	U	I	Mean	U	I	Mean
NPK	24.2	28.4	26.3	14.2	18.7	16.4
NPK+ZnSO4	27.0	29.8	28.4	18.1	21.8	19.9
NPK+FYM	23.2	24.4	23.8	14.6	20.9	17.7
NPK+ZnSO4+FYM	24.1	25.3	24.7	14.5	17.8	16.1
Mean	24.6	27.0		15.3	19.8	
CD (P=0.05)	1.8	1.6	2.1	1.7	1.5	2.3

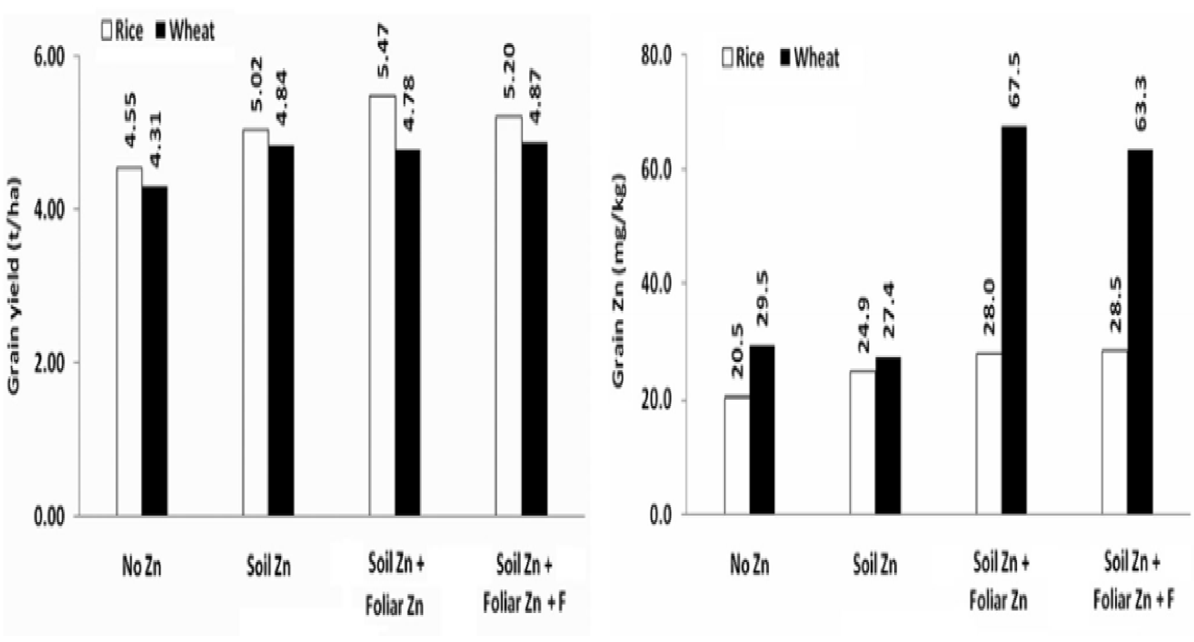
Prasad *et al.*, (2013) Zn enrichment of urea @ 2% Zn as zinc sulphate increased the grain Zn concentration by 61.8% in rice and by 51.1% in wheat (Fig 6).

Fig: 6. Grain Zn concentration in rice due to degree of Zn enrichment of urea.



Hari Ram *et al.*, (2015) studied that soil Zn application increased wheat grain yield up to 50% and rice grain yield only up to 14.8%. Soils rich in Zn showed no or little effect on grain yield when Zn was applied. The spraying of 3.0 kg ZnSO4.7H_2O in two applications at the flowering and early grain development stages increased the grain zinc significantly irrespective of the soil types. Application of foliar Zn with or without propiconazole resulted in significant increase in grain Zn, which was 38–40% in rice and 87–150% in wheat, over no Zn (Fig 7).

Fig: 7. Effect of various zinc fertilizer treatments on the grain yield of rice and wheat.



Zou *et al.*, (2012) reported that 83.5 and 89.7 % increases in grain Zn concentration by foliar Zn alone and soil + foliar Zn application, respectively while soil Zn application was less effective (Table 8). The increments in grain Zn associated with only foliar Zn spray was more than 10 mg Zn kg^{-1} grain. In the case of soil + foliar Zn application, an increment of 20 mg kg^{-1} grain was found in half of the field tests.

Table: 8. Grain Zn concentration of wheat grown with Zn fertilizer treatments.

Country	Location	pH and DTPA-Zn ($mg\ kg^{-1}$)	Year	Grain Zn concentration ($mg\ kg^{-1}$)				CD (P=0.05)
				Nil	Soil Zn	Foliar Zn	Soil + foliar Zn	
China	Quzhou	7.8 & 0.32	2009	27.7	32.4	47.2	53.9	3.5
			2010	29.5	40.9	44.3	52.0	6.8
	Yongshou	8.0 & 0.31	2009	18.8	21.0	26.5	22.5	3.8
			2010	19.5	22.1	31.4	34.0	3.5
India	Varanasi	7.7 & 0.86	2008	29.0	32.0	44.0	47.0	12.0
	Kapurthala	7.6 & 2.2	2010	49.0	52.0	64.8	65.3	7.4
			2011	31.4	30.2	51.1	49.1	7.9
	Ludhiana	7.6 & 6.5	2010	25.5	30.3	61.0	60.8	7.5
			2011	27.3	36.7	58.3	57.0	6.4
Pakistan	Faisalabad	- & 1.3	2008	29.0	29.0	60.0	59.0	9.0
Mean				27.4	30.5	48.0	49.0	
% increase over nil					12.3	83.5	89.7	

Mohsin *et al.*, (2014) Maximum grain yield (Table 9) were recorded in maize hybrid Pioneer 30-Y-87 with combined application of Zn as seed priming (2%) and foliar spray (2%) during both the years. Improvement in grain yield was 20% and 22%, during the year 2009 and 2010, respectively. Seed priming with Zn solution. Different Zn application methods improved kernel Zn contents, however, combined application of Zn as seed priming (2%) and foliage spray (2%) in maize hybrid DK-919 gave 43.61% and 36.56% more grain Zn content, respectively during the year 2009 and 2010 than any other.

Table: 9. Influence of zinc application through seed treatment and foliar spray on grain yield and grain Zn content of maize.

Treatment	Grain yield ($t\ ha^{-1}$)		Grain Zn content ($mg\ kg^{-1}$)	
	2009	2010	2009	2010
CK	5.80 g	5.52 g	19.88 g	21.44 g
SP_1	6.09 f	5.83 f	22.52 f	23.52 f
SP_2	6.14 f	5.85 ef	22.57 f	23.66 f
F_1	6.24 ef	5.94 e	23.11 e	24.10 e
F_2	6.60 cd	6.35c	27.04 b	27.74 b
$SP_1 + F_1$	6.41 de	6.14d	23.98 d	25.15 d
$SP_1 + F_2$	6.81 ab	6.56b	27.06 b	27.79 b
$SP_2 + F_1$	6.66 bc	6.35c	25.55 c	26.89 c
$SP_2 + F_2$	6.96 a	6.73a	28.55 a	29.28 a

Shivay and Prasad (2014) In both the years of study One to three foliar applications of 0.2 or 0.5% solutions of ZSHH or one to three foliar applications of 0.2% solution of Zn–EDTA or a single application of 0.5% solution of Zn–EDTA were equally effective and at par with soil application of ZnSHH or Zn–EDTA. In both the years of study two or three foliar applications of 0.5% solution of Zn– EDTA produced the highest yield and yield attributes of Basmati rice, significantly more than soil application of ZnSHH or Zn–EDTA and most other treatments of foliar spray. (Table 10).

Table: 10. Effect of source and method of Zn application on yield attributes and yield of maize.

Treatment ($Zn\ ha^{-1}$)	Cob length (cm)	Grain weight (g cob^{-1})	1,000-grain wt (g)	Grain yield ($t\ ha^{-1}$)	Stover yield ($t\ ha^{-1}$)
Control	13.0	70.6	190.0	4.00	6.10
5 kg to soil	14.0	74.9	199.3	4.70	6.68
1 kg foliar	13.5	72.8	193.3	4.42	6.50
5 kg to soil + 1 kg foliar	15.2	76.5	201.5	5.10	7.03
2.83 kg through Zn-coated urea (to soil)	14.4	75.2	200.5	4.80	6.90
CD (P=0.05)	2.1	4.1	NS	0.38	0.62

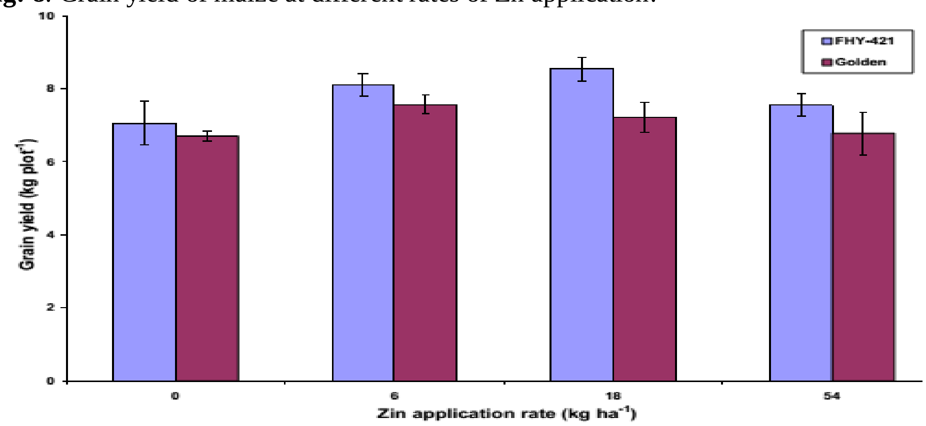
Shivay and Prasad (2014) found that significant increase in Zn concentration in grain and straw over NPK fertilization was recorded with soil application of ZnSHH or Zn–EDTA, which were at par with two or three applications of 0.2% solutions of ZnSHH or Zn–EDTA or one or two applications of 0.5% solutions of ZnSHH or Zn–EDTA. A single application of 0.2% of ZSHH or Zn–EDTA was inferior to soil application of Zn and did not significantly increase Zn concentration in rice grain and straw. The highest Zn concentration in grains and straw of Basmati was recorded with three foliar applications of 0.5% solution of Zn–EDTA, significantly more than soil application of ZnSHH or Zn–EDTA and most other foliar application treatments (Table 11).

Table: 11. Effect of source and method of Zn application on Zn concentration in grain and stover and mobilization efficiency index in maize.

Treatment (Zn ha^{-1})	Grain Zn concentration (mg kg^{-1}grain)	Stover Zn concentration (mg kg^{-1}DM)	Zn Mobilization efficiency index
Control	40.2	45.0	0.89
5 kg to soil	44.2	49.2	0.90
1 kg foliar	46.0	59.2	0.78
5 kg to soil + 1 kg foliar	49.2	64.5	0.76
2.83 kg through Zn-coated urea (to soil)	45.8	58.2	0.79
CD (P=0.05)	2.0	2.7	0.03

Kanwal *et al.,* (2010) studied that Zinc application to soil had a significant ($p<0.05$) effect on grain yield of both the maize hybrid FHY-421 and synthetic variety Golden (Fig. 8). Cultivars also differed significantly ($p<0.01$) for the grain yield. Maximum increase (21%) in grain yield of hybrid (FHY-421) was observed when the Zn was applied @ 18 kg ha^{-1}, while synthetic variety (Golden) exhibited maximum increase (13%) in grain yield when Zn was applied @ 6 kg ha^{-1} to the soil.

Fig: 8. Grain yield of maize at different rates of Zn application.



Effect of iron deficiency:-

Shing *et al.,* (2013) Three foliar sprays of FeSO4.7H2O @ 0.5 and 1% levels at different growth stages of rice crop significantly increased the yield of rice cultivars (Table 12). The data showed that the maximum grain yield was reported in PAU 201 cultivar (82.8 q ha^{-1}) and minimum grain yield was reported in PR120 cultivar (78.7 q ha^{-1}) with 0.5 percent level of FeSO4.7H2O spray. The grain yield of PR113, PR116 and PR118 cultivars with 0.5 per cent level of FeSO4.7H2O spray ranged from 79.0- 80.1 q ha^{-1}. With three foliar sprays of FeSO4.7H2O with 0.5 per cent level at different growth stages of rice crop, the grain yield of PR113, PR116, PR118, PR120 and PAU 201 cultivars increased by 5.1, 4.3, 2.9, 3.2 and 3.8 per cent respectively, over control.

Table: 12. Grain yield (q ha^{-1}) of different rice cultivars as affected by foliar spray of $FeSO_4.7H_2O$.

Treatment	Rice cultivars				
	PR 113	PR 116	PR 118	PR 120	PAU 201
Control	75.3	75.7	77.8	76.2	79.7
0.5 % $FeSO_4$	79.2	79.0	80.1	78.7	82.8
% increase over control	5.1	4.3	2.9	3.2	3.8
1 % $FeSO_4$	81.2	80.4	80.7	79.5	84.0
% increase over control	7.8	6.2	3.7	4.3	5.3
CD (P=0.05)	0.2	2.2	0.1	0.1	0.2

Shing *et al.,* (2013) The data showed that three foliar sprays of FeSO4.7H2O with 0.5 per cent and 1 per cent levels at different growth stages of rice crop (maximum tillering, pre-anthesis and post-anthesis stages) significantly increased Fe concentration in brown rice of different rice cultivars as compared to control but results shown by PR113 cultivar were non- significant (Table 13).

Table: 13. Effect of foliar spray of $FeSO_4.7H_2O$ on Fe concentration in brown rice of different rice cultivars.

Treatment	Rice cultivars				
	PR 113	PR 116	PR 118	PR 120	PAU 201
	Fe concentration in brown rice (mg kg^{-1})				
Control	15.2	14.8	13.0	17.8	12.5
0.5 % $FeSO_4$	18.8	20.5	19.7	20.2	19.8
% increase over control	23.6	38.5	51.5	13.4	58.4
1 % $FeSO_4$	26.4	25.8	26.5	28.2	28.8
% increase over control	73.6	74.3	103.8	58.4	130.4
CD (P=0.05)	NS	3.1	1.1	6.2	5.7

Aciksoz *et al.,* (2011) examined that application of urea alone and with iron fertilizer found non-significant grain yield over all treatment. And highest iron concentration in grain with the application of FeSO4 + Urea and at par with FeEDTA + Urea and Fe EDDHA + Urea as compare to over treatments (Table 14).

Table: 14. Effect of Fe with urea in grain yield and Fe concentrations in grain.

Foliar applications	Grain Yield (g $plant^{-1}$)	Fe concentration in Grain (mg kg^{-1})
Control	2.71	36
Control + Urea	3.34	36
FeSO4	2.73	38
FeSO4+ Urea	2.69	43
FeEDTA	3.07	38
FeEDTA + Urea	3.38	42
FeEDDHA	3.11	35
FeEDDHA + Urea	2.61	39
Fe Citrate	2.54	36
Fe Citrate + Urea	2.97	37
CD (P=0.05)	N.S.	5

Effect of both the zinc and iron deficiency:-

Dhaliwal *et al.,* (2014) revealed that foliar application of Zn raised the maximum grain yield up to 49.58 q ha^{-1} (PBW 550) and 47.82 q ha^{-1} (PBW 17), which were 9.22 and 6.30 per cent higher than control, respectively. The yield of wheat with foliar application of Zn varied from 5.11 to 9.68% in different cultivars. Similarly, the application of Fe increased the grain yield up to 49.35 q ha^{-1} (PBW 550) and 48.52 q ha^{-1} (PBW 17), which were 8.69 and 7.87 per cent higher than control, respectively (Table 15). And also showed that application of foliar sprays enhanced the content of Zn and Fe in wheat grains (Table 16) in all the varieties to varying degree of magnitude depending on the cultivars. The average concentration of Zn in wheat grains was 21.91 µg g^{-1} (range 20.35-23.89 µg g^{-1}) in control treatments which increased to 24.74 µg g^{-1} (range 21.60-26.39 µg g^{-1} dry weight) with foliar application of zinc. With foliar application of iron and the per cent increase in Fe content in grains ranged from as low as 9.27% in PDW-291 and high as 28.00% in PBW-343. Wide variation in percent increase in Fe concentration with foliar application was observed as it ranged from as low as 9.27% in variety PDW 291 to as high as 28.00 in variety PBW343.

Table: 15. Effects of foliar applications of Zn and Fe on grain yields (q ha^{-1}) in different wheat cultivars.

Treatment	PBW 343	PBW 550	PBW 17	PDW 233	PDW 274	PDW 291	Average
	Grain yield (q ha^{-1}) with foliar Zn						
-Zn	43.37	45.42	44.98	43.32	42.25	41.00	43.39
+Zn (F)	47.57	49.58	47.82	45.92	44.40	43.30	46.43
% increase	9.68	9.22	6.30	6.04	5.11	5.62	7.00
	Grain yield (q ha^{-1}) with foliar Fe						
-Fe	43.37	45.42	44.98	43.32	42.25	41.00	43.39
+Fe (F)	46.43	49.35	48.52	46.25	43.40	42.12	46.01
% increase	7.10	8.69	7.87	6.76	2.71	2.76	5.98

Table: 16. Effect of Zn and Fe sprays on their respective concentration in grains of different wheat cultivars

Treatment	PBW 343	PBW 550	PBW 17	PDW 233	PDW 274	PDW 291	Average
	Concentration of Zn (mg kg^{-1}) in wheat grains with foliar Zn						
-Zn	21.42	20.56	21.38	20.35	23.89	23.36	21.91
+Zn (F)	24.18	26.14	26.39	21.60	25.56	24.56	24.74
% increase	12.62	27.15	20.81	6.16	7.07	5.15	13.16
	Concentration of Fe (mg kg^{-1}) in wheat grains with foliar Fe						
-Fe	37.42	39.14	40.47	38.90	39.14	41.99	39.51
+Fe (F)	47.70	45.27	48.90	44.27	46.65	45.89	46.45
% increase	28.00	15.66	20.99	13.76	19.17	9.27	17.81

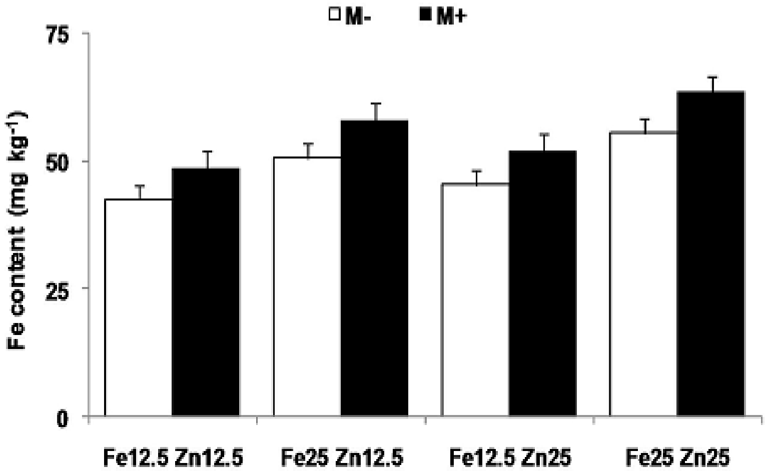
Table: 17. Effect of nitrogen levels on Zn and Fe content in grain, stover and harvest index of Zn and Fe in maize.

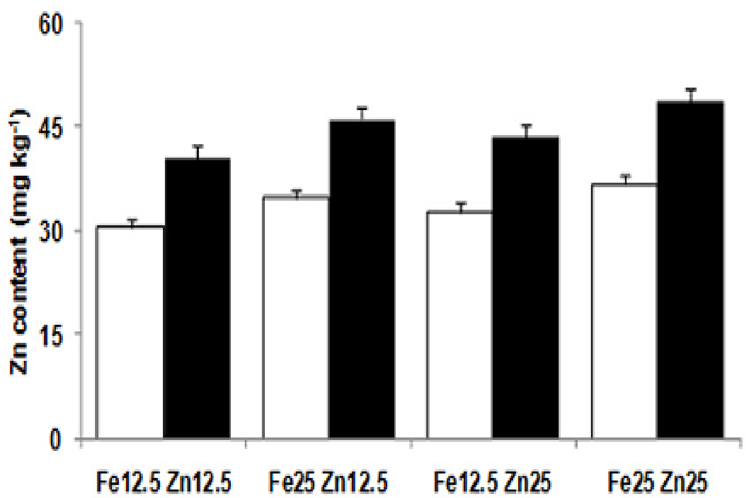
Parameters	N levels			
	N-0	N-low	N-opt	N-over
GZnC (mg kg^{-1})	15.2b	15.2b	16.6ab	17.3a
SZnC (mg kg^{-1})	18.2ab	17.6b	20.9ab	21.8a
ZnHI (%)	48a	49a	49a	48a
GFeC (mg kg^{-1})	13.4c	15.5b	16.0ab	17.2a
SFeC (mg kg^{-1})	63.3a	69.6a	58.3a	60.6a
FeHI (%)	19b	21ab	25a	25a

Xue *et al.,* (2014) found that over application of nitrogen significantly higher Zn and Fe concentration in grain stover and harvest index

Balakrishnan and Subramanian (2012) reported that Higher Fe concentrations in grains of Mycorrhiza+ plants may be attributed to the hyphal transport of Fe and besides improved plant available Fe that may have supported Fe nutrition of maize plants and fortification of grains calcareous (Mycorrhiza- 23.6; Mycorrhiza+ 35.2 mg kg-1). Similarly, Zn concentrations (Figure 10) of maize grains were significantly higher for mycorrhizal treatments in both calcareous (36.3 mg kg^{-1}) and non-calcareous (39.7 mg kg^{-1}) soils than Mycorrhiza- treatments (Calcareous 22.6; non-calcareous 27.2 mg kg^{-1}) (Fig 9).

Fig: 9. Effect of Zn and Fe concentration of grain (mg kg^{-1}) of Arbuscular mycorrhizal fungus inoculated (AMF^{+}) and uninoculated (AMF^{-}) in maize plants.





Health problems associated with zinc and iron deficiency:-

Zn deficiency:-

- Pregnancy complications, infertility, low birth weight.
- Impairments in brain development and function, decreased nerve conduction, skin lesions.
- Growth faltering, diarrhoea and pneumonia etc.

Fe deficiency:-

- Anaemia and neurodegenerative diseases, mental retardation.
- Impaired immune system, tendency toward bleeding, depression.
- Women and children are at more risk.

Challenges for agronomic biofortification of staple foods:-

- Setting appropriate target levels for the zinc content of biofortified staple foods.
- Retention of zinc in biofortified staple food.
- Bioavailability of zinc from agronomic biofortified staple food.
- Determining biological impact of biofortified staple crops.
- Creating awareness among the farmers regarding biofortification.
- Increased cost of fertilization.

Conclusions:-

- Zinc and iron are essential micronutrients for human health and deficiency of these micronutrients leads to malnutrition and diseases.
- Zinc and iron application increases concentration in produces as well as grain yield under nutrient deficient soil.
- Foliar sprays of Zn and Fe (0.5% $ZnSO_4$ and $FeSO_4$) with two-three times is a most effective level of bio-fortification in cereals as compare to soil application.

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