AGRONOMIC BIOFORTIFICATION OF ZINC IN RICE
(*Oryza sativa* L.)

by

AMAL JOSE

(2017-11-131)

THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"AGRONOMIC BIOFORTIFICATION OF ZINC IN RICE (*Oryza sativa* L.)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

@	At the rate of
^{o}C	Degree Celcius
%	Per cent
₹	Rupees
B:C ratio	Benefit : cost ratio
CD (0.05)	Critical difference at 5 per cent level
NS	Not significant
cm	Centimetre
m	Metre
mm	Millimetre
RBD	Randomized block design
dS m^{-1}	Deci Siemens per meter
EC	Electrical conductivity
et al.	Co- workers/ co-authors
Fig.	Figure
FYM	Farmyard manure
SA	Soil applied
FS	Foliar spray
Kg	Kilogram
ha^{-1}	Per hectare
kg ha^{-1}	Kilogram per hectare
g kg^{-1}	Gram per kilogram
mg kg^{-1}	Milligram per kilogram
LAI	Leaf area index
mg	Milligram
cm^2	Square centimetre
m^{-2}	Per meter square
cc	Cubic centimetre
DAS	Days after sowing
DAT	Days after transplanting
No.	Number
SE m	Standard error of means
t ha^{-1}	Tonnes per hectare
Rs	Rupees
KAU	Kerala Agriculture University
POP	Package of practices
RDF	Recommended dose of fertilizer
N	Nitrogen

vH

P	Phosphorus
K	Potassium
Ca	Calcium
Mg	Magnesium
S	Sulphur
Zn	Zinc
$ZnSO_4$	Zinc sulphate
ppm	Parts per million
MT	Maximum tillering
PI	Panicle Initiation
B	Booting
M	Milking
Fe	Iron
Mn	Manganese
Cu	Copper
BRE_{Zn}	Biofortification recovery efficiency
CRE_{Zn}	Crop recovery efficiency
M t	Metric tonne
m ha	Million hectare

Introduction

1. INTRODUCTION

Rice is a major cereal crop which plays a key role in food security of India. In India rice, contributes a major part of total food grain production. Rice production in India is 110.17 M t from an area of 43 M ha with a productivity of 2550 kg ha^{-1} during 2016-17 (DACFW, 2018). Rice is consumed as major staple food grain in south and east India. West Bengal, Uttar Pradesh, Andhra Pradesh, Punjab and Tamil Nadu are the predominant rice growing states in India. Kerala recorded production of 0.43 M t from an area of 0.17 m ha with productivity of 2546 kg ha^{-1}during 2016-17 (FIB, 2019).

Intensive rice cultivation with high yielding varieties, lesser application of organic manures, excessive use of high analysis fertilizers and neglecting application of Zn had widespread zinc (Zn) deficiency in Indian soil. Application of Zn fertilizer in Zn deficient soil had increased rice grain yield by 31 per cent (Gogoi *et al.*, 2016). Zn deficiency is expected to increase from 49 to 63 per cent by 2025 (Singh, 2009). Zn deficiency ranges from 2.3 to 50 per cent in 10 districts of Kerala (Mathew and Aparna, 2012). Zn was found deficient in 12 per cent out of total 154531 soil samples analyzed in Kerala. Localized Zn deficiency in soil was reported from Thiruvananthapuram, Kollam, Alappuzha, Ernakulam, Thrissur, Kozhikode and Idukki districts of Kerala state (KSPB, 2013).

Zn is an important micronutrient and an essential element necessary for normal growth and development of plants. Zn is a cofactor of enzymes within plant concerned with carbohydrate metabolism, photosynthesis, conversion of sugars to starch, protein metabolism, auxin metabolism, pollen formation and membrane integrity. Zn deficiency in soil ultimately results in Zn deficiency in plants which causes retardation of photosynthesis and nitrogen (N) metabolism, reduced flowering and fruit development, prolonged growth periods, delayed maturity, lower yield, poor produce quality and sub optimal nutrient use efficiency (Das and Green, 2016a). Zn is an essential element in human nutrition. It is an integral component of about 300 enzymes within human body performing catalytic, co-catalytic and structural function within enzymes. It is important for growth and development, immune function, insulin action and reproductive health.

Recommended dietary allowance of Zn for an Indian adult is 12 mg day^{-1}. Habitual intake of Zn by a moderately active Indian adult male is only 9 to 11 mg day^{-1}. Thus Indian population is exposed to a marginal risk of inadequacy of Zn (NIN, 2009). Polished rice grains have inherently low Zn concentration 6 to 9 mg kg^{-1}(Sudha and Stalin, 2015). Further decrease in grain Zn concentration was noticed when rice was grown in Zn deficient soil. Recommended dietary allowance of cereals and millets for a moderately active Indian adult male is 400 g day^{-1}. Daily intake of all foods except cereals and millets (396 g day^{-1}) in Indian households is lower than recommended dietary allowance. Hence a strategy to improve dietary intake of Zn by balancing cereal based diets with expensive vegetables and animal products to alleviate Zn deficiency in Indian population is futile.

High per capita consumption rate of rice 238 to 586 g day^{-1} requires development of rice with high concentration of Zn in grain, a wise biofortification strategy. Biofortification is a process for increasing the bioavailable concentrations of essential elements in the edible portions of crops through agronomic intervention or genetic selection. Breeding of new rice varieties with genetic potential to accumulate high concentration of Zn in grain through genetic biofortification is the most cost effective approach to overcome Zn malnutrition in the long run. However agronomic biofortification through the use of Zn fertilizers, the fastest route to improve Zn concentration in rice grain of a variety widely accepted by farmers would help to overcome Zn malnutrition.

Most of the earlier studies involving Zn fertilization in rice were done to understand the effects of Zn fertilizer application either as basal or foliar or both on grain yield to overcome Zn deficiency in soil. More recent studies have assessed the effect of foliar Zn application on grain Zn concentration, showing that foliar Zn application had significantly enhanced grain Zn concentration over control treatments in rice. Phytic acid present in cereals was found to reduce absorption of Zn in humans and thereby reduce Zn bioavailability. In this context, present study was formulated with the following objectives

- To study the effect of time and method of Zn application on growth, yield, Zn biofortification and Zn bioavailability in transplanted rice

Review of Literature

2. REVIEW OF LITERATURE

2.1 ESSENTIALITY OF ZINC IN PLANT NUTRITION

Zinc is a predominant micronutrient and an essential element necessary for the normal growth and development of plants. The major role of Zn in higher plants is to take part in enzymatic and hormonal activities (Brown *et al.*, 1993). Approximately 2,800 enzymes need Zn for their activation and structural stability (Shuman *et al.*, 1995). Cakmak (2000) reported that Zn helps to protect the cells by both controlling the regeneration and detoxification of reactive oxygen species (ROS). Zn is the only element, which is the cofactor for all six classes of enzymes like lyases, ligases, isomerases, oxidoreductase, hydrolases and transferases (Broadley *et al.*, 2007). Basic biochemical processes in plants like enzyme activation, protein synthesis, carbohydrate and enzyme mechanism, chlorophyll production, pollen formation, synthesis of cytochrome and nucleotide, energy dissipation and membrane integrity maintenance are made of Zn through acting as a cofactor (Alloway, 2009). (Andreini *et al.*, 2009) stated that Zn induces the stomatal openings and influx of K^+ to guard cells and it is the major constituents in carbonic anhydrase. Zn also has an important role in the absorption of Fe in the human intestine and a sufficient quantity of Zn along with Fe is a mandatory rectifying Fe deficiency in the human body (Graham *et al.*, 2012). Zn is a cofactor of enzymes within the plant concerned with photosynthesis, conversion of sugars to starch, auxin metabolism, pollen formation and membrane integrity. Zn deficiency in soil ultimately results in Zn deficiency in plants which causes retardation of photosynthesis and N metabolism, reduced flowering and fruit development, prolonged growth periods, delayed maturity, lower yield, poor product quality and suboptimal nutrient use efficiency (Das and Green, 2016b).

2.2 ESSENTIALITY OF ZINC IN HUMAN NUTRITION

Apart from biological activities in plants, Zn has a critical role in human nutrition. It is a single metal element in whole enzyme groups which is required for normal growth and development of all living organisms, especially humans (Broadley *et al.*, 2007).

According to the International Zn Association, 26 per cent of the Indian population is under Zn deficiency, means 312 million people are suffering from problems Zn deficiency. National Institute of Nutrition (NIN, 2011) stated that the recommended dietary allowance for Zn was 12 mg day^{-1} and Zn deficiency mainly damages our skin, nail and hair. Dry skin and discolouration in nails are the main symptoms of Zn deficiency. Zn has an epic role in major body functions such as growth, immunity, vision, cognition, cell reproduction and disease resistance (Kawade, 2012).

2.3 STATUS OF ZINC IN SOIL

2.3.1 India

Half of the Indian soils are deficient in Zn. Available and total Zn concentration in Indian soils ranges from 0.1 to 24.6 mg kg^{-1} and 7 to 2960 mg kg^{-1} respectively with an average deficiency of 12 to 87 per cent. Crops grown in this area have low Zn content in the stem and grains. (Singh, 2009). Augmented cropping intensity in marginal lands, followed by a lesser use of micronutrients further intensified the magnitude of Zn deficiency in various states like Tamil Nadu, Karnataka, Kerala, Chhattisgarh and Maharashtra. Singh (2009) reported that because of intensive cultivation without adequate micronutrient supplementation, overall Zn deficiency is expected to increase from 48 per cent found in the year 1970 to 63 per cent by the year 2025. It is found that to rectify the Zn deficiency, we need 324 t ha^{-1} per year of fertilizer Zn by the year of 2025. The application of Zn fertilizer in Zn-deficient soil increased rice grain yield by 31 per cent (Gogoi *et al.*, 2016).

2.3.2 Kerala

Kerala soils are deficient in Zn by 34 per cent and Zn deficiency is expected to increase from 49 to 63 per cent by 2025 (Singh, 2009). Zn deficiency ranges from 2.3 to 50 per cent in ten districts of Kerala (Mathew and Aparna, 2012). From the analysis of 154531 soil samples in Kerala, 12 per cent deficiency has been reported. Localized Zn deficiency in soil was noticed from Thiruvananthapuram, Kollam, Alappuzha, Ernakulam, Thrissur, Kozhikode and Idukki districts of Kerala state (KSPB, 2013).

2.4 ZINC STATUS IN FOOD GRAINS

Major research on the availability of Zn is mainly confined to grains only since the pulses are rich in micronutrients. The availability of Zn in grains decreased due to anti-nutritional factors like phytate and polyphenols (Nieto *et al.*, 2007).

Rice is considered as the staple food in most of the Asian countries, where almost 90 per cent of it is grown and consumed (IRRI, 2006). The bioavailability and Zn concentration in rice and other cereal grains are comparatively low when they are grown on Zn deficient soils (Bell and Dell, 2008). Cakmak (2008) stated that in various Asian countries like India, Pakistan, Turkey and China, the low availability of Zn in alkaline calcareous soils is becoming a serious problem. Zn availability is very low in Indian soil (Prasad *et al.*, 2014), which is leading to low Zn content in rice grains.

The high occurrence of Zn deficiency in human populations is due to very low Zn content in rice and its high consumption relative to other food grains (Stein *et al.*, 2007). Both polished and unpolished rice contains a low Zn concentration, which is too low to meet human demands for Zn (Lee *et al.*, 2009). In a screening study, various methods have been developed to increase the Zn content in rice grains like

i) biofortification with popular Zn fertilizers (Cakmak, 2009), ii) manipulating Zn transporters and ligands in rice plants (Borrill *et al.*, 2014) and iii) efficient germplasm screening for higher bioavailable Zn.

Khan (2002) conducted a pot culture experiment to study the effect of different levels of Zn (0, 5,10 and 15 kg ha^{-1}) along with a basal dose of NPK. Zn content in leaves, root, grain and straw of rice increased significantly with an increase in the level of Zn fertilizers. The Zn concentration in the treatment receiving 150 kg ha^{-1} Zn was 85.10 and 46.64 ppm in straw and grain respectively. Zn content of rice leaves increased significantly with Zn addition, both before flowering and after harvest over control. Maximum Zn accumulation was noted with 15 kg Zn ha^{-1} and also available Zn content of soil increased significantly with the addition of Zn over control, at both panicle initiation (PI) and after the harvest of rice.

Zn accumulation mechanisms in rice grain can be grouped into two categories according to the predominant sources of Zn loading: as continued root uptake during the grain-filling stage (Jiang *et al.*, 2007) and remobilization of Zn from shoots or roots (Wu *et al.*, 2010).

2.5 BIOAVAILABILITY OF ZINC IN HUMAN NUTRITION

The bioavailability of major and trace minerals is defined as the proportion of the ingested minerals, which is absorbed and available for metabolic functions. (Nosratpou *et al.*, 2015).

According to Oberleas *et al.* (1961), the availability of minerals in the human body will decrease due to high phytic acid content. In the majority of cereals, minerals become unavailable due to the chelating property of phytic acid, which is readily bound to mineral ions as a result absorption of major divalent cations like iron, Zn, calcium, magnesium and manganese has been inhibited due to its action (Hallberg *et al.*, 1989). Phytate: Zn ratio is the major index for measuring the bioavailability. Low, medium and high bioavailability is accompanied by phytate: Zn ratio of >15: 1, 5-15: 1 and <5: 1 respectively (Graham, 1984). In rice bran, the phytic acid concentration is reported up to 8.7 per cent (Lehrfeld, 1994). Phytates or phytic acids present in seeds or grains of every plant which are reserve form of P also an anti-nutritional factor which limits the bioavailability of particularly divalent and trivalent minerals (Hurrel, 2004) especially, Zn. Bioavailability of Zn was limited to 50 per cent from different sources of our common diet which we are consuming (Welch and Graham, 2005). Miller (2007) reported that major cereals and pulses contain 1 to 3 per cent phytic acid. It is also present in tuber crops, vegetables, etc. Hence, animal-based food has more bioavailability than grain-based foods. Among the major cereals like rice and wheat, phytic acid is present in bran fraction such as aleurone layer.

2.6 AGRONOMIC BIOFORTIFICATION OF RICE THROUGH MINERAL FERTILIZATION

The study conducted by Dhanya (2014) reported that biofortification recovery efficiency (BRE_{Zn}) was increased with the foliar application of Zn rather than soil application in yard long bean. Cakmak (2016) stated that crop recovery efficiency and (CRE_{Zn}) and BRE_{Zn} got decreased with an increase in the number of sprays.

Gogoi *et al.* (2016) concluded that soil and foliar application of Zn through $ZnSO_4$ increased the Zn concentration from 23.9 mg kg^{-1} to 29.3 mg kg^{-1}.

Zn-EDTA applied to t rice, at active tillering, booting and grain filling stages at 0.5 per cent concentration was effective and recorded the best results and gave results as that of ZnSO4. The Zn concentration increased from 21.2 mg kg^{-1} to 30.3 mg kg^{-1} (Shivay *et al.*, 2016).

Phattarakul (2012) reported that application of ZnSO4 increased yield from 7.4 to 7.8 t ha^{-1} and combined application by soil and foliar increased grain content from

23.6 mg kg^{-1} to 30.3 mg kg^{-1}. Saha *et al.* (2017) reported that when Zn was applied as soil, Zn concentration in the soil increased from 12.5 to 29 ppm.

2.6.1 Source and Time of Zn Application

Various sources of Zn fertilizers are used to rectify the Zn deficiency in crops. Zn fertilizers will differ based on their Zn content, chemical composition and effectiveness. According to Mortvedt and Gilkes (1993), there are four types of Zn sources like inorganic compound, synthetic chelate, natural organic complexes and inorganic complexes. Zn fertilizers are available both in liquid and solid form.

Foliar application of Zn was considered as the best method of Zn application - compare with soil application. It is more beneficial when the Zn deficiency has appeared on the crop, but the disadvantage is that we have to apply it on crops at various stages of the crop or multiple times (Swietlik, 2002).

The stage or growth phase of the foliar Zn application has a critical role in the effectiveness of increasing grain Zn concentration. Foliar Zn applications at stem elongation plus booting stage had no significant effect on rice grain yield and resulted in a marginal effect on rice grain Zn concentration. Zn concentration is reached up to two-fold in husked rice and 30 per cent in white rice, when Zn was applied as foliar at the milk stage. But increased concentration of Zn in unhusked rice might also lead to Zn contamination (Phattarakul *et al.,* 2012).

Shivay *et al.* (2016) conducted an experiment to study the effect of NPK fertilizers with two sources of Zn mainly $ZnSO_4.7H_2O$ and Zn–EDTA on growth and yield, Zn concentration and uptake and Zn use efficiency in Basmati rice cultivar. The effect of Zn-EDTA and $ZnSO_4.7H_2O$ in the soil was the same. Three foliar applications of Zn-EDTA at various stages of rice such as tillering, booting and grain filling was the best treatment and resulted in significantly better growth, higher yield attributes, grain and straw yield and concentration and uptake of Zn in grain and straw of Basmati rice than soil application of $ZnSO_4.7H_2O$ or Zn-EDTA.

Kulhare *et al.* (2017) conducted a study to determine the effect of foliar spray of 0.5 and 1.0 per cent Zn salts and 1.0 per cent Zn salts + 0.5 per cent lime with different sources of Zn viz., $ZnSO_4.7H_2O$, $ZnCl_2$, $Zn_3(PO_4)_4$, ZnO and Na_2Zn-EDTA, on yield, Zn content, uptake and Zn use efficiency in rice. The best treatment on the basis of grain yield was the foliar application of Zn-EDTA over all the other sources of Zn but, the other Zn sources were on par amongst themselves. In the case of uptake by grain, Zn sources like $ZnSO_4.7H_2O$, Zn3 $(PO_4)_4$, ZnO and Zn EDTA also had significant results and was superior to $ZnCl_2$. However, foliar application of Zn as 1 per cent salt along with 0.5 per cent lime was ineffective compared with foliar spraying of Zn as Zn salt 1 per cent alone. Similarly, the Zn use efficiency with 0.5 per cent Zn salt was significantly higher than 1.0 per cent Zn salts + 0.5 per cent lime but 0.5 per cent Zn salt was on a par with 1.0 per cent Zn salts for available Zn. The reduced grain yields and lower Zn concentration of Zn during the foliar spray of Zn along with lime is due to inhibition of Zn uptake by Calcium.

2.7 EFFECT OF TIME AND METHOD OF ZINC APPLICATION ON RICE

2.7.1 Effect on Growth Characters

Singh *et al.* (1978) based on field trial concluded that foliar spray of various micronutrients such as Zn, Mn and combination of Zn + Mn + Cu + B + Mo at their active growth stages *i.e.*, tillering and booting stages leads to luxuriant vegetative growth and greater plant height. Fageria *et al.* (2003) concluded that the plant height of rice showed significant improvement with Zn fertilization either as $ZnSO_4$ or ZnO. Split application of Zn fertilizer was better in terms of growth and yield characters of rice (Naik and Das, 2008).

Based on a field study conducted in lateritic sandy loam soils of Kharagpur by Subbaiah and Mittra (1997) concluded that foliar spray of micronutrients such as Zn, B and Mo has significantly increased the growth characters such as LAI in rice.

According to Ghatak *et al.* (2005), various growth characters of rice such as plant height, effective tillers and LAI increased with the application of ZnSO4 @ 30 kg ha^{-1}. Dry matter accumulation of rice has been improved by the application of Zn @ 13.5 kg ha^{-1} (Slaton *et al.*, 2005).

Shivay *et al.* (2010) reported that plant height and other growth characters of rice increased with the application of Zn fertilizers compared to no Zn application.

Wijebandara and Iranie (2008) observed that application of 75 per cent RDF + biofertilizers with 25 kg $ZnSO_4$ resulted in taller plants, higher number of tillers, higher dry matter and higher grain and straw yield and yield attributes.

Root characters like root volume, root length and root weight increased significantly by the application of $ZnSO_4$ @ 10 mg kg^{-1} as foliar application in amaranthus (Sakthidharan, 2013). Foliar spray of 0.05% $ZnSO_4$ improved root length root volume and root weight in yard long bean (Dhanya, 2014)

2.7.2 Effect on Yield Attributes and Yield

From a study conducted by Khan (2002) with various levels of Zn (0, 5, 10 and 15 kg ha^{-1}) in the form of $ZnSO_4$ along with the basal application of N, P and K, it wascfound that the yield and yield attributes increased with elevated Zn fertilization. Other characters like plant height, average number of productive tillers per plant, panicles per plant, spikelets per panicle, test weight, grain and straw yield were maximum when Zn was applied at 10 kg ha^{-1}.

Prasad *et al.* (2002) conducted a field experiment in Bihar for five years to study the optimal frequency of Zn fertilizer application on Zn-deficient soil in the rice-wheat cropping system. The result indicated that the rate of increase in the yield of rice and wheat was 52.40 and 21 kg per kg $ZnSO_4$ respectively and the per cent increase in the yield of rice was 46.60 and 38.10 in wheat- rice and wheat cropping system and there was significant correlation with Zn removal.

Sharma *et al.* (2005) conducted a study on the effect of levels and methods of Zn fertilization on yield, concentration and uptake of Zn by rice. Zn was applied in the soil at different levels such as 0, 5.6, 11.2 and 22.4 kg ha^{-1}, foliar spray at 1 per cent and 2 per cent $ZnSO_4$ solution and roots of rice seedlings were dipped in 2 per cent and 4 per cent ZnO suspensions in water. Grain yield, Zn content and its uptake increased in all the experiments up to 22.4 kg ha^{-1} Zn. Soil applied Zn was significantly correlated with the yield of rice and Zn uptake. Zn content in 45 day old plants gave a significantly higher correlation with grain yield than the Zn content of rice straw.

Khan (2002) found that plant height, tillers per plant, panicles per plant, spikelets per panicle and yield were positively related to Zn fertilizers.

Saha *et al.* (2013) obtained an increase of 29 per cent grain yield of rice compared to control with soil and foliar application of Zn. They stated that soil application alone will increase the yield significantly, but the combined application of Zn via foliar and soil will enhance the Zn content and quality along with the yield.

Guo *et al.* (2014) carried out a study to compare the effects of different levels of Zn fertilizers (0, 15, 30 kg ha^{-1} $ZnSO_4.7H_2O$) and different application methods (soil and foliar) on yield. The results indicated that Zn fertilizer application significantly improved the rice grain yield by about 0.3 to 13 per cent. The yield improving efficiency of soil application was higher than that of foliar spray.

A work was conducted by Shivay *et al.* (2016) on a sandy clay-loam soil at New Delhi to study the effect of NPK fertilization and to compare the effect of $ZnSO_4$

and Zn-EDTA on growth and yield attributes, grain and straw yield, Zn concentration and uptake and Zn use efficiency in Basmati rice cultivar 'Pusa Sugandh 5'. Among the treatments, foliar applications of Zn-EDTA at three stages (tillering, booting and grain filling stages) were better and recorded significantly better growth, higher values for yield attributes, higher grain and straw yield and higher concentration and uptake of Zn in grain and straw of Basmati rice than soil application of $ZnSO_4$ or Zn-EDTA and two or a single foliar application of $ZnSO_4$ or Zn-EDTA.

According to Suresh and Salakinkop (2016) combined soil application of $ZnSO_4$ and $FeSO_4$ each at 25 kg ha^{-1} and foliar spray of $ZnSO_4$ and $FeSO_4$ each at 0.5 per cent were found to be best treatment, which resulted in significantly higher yield and yield attributing characters like productive tillers per meter row length, number of filled grains per panicle, grain yield (3,739 kg ha^{-1}), straw yield (5,539 kg ha^{-1}) and growth attributes like number of tillers per meter row length, LAI, SPAD value and dry matter production.

Ram *et al.* (2016) studied the effect of Zn through foliar application at eight field sites in three countries (India, China and Thailand) with three crops: rice, wheat and common bean. Rice grain yields exhibited a large variation among the locations of three countries. It varied from 10.45 t ha^{-1} at Anhui-Changfeng in 2013 to 4.57 t ha^{-1} at Ludhiana (India) in 2012. However, rice grain yield was not significantly influenced by any of the Zn treatments at all locations and during all years, except at Anhui-Changfeng location of China in 2013.

2.7.3 Effect on Quality Characters in Whole Grain and its Milled Fractions

Starch synthetase enzyme activity decreased under the stress of Zn deficiency and it was significantly reduced up to 36 per cent of the normal level in *Phaseolus vulgaris* L. (Jyung *et al.*, 1975). Hemantaranjan and Garg (1988) stated that an increase in total carbohydrate, starch and protein contents of wheat grain also improved with soil or foliar application of Zn.

According to Marschner (1995), poor Zn content in plants will significantly reduce the rate of protein synthesis as well as protein content, but increase the accumulation of amino acids.

Khan (2002) reported that the application of Zn increased grain protein and enhanced grain Zn concentration, while simultaneously reduced grain P concentration. Application of $ZnSO_4$ @ 50 kg ha^{-1} through soil improved the protein content (7.6 per cent) of rice grain and a further increase was noticed at higher levels of Zn (Chaudary and Sinha, 2007). All the quality parameters in rice, especially protein content, increased due to foliar spray of 1.5 per cent Zn enriched urea in sandy loam soils of New Delhi (Yadav *et al.*, 2010).

Veerendradixit *et al.* (2012) reported that protein content in rice increased up to 11.56 per cent with the application of $ZnSO_4$ @ 15 kg ha^{-1}. Application of $ZnSO_4$ @ 50 kg ha^{-1} along with a foliar spray of 0.5 per cent $ZnSO_4$ increased the protein content in whole grain as well as brown rice (Rani, 2013). The study conducted by Kumar *et al.* (2017) at Faizabad found that the soil application of $ZnSO_4$ @ 50 kg ha^{-1} increased the protein content of rice grain

The highest amylose (15.8 per cent) content and protein content (8.03 per cent) were reported in rice by the application of $ZnSO_4$ @ 30 kg ha^{-1} (Kumar *et al.*, 2017)

2.7.4 Effect on Zn Content in Whole Grain and its Milled Fractions

Reddy *et al.* (1987) concluded that maximum Zn concentration in grain was achieved by the foliar application of 0.2 per cent $ZnSO_4$ at three stages *i.e.*, 25^{th} day, 35^{th} and 45^{th} day and they concluded that foliar spray was comparable to soil application of 100 kg $ZnSO_4$. As per the report of Cakmak *et al.* (1999), Zn concentration in the shoot, as well as grain increased significantly by the combined application of 23 kg $ZnSO_4.7H_2O$ ha^{-1} and foliar spray of 220 g $ZnSO_4$ at two stages, tillering and stem elongation in wheat, which was sown in Zn-deficient calcareous soil.

According to the study of Rajkumar *et al.* (2002), Zn concentration in the grain increased with the foliar spray of $ZnSO_4$, but the Zn concentration in straw and grain together increased by the application of 30 mg kg^{-1} Zn in the soil. Khan *et al.* (2003) reported that in rice, whole grain concentration of Zn became very high (32.5 ppm) due to the foliar spray of 0.2 per cent $ZnSO_4$ compared to control and it was comparable to soil application of Zn @ 10 kg ha^{-1}, whereas, foliar application of 0.5 per cent Zn along with root dip has increased the grain concentration (Rao, 2003). According to Yadav *et al.* (2011), foliar spray of 0.2 per cent $ZnSO_4$ increased the grain concentration but it was comparable to soil-applied $ZnSO_4$ and 2 per cent Zn enriched urea.

A study conducted by Dhaliwal *et al.* (2010) in Ludhiana indicated that foliar application of 0.5 per cent of Zn before flowering, at active flowering and maturity stages, significantly increased the concentration of Zn in brown rice. Zhang *et al.* (2012) observed that there was a contribution of high Zn concentration in brown rice by the foliar application of $ZnSO_4$ at 15 days after anthesis.

Zn concentration in white rice or polished rice gets increased to a higher value compared to control in the foliar spray of 1 per cent $ZnSO_4$ at 50 per cent flowering stage and one week after 50 per cent flowering. The Zn content was higher than soil application @ 50 kg ha^{-1} as soil (Ravikiran and Reddy, 2004). The result of work conducted by Phattarukul *et al.* (2012), in sandy loam soils of Thailand indicated that the foliar spray during panicle initiation and one week after flowering in rice increased the Zn concentration in white rice or polished rice by 20-30 per cent than control.

Dhaliwal *et al.* (2010) concluded that Zn concentration in the husk became high *i.e.*, 27.9 to 30.2 mg kg^{-1} due to foliar spray of Zn at different growth stages of rice like before and after flower initiation stage and maximum flowering stage. Jena *et al.* (2011) reported that the application of $ZnSO_4$ @ 50 kg ha^{-1} along with 0.5 per cent foliar spray at pre-flowering and pre-milking stage increased the concentration of Zn in the husk.

Not only $ZnSO_4$ can affect the Zn concentration in plants, but Zn-EDTA can also affect the Zn concentration in straw. Das *et al.* (2004) reported that Zn concentration in straw became significantly highest in the treatment which received the foliar spray of Zn-EDTA twice. Poonia and Shivay (2011) stated that the foliar spray of $ZnSO_4$ at maximum tillering, pre-flowering and flowering stage increased the concentration of Zn in straw up to 178.5 mg kg^{-1}.

2.7.5 Effect on Nutrient Content and Uptake of Rice

Keram *et al.* (2012) studied the effect of application of Zn @ 0, 1.25, 2.5, 5, 10 and 20 kg ha^{-1} as $ZnSO_4$ at the time of sowing along with the recommended doses of N, P and K in rice. In general, all the parameters like yield, harvest index, total nutrient uptake and quality increased up to the highest level of Zn, except total P uptake. Total nutrient uptake of N-123.19 kg ha^{-1}, K-90.86 kg ha^{-1} and Zn-327.74 g ha^{-1} was recorded with the application of 20 kg Zn ha^{-1} with the recommended NPK as compared to control and other treatments, while the total P uptake declined with increasing levels Zn due to their antagonistic effect.

Nayyar *et al.* (2001) stated that the application ZnO improved the N uptake in rice compared to the application of $ZnSO_4$. A study conducted by Fageria (2003) found that the application of $ZnSO_4$ @ 30 kg ha^{-1} improved the uptake of N and K. The application of Zn either through foliar or soil had increased the N content of grain and straw (Khan, 2002).

Total grain and straw uptake of N, P and K increased with the application of 10 t FYM + foliar spray of 0.5 per cent $ZnSO_4$ (Rani, 2013). With the application of Zn @ 10 and 15 mg kg^{-1}, N, P and K uptake increased significantly over control and application of Zn @ 15 mg kg^{-1} increased the uptake of Zn in rice straw as well as rice grains (Hussain, 2015).

2.7.6 Effect on Availability of Nutrients in Soil

Application of FYM @ 10 t ha^{-1} + soil application of $ZnSO_4$ @ 50 kg ha^{-1} increased the soil Zn content as well as N content whereas, availability of other nutrients like P and Mg was decreased (Rani, 2013).

The availability of Zn increased with the application of $ZnSO_4$ @ 20 kg ha^{-1} in the soil for correcting the Zn deficiency (White and Broadley, 2011).

2.7.7 Effect on Economics of Cultivation

Sharma *et al.* (1999) reported that the net income and B: C ratio were the highest with two foliar sprays of Zn along with soil application of $ZnSO_4$ @ 36 kg ha^{-1}. Ghatak *et al.* (2005) reported the highest net income of Rs. 4, 832 ha^{-1} by the application of recommended NPK + soil Zn application.

Foliar application of 0.5 per cent $ZnSO_4$ at panicle initiation and heading stages increased net returns of rice up to Rs. 54, 180 ha^{-1} (Rani, 2013).

A study conducted by Hussain (2015) with different Zn sources revealed that the application of Zn @ 6 kg ha^{-1} increased the net returns compared to Zn-EDTA.

Application of Zn in under deficient soil condition is likely to enhance the growth characters and yield attributes of rice, apart from these timely and precise application of Zn through different methods helps to overcome Zn malnutrition in the long run via increase the Zn content in edible part of rice.

Materials and Methods

3. MATERIALS AND METHODS

The field experiment entitled "Agronomic biofortification of zinc in rice (*Oryza sativa* L.) was conducted during the season of *kharif* 2018 in farmer's field at Chirayinkeezhu, Thiruvananthapuram. The materials used and the methods followed during the conduct of experiment are presented in this chapter.

3.1 EXPERIMENTAL SITE

3.1.1. Location

The experimental site was geographically situated in Keezhuvillam village of Chirayankeezhu block, Thiruvananthapuram district at 8°39'21'' N latitude and 76°48' 5'' E longitude, at an altitude of 9 m above mean sea level.

3.1.2 Weather

Weather data recorded during crop period (08-05-2018 to 06-09-2018) are presented in Appendix I and graphically represented in Fig 1. Maximum temperature ranged from 29.5° C to 33.2° C, with an average of 31.3°C. Minimum temperature ranged from 22.6° C to 25.7° C, with an average of 24.1°C. During the corresponding period, relative humidity ranged from 71 to 97 per cent with an average of 84 per cent. Total rainfall of 1198 mm was received during the crop growth period of 121 days, which was insufficient for crop growth and therefore supplemented with need based irrigation. Total number of rainy days during the crop period was 61.

3.1.3. Soil

A composite soil sample was collected at a depth of 15 cm before commencement of the experiment and analyzed for chemical composition and physico-chemical properties. Data on analysis of soil of the experimental site are presented in Tables 1 and 2. Chemical properties of soil were rated as per the Package of Practices recommendations of Kerala Agricultural University (KAU, 2016). The soil of the experimental site was clay loam in texture, very strongly acidic in reaction, low in organic carbon and available N, deficient in available Ca, Mg and Zn, medium in available S and K and high in available P.

3.1.4. Cropping History

The experimental site was lying fallow during summer months before the experiment and prior to that it was under bulk crop of rice during *kharif* and *rabi* seasons.

3.2. MATERIALS

3.2.1. Crop and Variety

The rice variety tested was Uma (MO 16) released from Rice Research Station, Moncompu. It is a medium duration variety (120 to 135 days) during *kharif* and 115 to 120 days during *rabi* and suitable for cultivation in all three rice growing seasons of Kerala. Grains are medium bold with red coloured bran. The variety is non lodging, resistant to brown plant hopper and gall midge biotype 5 (Devika *et al.*, 2006).

3.2.2. Manures and Fertilizers

Urea (46% N), Rajphos (20% P_2O_5) and Muriate of potash (60% K_2O) were used as sources of N, P and K respectively. Zn was applied to the crop through fertilizer grade zinc sulphate heptahydrate ($ZnSO_4.7H_2O$) containing 22% Zn.

Table 1. Mechanical composition of soil of the experimental site

Sl. No	Soil Fraction	Content	Method
1	Sand, %	34.6	Bouyoucos hydrometer method (Bouyoucos, 1962)
2	Silt, %	36.8	
3	Clay, %	28.6	
Textural class: Clay loam			

Table 2 Physico - Chemical properties of the soil of the experimental site

Sl. No	Parameter	Content	Rating	Method and reference
1	pH	4.56	Very strongly acidic	1:2.5 soil solution ratio using potentiometric method with pH meter (Jackson, 1973)
2	Electrical Coductivity (dS m^{-1})	0.09	Normal	Digital electrical conductivity meter (Jackson, 1973)
3	Organic carbon (%)	0.66	Low	Walkley and Black rapid titration method (Jackson, 1973)
4	Available N (kg ha^{-1})	168	Low	Alkaline permanganate method (Subbiah and Asija, 1956)
5	Available P (kg ha^{-1})	45.2	High	Dickman and Bray's molybdenum blue method using a spectrophotometer (Jackson, 1973)
6	Available K (kg ha^{-1})	198	Medium	Ammonium acetate method (Jackson, 1973)
7	Available Ca (mg kg^{-1})	130	Deficient	EDTA titration method (Tucker and Kurtz,1960)
8	Available Mg (mg kg^{-1})	89	Deficient	EDTA titration method (Tucker and Kurtz,1960)
9	Available S (mg kg^{-1})	5.7	Sufficient	Turbidimetric method (Chesnin and Yien, 1950)
10	Available Zn (mg kg^{-1})	0.79	Deficient	Extraction using 0.5 N HCl and atomic absorption spectroscopy (Sims and Johnson,1991)

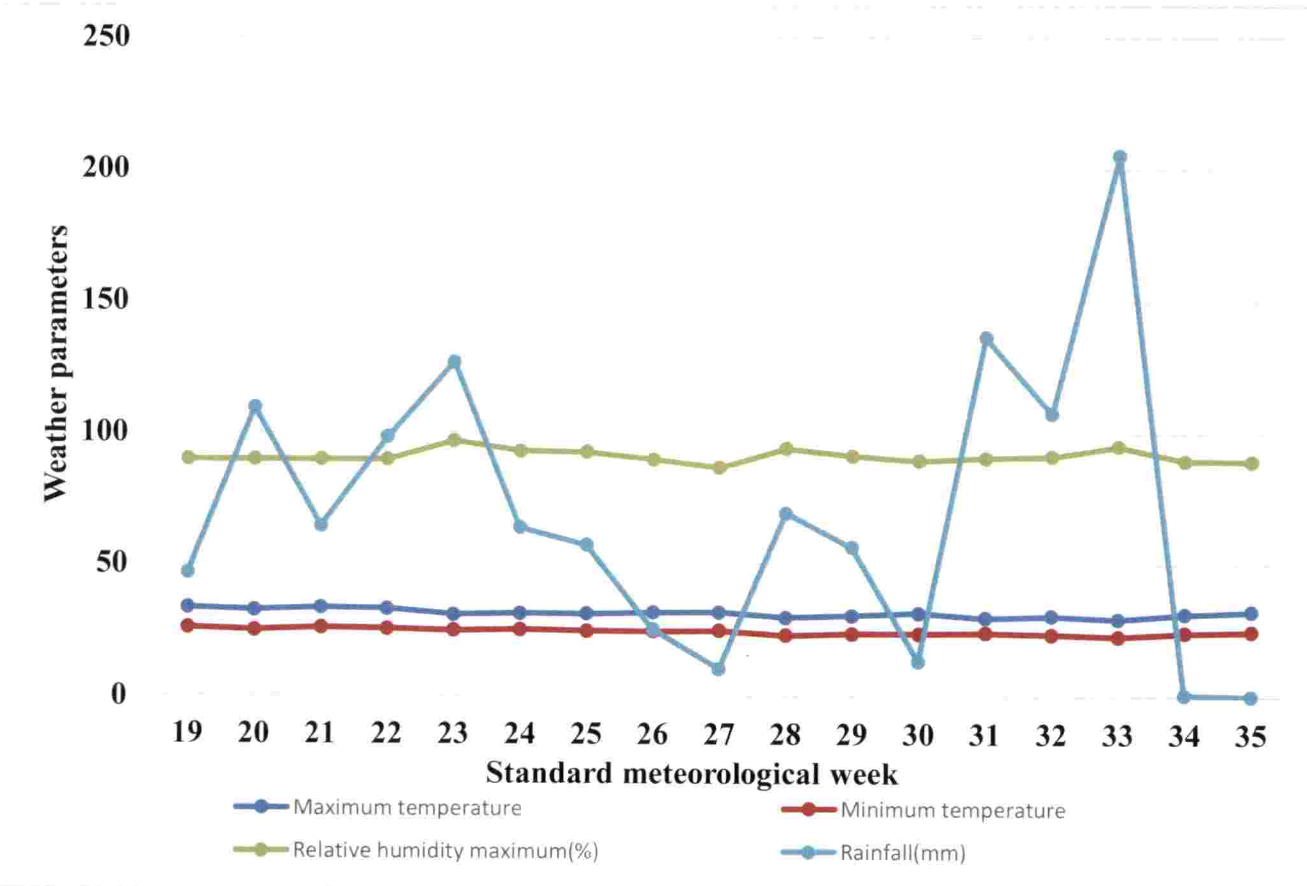


Fig.1. Weather data during *kharif* cropping period (May to September 2018)

3.3 METHODS

3.3.1 Design and Layout

Design : RBD

Treatments : 8

Replication : 3

Plot size : 5 m x 4 m

Treatments

T_1 : Control (without Zn)

T_2 : $ZnSO_4$ @ 20 kg ha^{-1} basal (*adhoc* recommendation)

T_3 : $ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages (*adhoc* recommendation)

T_4 : $ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages

T_5 : $ZnSO_4$ @ 0.1% foliar at PI, B and M stages

T_6 : $ZnSO_4$ @ 0.1% foliar at B and M stages

T_7 : $ZnSO_4$ @ 0.1% foliar at MT and M stages

T_8 : $ZnSO_4$ @ 0.1% foliar at M stage

MT -Maximum tillering
PI -Panicle initiation
B -Booting
M -Milking

*All treatments will receive recommended lime @ 600 kg ha^{-1}, farm yard manure @ 5 t ha^{-1} and N: P_2O_5: K_2O @ 90: 45: 45 kg ha^{-1}(KAU, 2016)

3.3.2. Crop Management

3.3.2.1. Nursery

The nursery area was ploughed, levelled and made weed free. FYM @ 1 kg m^{-2} was applied and incorporated. Pre-germinated seeds of rice (variety Uma) was sown @ 70 kg ha^{-1}. The nursery was managed as per the KAU POP.

3.3.2.2. Main Field

The experimental area was ploughed twice, puddled and levelled. Weeds and stubbles were removed. The experimental area was divided into three blocks of eight plots each. The blocks and plots were separated with bunds of 30 cm width. Irrigation and drainage channels were provided for all the plots.

3.3.2.3. Application of Lime

Lime @ 600 kg ha^{-1} was applied in two split doses i.e. 350 kg ha^{-1} just after the second tillage and the remaining at tillering stage (25 DAT).

3.3.2.4. Manures and Fertilizers

Well decomposed farmyard manure (FYM) @ 5 t ha^{-1} was applied uniformly to all the plots at the time of land preparation. Urea, rock phosphate and muriate of potash were applied to all the plots to supply the major nutrients @ 90:45:45 kg NPK ha^{-1} as per KAU POP (KAU, 2016). Half the dose of N, entire dose of P and half K were applied basally. The remaining N and K fertilizers were applied five days before PI stage. Foliar spray of $ZnSO_4.7H_2O$ was done at various stages of rice as such as MT, (25 DAT), 5 days before PI (40 DAT), booting (60 DAT), milking (75 DAT) as per the treatments. Basal soil application of $ZnSO_4.7H_2O$ @ 20 kg ha^{-1} was applied as per the treatment.

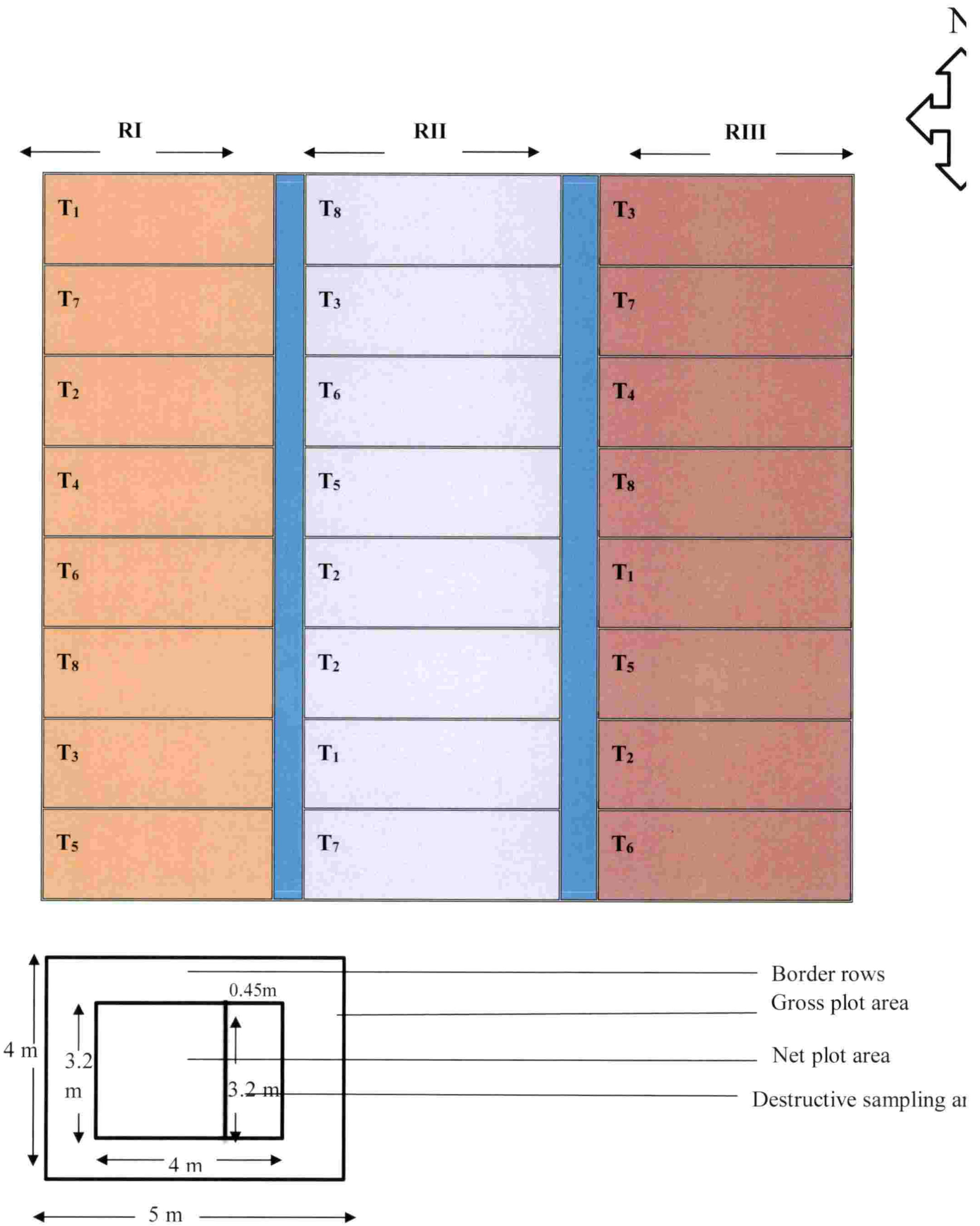


Fig. 2 Lay out of the experiment

Plate: 1 General view of experimental plot

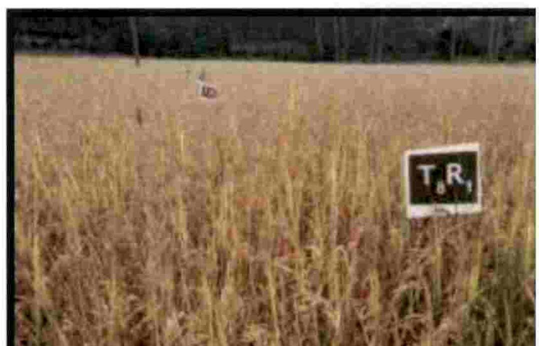
Transplanting stage

Maximum tillering stage

Panicle initiation stage

Boot leaf stage

Milking stage



Harvest Stage

Plate: 2 Different growth stages of experimental crop of rice

3.3.2.5 Transplanting

Twenty one days old seedlings were uprooted from the nursery and transplanted @ 2 to 3 seedlings per hill at a spacing of 20 cm x 15 cm, at a depth of 3-4 cm. Gap filling was done one week after transplanting so as to maintain uniform plant population, maintaining two seedlings per hill.

3.3.2.6 Water Management

The water level was maintained at about 1.5 cm during transplanting. Thereafter the water level was increased gradually to about 5 cm throughout the growth period, with occasional drainage. Water was drained 10 days before harvest.

3.3.2.7 Weed Management

The field was maintained weed free upto 45 DAT with two hand weeding @ 20 DAT and 40 DAT.

3.3.2.8 Plant Protection

Rice bug (*Leptocorisa acuta*) was observed in the field during flowering stage. Recommended prophylactic measure was undertaken to control the pest. Incidence of disease was not observed in the plot.

3.3.2.9 Harvest

The crop in the individual plots was harvested leaving two rows on all sides as border rows. The net plot area was harvested separately, threshed, winnowed and weight of grain and straw were recorded separately from the individual plots.

3.4 OBSERVATIONS

3.4.1 Growth Characters

Two rows from all sides of gross plot (5m x 4m) were left as border rows. Six hills were selected randomly from the net plot area (4.0 m x 3.2 m) inside the gross plot and tagged as sample hills. Observations on plant height, tillers m^{-2} and LAI were recorded from the sample plants and the mean values were worked out. Six hills were selected randomly from destructive sampling area (0.45 m x 3.2 m) outside the net plot area leaving border rows and tagged as sample hills. Observations on root length, root weight, root volume and dry matter production were recorded from the sample hills and mean values worked out.

3.4.1.1 Plant Height

Plant height was recorded at PI, MT and at harvest stages using the method described by Gomez (1972). The height was measured from the base of the plant to the tip of the longest leaf or tip of the longest ear head, whichever was longer and the average was recorded in centimeters (cm).

3.4.1.2 Tillers m^{-2}

Tiller count was taken from six tagged observation hills at MT, PI and harvest stages and the mean values recorded as number of tillers m^{-2}.

3.4.1.3 Leaf Area Index

The LAI was calculated at the PI stage using method suggested by Yoshida *et al.* (1976). The maximum length ‘l’ and width ‘w’ of all leaves of the middle most tiller of the six sample hills were recorded and LAI was calculated.

Leaf area of single leaf = k x l x w;

where 'k' is adjustment factor taken as 0.75 at PI stage (Yoshida *et al.*, 1976)

$$\text{LAI} = \frac{\text{Sum of leaf area of six sample hills (cm}^{2)}}{\text{Area of land covered by six sample hills (cm}^2)}$$

3.4.1.4 Root Length

Six hills were randomly selected from destructive sampling area. The sample hills from destructive sampling area of each plot were uprooted during MT and harvest stages, washed well and root length was measured (Misra and Ahmed, 1989). The mean value expressed in cm.

3.4.1.5 Root Weight

The fresh weight of the washed roots from six hills plants were noted during MT and harvest stages and the mean value expressed in g per hill (Misra and Ahmed, 1989).

3.4.1.6 Root Volume

Root volume per plant was found out by water displacement method noted during MT and harvest stages and the mean value was expressed in cubic centimeter per hill (Misra and Ahmed, 1989)

3.4.1.7 Dry Matter Production

At MT and PI stages, six sample hills were randomly selected and uprooted from the area demarcated for destructive sampling outside the net plot area leaving the border rows. The plant samples were washed, air dried in shade and then oven dried to a constant weight. The total dry matter production was computed and was expressed in g per hill. At harvest stage, six sample hills were uprooted, separated into grain and straw, air dried under shade and later oven dried to a constant weight. The dry weight of each sample plant was recorded separately as grain, straw and total dry matter using an electronic weighing balance and expressed in g per hill.

3.4.2 Yield Attributes and Yield

3.4.2.1 Days to 50 per cent Flowering

Number of days taken by 50 per cent of the hills to flower was recorded.

3.4.2.2 Productive Tillers m^{-2}

Tiller count was taken from six tagged observation hills at MT, PI and harvest stages and the mean values recorded as number of tillers m^{-2}.

3.4.2.3 Length of the Panicle

Ten panicles were selected at random from net plot area and panicle length was measured as the length from the neck of the panicle to the tip. The mean panicle length was expressed in cm.

3.4.2.4 Grain Weight per Panicle

The grains from the 10 randomly selected panicles were removed, dried, weighed and the weight was recorded as grain weight per panicle in g.

3.4.2.5 Filled Grains per Panicle

The filled grains were counted from the 10 randomly selected panicles from each plot and expressed as the mean number of filled grains per panicle.

3.4.2.6 Percent Filled Grains per Panicle

The total number of spikelets and number of filled grains were counted from 10 randomly selected panicles and the per cent filled grains per panicle was expressed using the formula:

$$\text{Per cent filled grains per panicle} = \frac{\text{Number of filled grains per panicle}}{\text{Total number of grains per panicle}} \times 100$$

3.4.2.7 Thousand Grain Weight

One thousand grains were counted from the cleaned and dried produce from the net plot area of each plot and the weight of the grains was recorded in g.

3.4.2.8 Grain Yield

The net plot area was harvested individually, threshed, cleaned, dried and weighed to express the grain yield in kg ha^{-1} at 14 per cent moisture.

3.4.2.9 Straw Yield

The straw harvested from net plot area was dried to constant weight under sun and then weighed to express the straw yield in kg ha^{-1}.

3.4.2.10 Total Dry Matter Production

At harvest stage, six sample hills were uprooted, separated into grain and straw, air dried and later oven dried to a constant weight. The dry weight of each sample plant was recorded separately as grain, straw and total dry matter using an electronic weighing balance and expressed in kg ha^{-1}.

3.5. CHEMICAL ANALYSIS

3.5.1 Plant Analysis

3.5.1.1 Quality Attributes of Whole Grain and its Milled Fractions

The whole grain rice from each plot was cleaned to remove foreign matter. It was then washed to remove dust, air dried and oven dried to a constant weight. Whole grain rice was milled to obtain different fractions viz. brown rice, white rice, bran and husk. The method used in analysis of whole grain and its milled fractions are presented in Table 3.

Table 3. Standard analytical methods followed for analysis of whole grain and its milled fractions.

Sl.No	Parameter	Method	Reference
1	Zinc	Nitric-Perchloric acid (9:4) digestion and Atomic absorption spectrometry	Jackson (1973)
2	Starch	Titrimetric method	Aminoff *et al.,(*1970)
3	Crude protein	The N content determined by micro Kjeldhals method multiplied by coefficient factor 5.95.This coefficient factor is based on nitrogen content (16.8%) of major rice protein glutelin	Juliano (1979)
4	Phytic acid	Phytic acid is extracted with trichloro acetic acid and precipitated as ferric salt. Fe content is determined colorimetrically and phytic acid P is calculated using 4Fe:6P molecular ratio in the precipitate	Sadasivam and Manickam (1992)

Table 4. Standard analytical methods followed for plant analysis

Sl.No	Parameter	Method	Reference
1	Nitrogen	Micro Kjedahl method	Jackson (1973)
2	Phosphorus	Nitric-Perchloric acid (9:4) digestion and spectrometry using Vanado molybdo phosphoric yellow colour method	Jackson (1973)
3	Potassium	Nitric-Perchloric acid (9:4) digestion and flamephotometry	Jackson (1973)
7	Calcium	Nitric-Perchloric acid (9:4) digestion and versanate titration	Piper (1967)
5	Magnesium	Nitric-Perchloric acid (9:4) digestion and versanate titration	Piper (1967)
6	Sulphur	Nitric-Perchloric acid (9:4) digestion and turbidimetry	Chesnin and Yien (1950)
7	Zinc	Nitric-Perchloric acid (9:4) digestion and Atomic absorption spectrometry	Jackson (1973)

3.5.1.2 Nutrient Content of Rice Grain and Straw

The grain and straw were collected from each plot and analyzed separately for total N, P, K, Ca, Mg, S, and Zn content. The methods used in plant analysis are presented in Table 4.

3.5.2 Soil analysis

After the harvest, soil samples were collected from each plot separately and analyzed for pH, EC, organic carbon, available N, P, K, Ca, Mg, S and Zn adopting the methods presented in Table 2.

3.6 COMPUTED INDICES

3.6.1 Harvest Index

Harvest index for each treatment was calculated from grain and straw yield using the formula put forth by Donald and Hanblin (1976)

$$\text{Harvest index} = \frac{\text{Economic yield}}{\text{Biological yield}}$$

3.6.2 Phytate: zinc molar ratio

Phytate: zinc molar ratio was calculated using the formula put forth by Rani (1991)

$$\text{Phytic acid: Zinc molar ratio} = \frac{\left(\dfrac{\text{Phytic acid concentration (mg kg}^{-1})}{660}\right)}{\left(\dfrac{\text{Zinc concentration (mg kg}^{-1})}{65.4}\right)}$$

Where 660 is molecular weight of phytic acid and 65.4 is atomic weight of zinc

Index of bioavailability	Phytic acid: zinc molar ratio
High	<5: 1
Medium	5-15: 1
Low	>15: 1

(Graham, 1984)

3.6.2 Nutrient Uptake

Nutrient uptake was calculated using the formula

$$\text{Nutrient uptake (kg ha}^{-1}\text{)} = \frac{\text{Nutrient content (\%) x Dry matter production (kg ha}^{-1}\text{)}}{100}$$

3.6.2 Crop Recovery Efficiency

CRE_{Zn} defined as increase in Zn uptake in Zn treated plant over Zn untreated plant per unit quantity of Zn applied, expressed as percentage. (Shivay *et al.*, 2016)

$$CRE_{Zn}(\%) = \frac{\text{Zinc uptake in whole plant of zinc fertilized plot (kg ha}^{-1}\text{)} - \text{Zinc uptake in whole plant of zinc unfertilized plot (kg ha}^{-1}\text{)}}{\text{Quantity of zinc applied (kg ha}^{-1}\text{)}} \times 100$$

3.6.2 Biofortification Recovery Efficiency

(BRE_{Zn} defined as increase in Zn uptake in edible part of the Zn treated plant over Zn untreated plant per unit quantity of Zn applied, expressed as percentage (Shivay *et al.*, 2008)

$$BRE_{Zn}(\%) = \frac{\text{Zinc uptake in grains of zinc fertilized plot (kg ha}^{-1}\text{)} - \text{Zinc uptake in grains of zinc unfertilized plot (kg ha}^{-1}\text{)}}{\text{Quantity of zinc applied (kg ha}^{-1}\text{)}} \times 100$$

3.7 ECONOMIC ANALYSIS

3.7.1 Net Income

Net income was computed using the formula

Net income (₹ ha^{-1}) = Gross income (₹ ha^{-1}) – Total expenditure (₹ ha^{-1})

3.7.2 Benefit Cost Ratio (B: C ratio)

Benefit cost ratio was calculated using the formula

$$\text{B: C ratio} = \frac{\text{Gross income (₹ ha}^{-1}\text{)}}{\text{Total expenditure (₹ ha}^{-1}\text{)}}$$

3.8 Statistical Analysis

The data generated from the experiment were statistically analyzed using Analysis of Variance technique (ANOVA) as applied to Randomized Block Design (Panse and Sukhatme, 1967) and the significance was tested using F test (Snedecor and Cochran, 1967). Wherever the F values were significant, critical difference was worked out at five per cent probability level. The significance of the control as compared against the treatments was also tested. The treatment *vs.* control comparison was denoted as "S" when significant and "NS" when not significant.

Results

4. RESULTS

The study entitled "Agronomic biofortification of Zn in rice (*Oryza sativa* L.)" was conducted in farmer's field in Chirayinkeezhu, Thiruvananthapuram district during May to September 2018. The main objective of the study was to assess the effect of time and method of Zn application on growth, yield, Zn biofortification and Zn bioavailability in transplanted rice. The results of the experiment are presented in this chapter.

4.1 GROWTH CHARACTERS

4.1.1 Plant Height

The results on effect of time and method of Zn application on plant height at MT, PI and harvest stages are presented in Table 5. Treatments did not have any significant influence on plant height.

4.1.2 Tillers m^{-2}

Data on number of tillers m^{-2} at MT, PI and harvest stages are presented in Table 6. There was no significant difference in number of tillers m^{-2} during different growth stages of the crop due to treatments.

4.1.3 Leaf Area Index

The result on LAI at PI stage is presented in Table 7. Among the different treatments, T_7 ($ZnSO_4$ @ 0.1% foliar at MT and M stages) produced higher LAI (5.26), which was on par with T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal), T_3 ($ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages) and T_4 ($ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages). The lowest LAI (4.05) was found in T_1 (Control-without Zn).

Table 5. Effect of time and method of Zn application on plant height, cm

Treatments	MT	Panicle initiation	Harvest
T_1: Control (without Zn)	73.9	82.1	109
T_2:$ZnSO_4$ @ 20 kg ha^{-1} basal (*adhoc* recommendation)	75.7	85.2	112
T_3: $ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages (*adhoc* recommendation)	74.6	84.9	111
T_4: $ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages	74.3	85.4	113
T_5: $ZnSO_4$ @ 0.1% foliar at PI, B and M stages	75.3	84.1	111
T_6: $ZnSO_4$ @ 0.1% foliar at B and M stages	74.9	84.1	110
T_7: $ZnSO_4$ @ 0.1% foliar at MT and M stages	74.0	85.5	114
T_8: $ZnSO_4$ @ 0.1% foliar at M stage	75.5	83.3	110
SEm (±)	0.4	1.8	1.4
CD (0.05)	NS	NS	NS

MT-Maximum tillering; PI-Panicle initiation; B-Booting; M-Milking; NS-not significant

Table 6. Effect of time and method of Zn application on number of tillers m^{-2}, nos.

Treatments	MT	Panicle initiation	Harvest
T_1: Control (without Zn)	541	492	439
T_2: $ZnSO_4$ @ 20 kg ha^{-1} basal (*adhoc* recommendation)	466	423	379
T_3: $ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages (*adhoc* recommendation)	480	439	391
T_4: $ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages	466	426	380
T_5: $ZnSO_4$ @ 0.1% foliar at PI, B and M stages	520	476	428
T_6: $ZnSO_4$ @ 0.1% foliar at B and M stages	518	470	423
T_7: $ZnSO_4$ @ 0.1% foliar at MT and M stages	459	420	374
T_8: $ZnSO_4$ @ 0.1% foliar at M stage	532	480	431
SEm (±)	31.0	28.6	25.6
CD (0.05)	NS	NS	NS

MT-Maximum tillering; PI-Panicle initiation; B-Booting; M-Milking; NS-not significant

Table 7. Effect of time and method of Zn application on leaf area index

Treatments	LAI at panicle initiation
T_1: Control (without Zn)	4.05
T_2: $ZnSO_4$ @ 20 kg ha^{-1} basal (*adhoc* recommendation)	5.23
T_3: $ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages (*adhoc* recommendation)	5.20
T_4: $ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages	5.22
T_5: $ZnSO_4$ @ 0.1% foliar at PI, B and M stages	4.14
T_6: $ZnSO_4$ @ 0.1% foliar at B and M stages	4.18
T_7: $ZnSO_4$ @ 0.1% foliar at MT and M stages	5.26
T_8: $ZnSO_4$ @ 0.1% foliar at M stage	4.09
SEm (±)	0.28
CD (0.05)	0.856

MT-Maximum tillering ; PI-Panicle initiation; B-Booting; M-Milking;

4.1.4 Root Length

Data on root length at MT and harvest stages are presented in Table 8. At MT stage, the longest roots (20.3 cm) was observed in the treatment T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal) which was significantly superior to all the other treatments.

At harvest stage, the highest root length (22.4 cm) was found in T_4 ($ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages), which was on par with all the treatments, except T_5, T_6, T_1 and T_8. The shortest roots (16.7 cm) were recorded in T_8 ($ZnSO_4$ @ 0.1% foliar at M stage).

4.1.5 Root Weight

Data obtained on root weight at MT and harvest stages are given in Table 9. At MT stage, significantly higher root weight was reported (4.5 g per hill) in T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal) .

At harvest stage, T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal) had showed the highest root weight (23.2 g per hill), which was on par with T_3, T_4, and T_7. The lowest root weight (17.2 g per hill) was observed in T_8 ($ZnSO_4$ @ 0.1% foliar at M stage) which was on par with T_1, T_5 and T_6.

4.1.6 Root Volume

A perusal of data on root volume at MT and harvest stages (Table 10) indicates that root volume at MT stage was significantly influenced by treatments and the significantly highest root volume (8.95 cc) was recorded in T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal).

At harvest stage, the highest root volume (40.1 cc) was found in treatment T_4 ($ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages) which was on par with T_2, T_3 and

T_7. The lowest root volume (30.9 cc) was observed in T_8 ($ZnSO_4$ @ 0.1% foliar at M stage) which was comparable to T_1, T_5 and T_6.

4.1.7 Dry Matter Production

Data on dry matter production (g per hill) at MT, PI and harvest stages are presented in Table 11. The dry matter production at MT stage was the highest (20.4 g per hill) in the treatment, T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal) which was superior to all the other treatments.

At PI stage, maximum dry matter production (28.2 g per hill) was found in T_7 ($ZnSO_4$ @ 0.1% foliar at MT and M stages) which was on par with T_2, T_3 and T_4. The lowest dry matter production (23.7 g per hill) was found in T_1 (Control-without Zn) was comparable with T_5, T_6 and T_8.

At harvest stage, significantly higher dry matter production (37.8 g per hill) was noticed in T_7 ($ZnSO_4$ @ 0.1% foliar at MT and M stages), which was on par with T_2, T_3 and T_4. The lowest dry matter production (29.3 g per hill) was found in T_1 (Control -without Zn) which was comparable with T_5, T_6 and T_8.

Table 8. Effect of time and method of Zn application on root length, cm

Treatments	Maximum tillering	Harvest
T_1: Control (without Zn)	15.1	17.2
T_2: $ZnSO_4$ @ 20 kg ha^{-1} basal (*adhoc* recommendation)	20.3	21.9
T_3: $ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages (*adhoc* recommendation)	15.8	21.7
T_4: $ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages	13.6	22.4
T_5: $ZnSO_4$ @ 0.1% foliar at PI, B and M stages	15.6	18.3
T_6: $ZnSO_4$ @ 0.1% foliar at B and M stages	15.5	17.9
T_7: $ZnSO_4$ @ 0.1% foliar at MT and M stages	15.8	22.0
T_8: $ZnSO_4$ @ 0.1% foliar at M stage	14.1	16.7
SEm (±)	0.7	0.8
CD (0.05)	2.06	2.30

MT-Maximum tillering; PI-Panicle initiation; B-Booting; M-Milking

Table 9. Effect of time and method of Zn application on root weight, g per hill

Treatments	Maximum tillering	Harvest
T_1: Control (without Zn)	2.7	17.6
T_2: $ZnSO_4$ @ 20 kg ha^{-1} basal (*adhoc* recommendation)	4.5	23.2
T_3: $ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages (*adhoc* recommendation)	3.2	22.9
T_4: $ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages	2.4	22.8
T_5: $ZnSO_4$ @ 0.1% foliar at PI, B and M stages	2.9	18.8
T_6: $ZnSO_4$ @ 0.1% foliar at B and M stages	2.9	18.0
T_7: $ZnSO_4$ @ 0.1% foliar at MT and M stages	3.2	23.1
T_8: $ZnSO_4$ @ 0.1% foliar at M stage	2.5	17.2
SEm (±)	0.2	1.0
CD (0.05)	0.65	3.18

MT-Maximum tillering; PI-Panicle initiation; B-Booting; M-Milking

Table. 10. Effect of time and method of Zn application on root volume, cc

Treatments	Maximum tillering	Harvest
T_1: Control (without Zn)	6.99	31.4
T_2: $ZnSO_4$ @ 20 kg ha^{-1} basal (*adhoc* recommendation)	8.95	39.4
T_3: $ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages *(adhoc* recommendation)	7.03	38.9
T_4: $ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages	7.34	40.1
T_5: $ZnSO_4$ @ 0.1% foliar at PI, B and M stages	7.01	33.2
T_6: $ZnSO_4$ @ 0.1% foliar at B and M stages	6.51	31.0
T_7: $ZnSO_4$ @ 0.1% foliar at MT and M stages	7.12	39.8
T_8: $ZnSO_4$ @ 0.1% foliar at M stage	6.62	30.9
SEm (±)	0.4	1.3
CD (0.05)	1.21	4.17

MT-Maximum tillering; PI-Panicle initiation; B-Booting; M-Milking

Table 11. Effect of time and method of Zn application on dry matter production, g per hill

Treatments	Maximum tillering	Panicle initiation	Harvest
T_1: Control (without Zn)	18.1	23.7	29.3
T_2: $ZnSO_4$ @ 20 kg ha^{-1} basal (*adhoc* recommendation)	20.4	28.1	37.6
T_3: $ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages *(adhoc* recommendation)	18.5	27.8	37.5
T_4: $ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages	18.0	28.2	37.7
T_5: $ZnSO_4$ @ 0.1% foliar at PI, B and M stages	18.4	24.8	29.6
T_6: $ZnSO_4$ @ 0.1% foliar at B and M stages	18.3	24.9	29.9
T_7: $ZnSO_4$ @ 0.1% foliar at MT and M stages	18.4	28.2	37.8
T_8: $ZnSO_4$ @ 0.1% foliar at M stage	18.5	23.7	29.6
SEm (±)	0.3	0.9	2.3
CD (0.05)	1.00	2.65	6.82

MT-Maximum tillering; PI-Panicle initiation; B-Booting; M-Milking

4.2 YIELD ATTRIBUTES AND YIELD

4.2.1 Days to 50 per cent Flowering

The result on days to 50 per cent flowering are given in Table 12. Data on days to 50 per cent flowering did not show any significant variation among treatments.

4.2.2 Productive Tillers m^{-2}

Productive tillers m^{-2} was not significantly influenced by time and method of Zn application (Table 12).

4.2.3 Length of the Panicle

Data on length of the panicle are presented in Table 12. The panicle length was significantly higher (20.9 cm) for the treatment T_7 ($ZnSO_4$ @ 0.1% foliar at MT and M stages), which was on par with T_2, T_3 and T_4. Least length of the panicle (17.4 cm) was observed in T_1 (Control -without Zn) which was comparable to T_5, T_6 and T_8.

4.2.4 Grain Weight per Panicle

Data on weight of the grains per panicle presented in the Table 12 indicated significantly higher grain weight per panicle (1.88 g) in T_7 ($ZnSO_4$ @ 0.1% foliar at MT and M stages) which was on par with T_2, T_3 and T_4. The lowest grain weight per panicle (1.25 g) was in treatment T_1 (Control -without Zn) which was comparable to T_5, T_6 and T_8.

Table 12. Effect of time and method of Zn application on days to 50 per cent flowering, productive tillers m^{-2}, length of the panicle and grain weight per panicle

Treatments	Days to 50 per cent flowering (in DAT)	Productive tillers m^{-2}	Length of the panicle (cm)	Grain weight per panicle (g)
T_1: Control (without Zn)	75.5	411	17.4	1.25
T_2:$ZnSO_4$ @ 20 kg ha^{-1} basal (*adhoc* recommendation)	74.4	354	20.3	1.87
T_3: $ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages *(adhoc* recommendation)	74.4	368	20.1	1.79
T_4:$ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages	74.3	355	20.8	1.85
T_5: $ZnSO_4$ @ 0.1% foliar at PI, B and M stages	75.2	398	17.8	1.30
T_6: $ZnSO_4$ @ 0.1% foliar at B and M stages	75.2	395	17.8	1.31
T_7: $ZnSO_4$ @ 0.1% foliar at MT and M stages	74.3	349	20.9	1.88
T_8: $ZnSO_4$ @ 0.1% foliar at M stage	75.5	405	17.7	1.27
SEm (±)	1.1	24	0.5	0.08
CD (0.05)	NS	NS	1.64	0.225

MT-Maximum tillering; PI-Panicle initiation; B-Booting; M-Milking; NS- not significant

4.2.5 Filled Grains per Panicle

Observed data on number of filled grains per panicle presented in Table 13 revealed that T_7 ($ZnSO_4$ @ 0.1% foliar at MT and M stages) resulted in higher number of filled grains per panicle (127 nos.), which was on par with T_2, T_3 and T_4. Significantly lower number of filled grains per panicle (84 nos.) was recorded with T_1 (Control-without Zn) which was comparable with T_5, T_6 and T_8.

4.2.6 Per cent Filled Grains per Panicle

The effect of time and method of Zn application on per cent filled grains per panicle is given in the Table 13. T_7 ($ZnSO_4$ @ 0.1% foliar at MT and M stages) had higher per cent filled grain per panicle (83.9%) which was on par with T_2, T_3 and T_4. The lowest per cent filled grains per panicle (74 %) was in T_1 (Control -without Zn) which was comparable to T_5, T_6 and T_8.

4.2.7 Thousand Grain Weight

Data on thousand grain weight furnished in the Table 13 revealed that higher thousand grain weight (26.2 g) was recorded in T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal) which was comparable to T_3, T_4 and T_7. The lower thousand grain weight (23.5 g) was recorded in T_8 ($ZnSO_4$ @ 0.1% foliar at M stage) which was on par to T_1, T_5 and T_6.

Table 13. Effect of time and method of Zn application on filled grains per panicle, percent filled grains per panicle and thousand grain weight

Treatments	Filled grains per panicle (nos.)	Per cent filled grains per panicle	Thousand grain weight (g)
T_1: Control (without Zn)	84	74.0	23.6
T_2:$ZnSO_4$ @ 20 kg ha^{-1} basal (*adhoc* recommendation)	125	83.7	26.2
T_3: $ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages *(adhoc* recommendation)	122	83.5	25.4
T_4:$ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages	125	83.7	25.9
T_5: $ZnSO_4$ @ 0.1% foliar at PI, B and M stages	89	74.2	23.7
T_6: $ZnSO_4$ @ 0.1% foliar at B and M stages	90	74.3	23.6
T_7: $ZnSO_4$ @ 0.1% foliar at MT and M stages	127	83.9	26.0
T_8: $ZnSO_4$ @ 0.1% foliar at M stage	87	74.2	23.5
SEm (±)	7	2.6	0.5
CD (0.05)	20.00	7.87	1.41

MT-Maximum tillering; PI-Panicle initiation; B-Booting; M-Milking

4.2.8 Grain Yield

A critical analysis of data on grain yield presented in Table 14 showed that treatments significantly affected grain yield. Maximum grain yield (6605 kg ha^{-1}) was recorded in T_7 ($ZnSO_4$ @ 0.1% foliar at MT and M stages) which was comparable to T_2, T_3 and T_4. The lowest grain yield (5093 kg ha^{-1}) was found in T_1 (Control-without Zn) was comparable with T_5, T_6 and T_8.

4.2.9 Straw Yield

Treatments had a significant effect on straw yield (Table 14). The treatment T_7 ($ZnSO_4$ @ 0.1% foliar at MT and M stages) produced significantly higher straw yield (7024 kg ha^{-1}) which was on par with T_2, T_3 and T_4. The lowest straw yield (5536 kg ha^{-1}) was found in T_1 (Control -without Zn), which was comparable to T_5, T_6 and T_8.

4.2.10 Harvest Index

Data on harvest index presented in Table 14 showed no significant difference due to treatments.

4.2.11 Total Dry Matter Production

Comparing data on total dry matter production among treatments, significantly higher total dry matter production (12585 kg ha^{-1}) was observed in T_7 ($ZnSO_4$ @ 0.1% foliar at MT and M stages), which was on par with treatments T_2, T_3, and T_4 (Table 14). The lowest dry matter production (9773 kg ha^{-1}) was recorded in T_1 (Control - without Zn) was comparable to treatments T_5, T_6 and T_8.

Table 14. Effect of time and method of Zn application on grain yield, straw yield, harvest index and total dry matter production

Treatments	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})	Harvest Index	Total dry matter production (kg ha^{-1})
T_1: Control (without Zn)	5093	5536	0.479	9773
T_2:$ZnSO_4$ @ 20 kg ha^{-1} basal (*adhoc* recommendation)	6566	7013	0.484	12542
T_3: $ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages (*adhoc* recommendation)	6538	7017	0.482	12483
T_4:$ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages	6587	7022	0.484	12561
T_5: $ZnSO_4$ @ 0.1% foliar at PI, B and M stages	5155	5577	0.480	9867
T_6: $ZnSO_4$ @ 0.1% foliar at B and M stages	5178	5581	0.481	9965
T_7:$ZnSO_4$ @ 0.1% foliar at MT and M stages	6605	7024	0.485	12585
T_8: $ZnSO_4$ @ 0.1% foliar at M stage	5128	5556	0.480	9858
SEm (±)	397	426	0.004	749
CD (0.05)	1207.2	1293.6	NS	2274.34

MT-Maximum tillering; PI-Panicle initiation; B-Booting; M-Milking; NS-not significant

4.3 CHEMICAL ANALYSIS

4.3.1 Plant Analysis

4.3.1.1 Quality Attributes of Whole Grain and its Milled factions

4.3.1.1.1 ***Starch, Crude protein, Phytate, Zn content and Phytate: Zn ratio in Whole Grain***

Data furnished in Table 15indicated that the treatments have significantly influenced the starch content of whole grain. Treatment T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal) resulted in significantly higher starch content (75.4%) which was on par with treatments T_3, T_4 and T_7. The least starch content (64.3%) was found in T_1 (Control - without Zn) was at par with treatments T_5, T_6 and T_8

Perusal of data (Table 15) showed that crude protein content of whole grain (6.78%) was highest in the treatment, T_7 ($ZnSO_4$ @ 0.1% foliar at MT and M stages) which was on par with treatments T_2, T_3 and T_4. Minimum crude protein content (4.6 %) found in treatment T_1 (Control -without Zn), which was on par with T_5, T_6 and T_8.

Data on phytate content in whole grain given in Table 15 did not differ significantly due to treatments.

Regarding Zn concentration of whole grain presented in Table 15, the data revealed that Zn concentration was significantly higher (32.1 mg kg^{-1}) in the treatment T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal), which was comparable with T_3, T_4 and T_7. Minimum Zn concentration of whole grain (23 mg kg^{-1}) was observed with T_1 (Control-without Zn) which was on par with T_5, T_6 and T_8.

Mean data regarding phytate: Zn ratio are presented in Table 15. Significantly low phytate: Zn ratio (28.4) was found in the treatment T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal) which was on par with T_3, T_4 and T_7. The treatment T_1 (Control-without Zn) showed highest phytate: Zn ratio (39.5) which was on par with T_5, T_6 and T_8.

4.3.1.1.2 Starch, Crude Protein, Phytate, Zn content and Phytate: Zn ratio in Brown rice

Data pertaining to starch content of brown rice are presented in Table 16. Treatment T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal) resulted in higher starch (86.2%) and it was found to be on par with treatments T_3, T_4 and T_7. Lowest starch content (72.4%) was exhibited by T_1 (Control -without Zn), which was comparable with T_5, T_6 and T_8.

Analyzed data on crude protein content of brown rice presented in Table 16 indicated that T_7 ($ZnSO_4$ @ 0.1% foliar at MT and M stages) resulted in maximum crude protein (8.65%) which was comparable to treatments T_2, T_3 and T_4. Treatment T_1 (Control-without Zn) resulted in minimum crude protein (6.40%), which was comparable with T_5, T_6 and T_8.

Data on phytate content of brown rice presented in Table 16 did not show any significant effect due to treatments.

Data in Table 16 indicated that concentration of Zn in brown rice (20.4 mg kg^{-1}) was significantly highest in T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal) which was comparable with treatments T_3, T_4 and T_7. The minimum Zn concentration (13.7 mg kg^{-1}) was found in T_1 (Control without Zn), which was statistically on par with T_5, T_6 and T_8.

Mean data on phytate: Zn ratio of brown rice are presented in Table 16. It indicated that significantly lower phytate: Zn ratio (63.1) was recorded in T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal) which was on par with treatments T_3, T_4 and T_7. The treatment T_1 (Control-without Zn) had maximum (84.3) phytate: Zn ratio which was comparable to T_5, T_6 and T_8.

4.3.1.1.3 Starch, Crude Protein, Phytate, Zn Content and Phytate: Zn ratio in Polished White Rice

Data on starch content in polished white rice are given in Table 17. Maximum starch content (92%) was exhibited in the treatment T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal) which was comparable to treatments T_3, T_4 and T_7. Minimum starch content (81.3%) was recorded in treatment T_1 (Control -without Zn) which was on par with T_5, T_6 and T_8.

Mean data on crude protein content in polished white rice are presented in Table 17. Treatments did not exert significant effect on crude protein content.

Anti-nutritional factor phytate content in polished white rice did not show any significant effect due to treatments and the data are presented in Table 17.

Data on Zn concentration of polished white rice are given in Table 17. It is clear from the table that significantly higher concentration of Zn (9.48 mg kg^{-1}) was recorded in the treatment, T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal) which was comparable to treatments T_3, T_4 and T_7. The treatment T_1 (Control-without Zn) recorded minimum Zn concentration (6.83 mg kg^{-1}) and was on par with T_5, T_6 and T_8.

Ratio between phytate and Zn was found significantly low (16.6) in the treatment T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal) which was on par with T_3, T_4 and T_7. Maximum phytate: Zn ratio (29.7) was noticed in T_1 (Control -without Zn), which was statistically on par with T_5, T_6 and T_8.

Table 15. Effect of time and method of Zn application on quality attributes of whole grain (Dry weight basis)

Treatments	Starch (%)	Crude protein (%)	Phytate ($g\ kg^{1}$)	Zn ($mg\ kg^{-1}$)	Phytate: Zn ratio
T_1: Control (without Zn)	64.3	4.60	9.17	23.0	39.5
T_2:$ZnSO_4$ @ 20 kg ha^{-1} basal (*adhoc* recommendation)	75.4	6.53	9.20	32.1	28.4
T_3:$ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages *(adhoc* recommendation)	75.3	6.34	9.26	31.4	29.2
T_4:$ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages	74.6	6.66	9.26	30.6	30.0
T_5: $ZnSO_4$ @ 0.1% foliar at PI, B and M stages	65.7	4.91	9.15	24.8	36.6
T_6: $ZnSO_4$ @ 0.1% foliar at B and M stages	65.1	5.06	9.10	23.9	37.7
T_7: $ZnSO_4$ @ 0.1% foliar at MT and M stages	74.3	6.78	9.21	29.8	30.6
T_8: $ZnSO_4$ @ 0.1% foliar at M stage	64.5	4.65	9.14	23.6	38.4
SEm (±)	2.59	0.4	0.7	1.4	1.5
CD (0.05)	7.85	1.09	NS	4.13	4.61

MT-Maximum tillering; PI-Panicle initiation; B-Booting; M-Milking; NS-not significant

Table 16. Effect of time and method of Zn application on quality attributes of brown rice (Dry weight basis)

Treatments	Starch (%)	Crude Protein (%)	Phytate (g kg^{-1})	Zn (mg kg^{-1})	Phytate: Zn ratio
T_1: Control (without Zn)	72.4	6.40	11.7	13.7	84.3
T_2:$ZnSO_4$ @ 20 kg ha^{-1} basal (*adhoc* recommendation)	86.2	8.60	13.0	20.4	63.1
T_3: $ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages *(adhoc* recommendation)	85.6	8.58	13.2	20.1	64.8
T_4:$ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages	84.7	8.62	13.1	19.5	66.6
T_5: $ZnSO_4$ @ 0.1% foliar at PI, B and M stages	74.4	6.46	12.4	14.9	82.3
T_6: $ZnSO_4$ @ 0.1% foliar at B and M stages	73.5	6.48	12.0	14.4	82.7
T_7: $ZnSO_4$ @ 0.1% foliar at MT and M stages	84.3	8.65	12.9	19.1	67.3
T_8: $ZnSO_4$ @ 0.1% foliar at M stage	72.9	6.43	12.0	14.1	84.0
SEm (±)	3.18	0.6	1.2	0.8	4.2
CD (0.05)	9.64	1.68	NS	2.54	12.47

MT-Maximum tillering; PI-Panicle initiation; B-Booting; M-Milking; NS-not significant

Table 17. Effect of time and method of Zn application on quality parameters of polished white rice (Dry weight basis)

Treatments	Starch (%)	Crude protein (%)	Phytate (g kg^{-1})	Zn (mg kg^{-1})	Phytate: Zn ratio
T_1:Control (without Zn)	81.3	6.16	2.05	6.83	29.7
T_2:$ZnSO_4$ @ 20 kg ha^{-1} basal (*adhoc* recommendation)	92.0	6.93	1.59	9.48	16.6
T_3: $ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages *(adhoc* recommendation)	91.9	6.92	1.64	9.38	17.3
T_4: $ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages	91.2	6.81	1.73	9.20	18.7
T_5:$ZnSO_4$ @ 0.1% foliar at PI, B and M stages	82.7	6.68	1.89	7.21	26.0
T_6:$ZnSO_4$ @ 0.1% foliar at B and M stages	82.1	6.47	1.97	7.16	27.3
T_7: $ZnSO_4$ @ 0.1% foliar at MT and M stages	90.9	6.73	1.73	9.11	18.8
T_8: $ZnSO_4$ @ 0.1% foliar at M stage	81.5	6.45	2.00	6.96	28.5
SEm (±)	2.6	0.5	0.1	0.4	1.7
CD (0.05)	8.02	NS	NS	1.33	5.32

MT-Maximum tillering; PI-Panicle initiation; B-Booting; M-Milking; NS-not significant

4.3.1.1.4 Starch, Crude Protein, Phytate, Zn Content and Phytate: Zn ratio in Rice Husk

Data on starch content of rice husk are presented in Table 18. Treatments did not show any significant effect on starch, crude protein and phytate contents of rice husk.

The effect of time and method of Zn application on Zn content of rice husk is presented in Table 18. Zn concentration in rice husk increased (5.94 mg kg^{-1}) due to treatment T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal) and was comparable to treatments T_3, T_4 and T_7. Lower Zn concentration in rice husk (4 mg kg^{-1}) was observed in the treatment T_1 (Control-without Zn) which was on par with T_5, T_6 and T_8.

Data on phytate: Zn ratio of rice husk is presented in Table 18. Lower phytate: Zn ratio (0.085) was noticed in the treatment T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal) which was on par with treatments T_3, T_4 and T_7. Higher phytate: Zn ratio (0.133) was found in T_1 (Control-without Zn) was comparable to T_5, T_6 and T_8.

4.3.1.1.5 Starch, Crude protein, Phytate, Zn Content and Phytate: Zn ratio in Rice Bran

Data on starch content in rice bran are presented in Table 19. Higher starch content in rice bran (49.9%) was obtained in the treatment T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal) which was comparable to T_3, T_4 and T_7. Lowest starch content (34.4%) was recorded in treatment, T_1 (Control-without Zn) was on par with T_5, T_6 and T_8.

Crude protein content in rice bran showed significant difference due to treatments and the data are presented in Table 19. Significantly higher crude protein content (14.3%) was recorded with treatment T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal) which was comparable to treatments T_3, T_4 and T_7. Minimum crude protein content (10.7%) was obtained in treatment T_1 (Control - without Zn) which was comparable with T_5, T_6 and T_8.

Data presented in Table 19 indicated that treatments showed no significant effect on phytate content of rice bran.

Analysis of treatment means revealed that significantly higher Zn concentration (80.5 mg kg^{-1}) was observed in treatment T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal), which was on par with T_3, T_4 and T_7 (Table19). Significantly lowest Zn concentration (57.8 mg kg^{-1}) recorded in the treatment T_1 (Control without Zn), was on par with T_5, T_6 and T_8.

Data on phytate: Zn ratio of bran given in Table 19 showed significantly lower value (34.4) in the treatment T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal), which was on par with T_3, T_4 and T_7. Maximum phytate: Zn ratio (51.3) recorded in T_1 (Control-without Zn) which was comparable to treatments T_5, T_6 and T_8.

Table 18. Effect of time and method of Zn application on quality attributes of rice husk (Dry weight basis)

Treatments	Starch (%)	Crude protein (%)	Phytate (mg kg^{-1})	Zn (mg kg^{-1})	Phytate: Zn ratio
T_1: Control (without Zn)	25.3	2.08	5.36	4.00	0.133
T_2:$ZnSO_4$ @ 20 kg ha^{-1} basal (*adhoc* recommendation)	29.0	2.56	5.09	5.94	0.085
T_3: $ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages *(adhoc* recommendation)	28.7	2.52	5.18	5.64	0.091
T_4:$ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages	28.5	2.56	5.27	5.56	0.094
T_5:$ZnSO_4$ @ 0.1% foliar at PI, B and M stages	26.4	2.36	5.19	4.36	0.118
T_6:$ZnSO_4$ @ 0.1% foliar at B and M stages	25.9	2.40	5.30	4.21	0.125
T_7: $ZnSO_4$ @ 0.1% foliar at MT and M stages	28.2	2.69	5.34	5.40	0.098
T_8: $ZnSO_4$ @ 0.1% foliar at M stage	25.7	2.19	5.22	4.06	0.127
SEm (±)	0.2	0.1	0.4	0.2	0.005
CD (0.05)	NS	NS	NS	0.85	0.008

MT-Maximum tillering; PI-Panicle initiation; B-Booting; M-Milking; NS-not significant

Table 19. Effect of time and method of Zn application on quality attributes of rice bran (Dry weight basis)

Treatments	Starch (%)	Crude protein (%)	Phytate (g kg^{-1})	Zn (mg kg^{-1})	Phytate: Zn ratio
T_1: Control (without Zn)	34.4	10.7	29.9	57.8	51.3
T_2: $ZnSO_4$ @ 20 kg ha^{-1} basal (*adhoc* recommendation)	49.9	14.3	28.0	80.5	34.4
T_3: $ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages *(adhoc* recommendation)	48.4	14.2	27.9	78.4	35.3
T_4: $ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages	47.3	14.1	28.6	76.3	37.1
T_5: $ZnSO_4$ @ 0.1% foliar at PI, B and M stages	39.1	11.0	29.5	60.8	48.1
T_6: $ZnSO_4$ @ 0.1% foliar at B and M stages	37.7	10.9	29.7	59.6	49.4
T_7: $ZnSO_4$ @ 0.1% foliar at MT and M stages	46.3	14.1	29.4	75.8	38.5
T_8: $ZnSO_4$ @ 0.1% foliar at M stage	36.3	10.8	29.9	58.6	50.5
SEm (±)	2.1	0.7	2.5	3.9	2.6
CD (0.05)	6.32	2.34	NS	11.80	7.79

MT-Maximum tillering; PI-Panicle initiation; B-Booting; M-Milking; NS-not significant

4.3.1.2 Nutrient Content of Rice Grain and Straw and Nutrient Uptake

4.3.1.2.1 Nitrogen Content and Uptake

Data on N content of grain and straw and total N uptake are presented in Table 20. Higher grain N content was recorded in T_7 (1.18%) ($ZnSO_4$ @ 0.1% foliar at MT and M stages) was on par with T_4, T_2 and T_3. Lower grain N content (0.94%) found in treatment T_1 (Control - without Zn) was comparable with T_8, T_5 and T_6.

Significantly higher straw N content (0.88%) was recorded in T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal) which was comparable to treatments T_4, T_7 and T_3. Lower N content (0.62%) of straw was found in T_8 ($ZnSO_4$ @ 0.1% foliar at M stage) and T_1 was on par with T_5 and T_6.

Perusal of data on total N uptake indicated that treatment T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal) contributed significantly higher N uptake (138.5 kg ha^{-1}) which was on par with T_4, T_7 and T_3. Minimum N uptake (82.2 kg ha^{-1}) recorded by T_1 (Control -without Zn) was comparable to T_8, T_5 and T_6.

4.3.1.2.2 Phosphorus Content and Uptake

The P content in rice grain and straw and total P uptake are presented in Table 21. Treatments did not exert significant effect in P content in rice grain and straw.

Total uptake of P (24.3 kg ha^{-1}) was highest in T_4 ($ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages), which was on par with T_2, T_3 and T_7. The lowest plant P uptake (14.6 kg ha^{-1}) showed by T_1 (Control - without Zn) was on par with T_5, T_6 and T_8.

Table 20. Effect of time and method of Zn application on N content and uptake

Treatments	N content (%)		N uptake (kg ha^{-1})
	Grain	Straw	
T_1: Control (without Zn)	0.94	0.62	82.2
T_2:$ZnSO_4$ @ 20 kg ha^{-1} basal (*adhoc* recommendation)	1.17	0.88	138.5
T_3: $ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages *(adhoc* recommendation)	1.16	0.80	131.9
T_4: $ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages	1.18	0.86	137.7
T_5: $ZnSO_4$ @ 0.1% foliar at PI, B and M stages	0.96	0.64	85.4
T_6: $ZnSO_4$ @ 0.1% foliar at B and M stages	0.97	0.66	87.1
T_7: $ZnSO_4$ @ 0.1% foliar at MT and M stages	1.18	0.83	135.8
T_8: $ZnSO_4$ @ 0.1% foliar at M stage	0.95	0.62	83.5
SEm (±)	0.061	0.044	8.1
CD (0.05)	0.184	0.135	24.48

MT-Maximum tillering; PI-Panicle initiation; B-Booting; M-Milking

Table 21. Effect of time and method of Zn application on P content and uptake of rice

Treatments	P content (%)		P uptake (kg ha^{-1})
	Grain	Straw	
T_1: Control (without Zn)	0.20	0.08	14.6
T_2:$ZnSO_4$ @ 20 kg ha^{-1} basal (*adhoc* recommendation)	0.24	0.11	23.6
T_3: $ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages (*adhoc* recommendation)	0.23	0.10	22.3
T_4: $ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages	0.25	0.11	24.3
T_5:$ZnSO_4$ @ 0.1% foliar at PI, B and M stages	0.22	0.08	15.5
T_6: $ZnSO_4$ @ 0.1% foliar at B and M stages	0.22	0.09	16.3
T_7: $ZnSO_4$ @ 0.1% foliar at MT and M stages	0.26	0.11	24.2
T_8: $ZnSO_4$ @ 0.1% foliar at M stage	0.21	0.08	15.3
SEm (±)	0.016	0.011	1.83
CD (0.05)	NS	NS	5.54

MT-Maximum tillering; PI-Panicle initiation; B-Booting; M-Milking; NS- not significant

4.3.1.2.3 Potassium Content and Uptake

Data furnished in Table 22 revealed that grain and straw K content were not significantly affected by the treatments

Treatments significantly influenced total uptake of K. Maximum uptake of K (140.4 kg ha^{-1}) showed by T_7 ($ZnSO_4$ @ 0.1% foliar at MT and M stages) was found to be on par with T_2, T_3 and T_4. The least uptake of K (90 kg ha^{-1}) noticed in treatment T_8 ($ZnSO_4$ @ 0.1% foliar at M stage) was comparable to T_1, T_5 and T_6.

4.3.1.2.4 Calcium Content and Uptake

The result of Ca content in rice grain and straw and total Ca uptake are presented in Table 23. The Ca content in rice grain and straw did not show any significant effect due to treatments.

Data on total uptake of Ca indicated that maximum Ca uptake (49.6 kg ha^{-1}) recorded in treatment T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal) was comparable to T_4, T_7 and T_3. Minimum plant uptake of Ca (32.5 kg ha^{-1}) was found with T_8 ($ZnSO_4$ @ 0.1% foliar at M stage), which was on par with T_1, T_5 and T_6 .

Table 22. Effect of time and method of Zn application on K content and uptake

Treatments	K content (%)		K uptake (kg ha^{-1})
	Grain	Straw	
T_1: Control (without Zn)	0.27	1.38	90.4
T_2:$ZnSO_4$ @ 20 kg ha^{-1} basal (*adhoc* recommendation)	0.35	1.67	139.8
T_3: $ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages *(adhoc* recommendation)	0.33	1.62	136.7
T_4: $ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages	0.37	1.65	138.5
T_5: $ZnSO_4$ @ 0.1% foliar at PI, B and M stages	0.29	1.40	93.2
T_6: $ZnSO_4$ @ 0.1% foliar at B and M stages	0.32	1.41	95.8
T_7: $ZnSO_4$ @ 0.1% foliar at MT and M stages	0.38	1.64	140.4
T_8: $ZnSO_4$ @ 0.1% foliar at M stage	0.28	1.36	90.0
SEm (±)	0.023	0.108	12.78
CD (0.05)	NS	NS	38.760

MT-Maximum tillering; PI-Panicle initiation; B-Booting; M-Milking; NS- not significant

Table 23. Effect of time and method of Zn application on Ca content and uptake

Treatments	Ca content (%)		Ca uptake (kg ha^{-1})
	Grain	Straw	
T_1: Control (without Zn)	0.29	0.32	32.9
T_2:$ZnSO_4$ @ 20 kg ha^{-1} basal (*adhoc* recommendation)	0.31	0.42	49.6
T_3: $ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages *(adhoc* recommendation)	0.31	0.40	48.0
T_4:$ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages	0.32	0.41	49.3
T_5: $ZnSO_4$ @ 0.1% foliar at PI, B and M stages	0.30	0.33	33.6
T_6: $ZnSO_4$ @ 0.1% foliar at B and M stages	0.30	0.33	34.2
T_7: $ZnSO_4$ @ 0.1% foliar at MT and M stages	0.32	0.40	49.3
T_8: $ZnSO_4$ @ 0.1% foliar at M stage	0.30	0.31	32.5
SEm (±)	0.005	0.026	3.19
CD (0.05)	NS	NS	9.66

MT-Maximum tillering; PI-Panicle initiation; B-Booting; M-Milking; NS- not significant

4.3.1.2.5 Magnesium Content and Uptake

Data on Mg content of rice grain and straw along with total Mg uptake are presented on Table 24. The treatment effects were not significant

Total plant uptake of Mg was maximum (27.9 kg ha^{-1}) in T_4 ($ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages), was on par with T_7, T_2 and T_3. Treatment T_8 ($ZnSO_4$ @ 0.1% foliar at M stage) exhibited the least uptake of Mg (17.4 kg ha^{-1}), which was comparable with T_1, T_5 and T_6.

4.3.1.2.6 Sulphur Content and Uptake

Data on grain and straw content of S and total S uptake are presented on Table 25. Treatments significantly affected grain and straw S content as well as total S uptake. Maximum value for S content in grain (0.13%) and straw (0.09%) found in treatment T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal) was comparable to T_4, T_3 and T_7. Lower grain S content (0.10%) was recorded in treatments T_1 (Control- without Zn) T_8, T_6 and T_5

Total uptake of S was highest (14.9 kg ha^{-1}) in treatment T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal) which was on par with the treatments T_4, T_3 and T_7. Minimum uptake of S (8.5 kg ha^{-1}) was found in the treatment T_1 (Control- without zinc), which was comparable to T_8, T_6 and T_5.

Table 24. Effect of time and method of Zn application on Mg content and uptake

Treatments	Mg content (%)		Mg uptake (kg ha^{-1})
	Grain	Straw	
T_1: Control (without Zn)	0.12	0.21	17.9
T_2:$ZnSO_4$ @ 20 kg ha^{-1} basal (*adhoc* recommendation)	0.15	0.25	27.5
T_3: $ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages *(adhoc* recommendation)	0.15	0.24	26.2
T_4: $ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages	0.16	0.25	27.9
T_5:$ZnSO_4$ @ 0.1% foliar at PI, B and M stages	0.13	0.22	19.1
T_6: $ZnSO_4$ @ 0.1% foliar at B and M stages	0.14	0.22	19.9
T_7: $ZnSO_4$ @ 0.1% foliar at MT and M stages	0.17	0.24	27.8
T_8: $ZnSO_4$ @ 0.1% foliar at M stage	0.12	0.20	17.4
SEm (±)	0.009	0.016	1.29
CD (0.05)	NS	NS	3.93

MT-Maximum tillering; PI-Panicle initiation; B-Booting; M-Milking

Table 25. Effect of time and method of Zn application on S content and uptake

Treatments	S content (%)		S uptake kg ha^{-1}
	Grain	Straw	
T_1: Control (without Zn)	0.10	0.07	8.5
T_2:$ZnSO_4$ @ 20 kg ha^{-1} basal (*adhoc* recommendation)	0.13	0.09	14.9
T_3: $ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages *(adhoc* recommendation)	0.12	0.08	14.2
T_4: $ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages	0.13	0.09	14.5
T_5: $ZnSO_4$ @ 0.1% foliar at PI, B and M stages	0.10	0.07	9.2
T_6: $ZnSO_4$ @ 0.1% foliar at B and M stages	0.10	0.07	9.1
T_7: $ZnSO_4$ @ 0.1% foliar at MT and M stages	0.12	0.08	13.8
T_8: $ZnSO_4$ @ 0.1% foliar at M stage	0.10	0.07	8.9
SEm (±)	0.005	0.003	0.93
CD (0.05)	0.014	0.010	2.82

MT-Maximum tillering; PI-Panicle initiation; B-Booting; M-Milking

4.3.1.2.7 Zinc Content and Uptake

Data on grain and straw Zn content as well as total Zn uptake are presented on Table 26. Treatment T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal) resulted in significantly higher Zn content in grain (32.1 mg kg^{-1}) and straw (44.8 mg kg^{-1}), which was on par with treatments T_3, T_4 and T_7. Lower Zn content in grain (23 mg kg^{-1}) and straw (26.9 mg kg^{-1}) was found in T_1 (Control - without Zn) which was comparable with T_8, T_6 and T_5 which were on par.

Higher total plant uptake of Zn (0.52 kg ha^{-1}) was recorded in treatment T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal), which was on par with treatments T_3, T_4 and T_7. Treatment T_1 (Control -without Zn) showed limited uptake of Zn (0.26 kg ha^{-1}) which was comparable with T_8, T_6 and T_5.

4.3.1.2.8 Crop Recovery Efficiency of Zn

Data given in Table 27 indicated that T_7 ($ZnSO_4$ @ 0.1% foliar at MT and M stages) had the highest CRE (130%) which was superior to all other treatments. This was followed by the treatment T_4 ($ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages) with a CRE of 59.6 % which was superior to all the rest.

4.3.1.2.9 Biofortification Recovery Efficiency of Zn

Data on BRE of Zn given in Table 27 indicated that the treatment T_7 ($ZnSO_4$ @ 0.1% foliar at MT and M stages) resulted in the highest BRE of Zn (43.8%) which was superior to all other treatments. The next best treatment was T_4 ($ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages) with a BRE of 20.6%, which was superior to all the treatments

Table 26. Effect of time and method of Zn application on Zn content and uptake

Treatments	Zn content (%)		Zn uptake kg ha^{-1}
	Grain	Straw	
T_1: Control (without Zn)	23.0	26.9	0.26
T_2:$ZnSO_4$ @ 20 kg ha^{-1} basal (*adhoc* recommendation)	32.1	44.8	0.52
T_3: $ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages *(adhoc* recommendation)	31.4	44.2	0.52
T_4: $ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages	30.6	43.8	0.51
T_5:$ZnSO_4$ @ 0.1% foliar at PI, B and M stages	24.8	29.3	0.29
T_6: $ZnSO_4$ @ 0.1% foliar at B and M stages	23.9	27.8	0.28
T_7: $ZnSO_4$ @ 0.1% foliar at MT and M stages	29.8	43.6	0.50
T_8: $ZnSO_4$ @ 0.1% foliar at M stage	23.6	27.8	0.28
SEm (±)	1.36	4.33	0.037
CD (0.05)	4.13	13.14	0.112

MT-Maximum tillering; PI-Panicle initiation; B-Booting; M-Milking

Table 27. Effect of time and method of Zn application on Crop Recovery Efficiency (CRE_{Zn}) and Biofortification Recovery Efficiency (BRE_{Zn}), %

Treatments	CRE_{Zn}	BRE_{Zn}
T_1:Control (without Zn)	0.0	0.0
T_2:$ZnSO_4$ @ 20 kg ha^{-1} basal (*adhoc* recommendation)	5.6	2.0
T_3:$ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages *(adhoc* recommendation)	12.4	4.3
T_4:$ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages	59.6	20.6
T_5:$ZnSO_4$ @ 0.1% foliar at PI, B and M stages	8.0	3.3
T_6: $ZnSO_4$ @ 0.1% foliar at B and M stages	7.5	3.4
T_7: $ZnSO_4$ @ 0.1% foliar at MT and M stages	130.0	43.8
T_8: $ZnSO_4$ @ 0.1% foliar at M stage	10.6	3.9
SEm (±)	15.0	5.7
CD (0.05)	45.52	17.28

MT-Maximum tillering; PI-Panicle initiation; B-Booting; M-Milking

4.3.2 Soil analysis after the experiment

4.3.2.1 Soil pH

Data on soil pH after the experiment are presented on Table 28. There was no significant variation in soil pH due to treatments.

4.3.2.2 Electrical Conductivity

Electrical conductivity of soil after the experiment was recorded and presented in Table 28. Electrical conductivity did not differ significantly among treatments.

4.3.2.3 Organic Carbon

Data on organic carbon status of soil presented in Table 28 indicated that treatments had no significant effect on organic carbon content of soil.

4.3.2.4 Available N

The result of available N in soil after the field experiment is shown in Table 29. Higher available N (206 kg ha^{-1}) was found in the treatment T_1 (Control - without Zn) which was on par with T_5 ($ZnSO_4$ @ 0.1% foliar at PI, B and M stages) and T_8 ($ZnSO_4$ @ 0.1% foliar at M stage). The least available N (153 kg ha^{-1}) was found in T_7 ($ZnSO_4$ @ 0.1% foliar at MT and M stages) and was comparable with T_4, T_2 and T_3.

4.3.2.5 Available P

Data on available P after the field experiment are given in Table 29. Treatment T_1 (Control-without Zn) had highest available P (65.5 kg ha^{-1}) which was on par with T_8, T_5 and T_6. The minimum available P (53.6 kg ha^{-1}) in T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal) was on par with T_4, T_7 and T_3.

Table 28. Effect of time and method of Zn application on pH, EC and organic carbon status of soil after the experiment

Treatments	pH	EC (dS m^{-1})	Organic carbon (%)
T_1:Control (without Zn)	5.73	0.120	0.55
T_2:$ZnSO_4$ @ 20 kg ha^{-1} basal (*adhoc* recommendation)	5.35	0.164	0.49
T_3:$ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages (*adhoc* recommendation)	5.39	0.161	0.51
T_4:$ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages	5.51	0.135	0.48
T_5:$ZnSO_4$ @ 0.1% foliar at PI, B and M stages	5.56	0.130	0.53
T_6: $ZnSO_4$ @ 0.1% foliar at B and M stages	5.66	0.129	0.52
T_7: $ZnSO_4$ @ 0.1% foliar at MT and M stages	5.63	0.131	0.47
T_8: $ZnSO_4$ @ 0.1% foliar at M stage	5.71	0.126	0.54
SEm (±)	0.42	0.0086	0.03
CD (0.05)	NS	NS	NS

MT-Maximum tillering; PI-Panicle initiation; B-Booting; M-Milking; NS-non significant

Table 29. Effect of time and method of Zn application on available N, P and K in soil after the experiment, kg ha^{-1}

Treatments	Available N	Available P	Available K
T_1:Control (without Zn)	206	65.5	210
T_2:$ZnSO_4$ @ 20 kg ha^{-1} basal (*adhoc* recommendation)	155	53.6	147
T_3: $ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages *(adhoc* recommendation)	157	54.0	151
T_4: $ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages	154	53.8	149
T_5: $ZnSO_4$ @ 0.1% foliar at PI, B and M stages	202	64.8	207
T_6: $ZnSO_4$ @ 0.1% foliar at B and M stages	199	64.2	205
T_7: $ZnSO_4$ @ 0.1% foliar at MT and M stages	153	53.9	148
T_8: $ZnSO_4$ @ 0.1% foliar at M stage	204	65.0	208
SEm (±)	13.4	3.15	16.6
CD (0.05)	40.7	9.54	50.5

MT-Maximum tillering; PI-Panicle initiation; B-Booting; M-Milking

4.3.2.6 *Available K*

Data on available K in soil after the experiment are presented in the Table 29. The results indicated that T_1 (Control-without Zn) recorded maximum available K (210 kg ha^{-1}) and was comparable with treatments T_8, T_5 and T_6. The minimum value of available K (147 kg ha^{-1}) was in T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal) was on par with T_7, T_4 and T_3.

4.3.2.7 *Available Ca*

Data on available Ca in soil after the experiment revealed that available Ca did not vary significantly due to treatments (Table 30).

4.3.2.8 *Available Mg*

Data obtained for available Mg in soil after the experiment are given in Table 30. There was no significant difference in available Mg due to treatments

4.3.2.9 *Available S*

The perusal of data on available S after the experiment shown in Table 30 revealed that highest available S (8.1 kg ha^{-1}) was in T_1 (Control – without Zn), which was on par with T_8, T_5 and T_6. Lowest value of available S (6.1 kg ha^{-1}) observed in treatments T_7 ($ZnSO_4$ @ 0.1% foliar at MT and M stages) and T_4 ($ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages) were comparable to T_2 and T_3.

Table 30. Effect of time and method of Zn application on available Ca, Mg, S and Zn in soil after the experiment, mg kg^{-1}

Treatments	Available Ca	Available Mg	Available S	Available Zn
T_1: Control (without Zn)	180	57	8.1	0.83
T_2:$ZnSO_4$ @ 20 kg ha^{-1} basal (*adhoc* recommendation)	162	51	6.2	2.83
T_3:$ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages *(adhoc* recommendation)	156	52	6.2	1.55
T_4:$ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages	153	49	6.1	1.15
T_5:$ZnSO_4$ @ 0.1% foliar at PI, B and M stages	175	54	7.8	1.20
T_6:$ZnSO_4$ @ 0.1% foliar at B and M stages	176	55	7.8	1.17
T_7:$ZnSO_4$ @ 0.1% foliar at MT and M stages	161	50	6.1	0.82
T_8: $ZnSO_4$ @ 0.1% foliar at M stage	182	58	7.9	0.85
SEm (±)	9.0	2.1	0.3	0.10
CD (0.05)	NS	NS	1.02	0.290

MT-Maximum tillering; PI-Panicle initiation; B-Booting; M-Milking; NS- Not significat

Table 31. Effect of time and method of Zn application on economics of rice cultivation

Treatments	Net income (₹ ha^{-1})	B: C ratio
T_1: Control (without Zn)	11412	1.11
T_2:$ZnSO_4$ @ 20 kg ha^{-1} basal (*adhoc* recommendation)	87125	1.85
T_3:$ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages *(adhoc* recommendation)	79846	1.74
T_4:$ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages	85721	1.80
T_5: $ZnSO_4$ @ 0.1% foliar at PI, B and M stages	19884	1.19
T_6: $ZnSO_4$ @ 0.1% foliar at B and M stages	23109	1.22
T_7: $ZnSO_4$ @ 0.1% foliar at MT and M stages	91213	1.88
T_8: $ZnSO_4$ @ 0.1% foliar at M stage	10166	1.10

MT-Maximum tillering; PI-Panicle initiation; B-Booting; M-Milking

4.3.2.10 Available Zn

Data on available Zn in soil after the experiment are presented in Table 30. Available Zn (2.83 kg ha^{-1}) was significantly highest the treatment T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal) which was superior to all other treatments. Lowest available Zn (0.82 kg ha^{-1}) was by T_7 ($ZnSO_4$ @ 0.1% foliar at MT and M stages), which was on par with T_1 and T_8.

4.5 ECONOMIC ANALYSIS

4.5.1 Net income

Data on net income are presented in Table 31. A perusal of data on net income indicated that T_7 ($ZnSO_4$ @ 0.1% foliar at MT and M stages) resulted in the highest net income (₹ 91213 ha^{-1}). This was followed by T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal) with net income of ₹ 87125 ha^{-1}.

4.5.2 Benefit Cost ratio (B: C ratio)

Data on B: C ratio as influenced by time and method of Zn application are presented in Table 31. Among the different treatments, T_7 ($ZnSO_4$ @ 0.1% foliar at MT and M stages) registered highest B: C ratio of 1.88. This was followed by T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal) with B: C ratio of 1.85.

Discussion

5 DISCUSSION

An investigation entitled "Agronomic biofortification of Zn in rice (*Oryza sativa* L.)" was conducted in farmer's field at Chirayinkeezhu to study the effect of time and method of Zn application on growth, yield, Zn biofortification and Zn bioavailability in transplanted rice. Salient results of the field study are briefly discussed in this chapter.

5.1 EFFECT OF TIME AND METHOD OF ZINC APPLICATION IN RICE

5.1.1 Effect on Growth Characters

Perusal of data (Table 5 and Table 6) indicated that time and method of Zn application did not have any significant influence on plant height and number of tillers m^{-2}. However treatments significantly influenced LAI, root length, root weight, root volume and dry matter production (Tables 7 to 11).

LAI at PI stage in T_7 ($ZnSO_4$ @ 0.1% foliar at MT and M stages) was higher (5.26), which was on par with T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal), T_3 ($ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages) and T_4 ($ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages) (Fig.3) Zn applied either basally in soil immediately after transplanting or foliar sprayed at MT were effective in increasing leaf growth. Initial soil nutrient status indicated that soil of the experimental site was deficient in available Zn (0.79 mg kg^{-1}) (Table 2). Hussain (2015) reported that application of Zn fertilizers in Zn deficient soil causes leaf area expansion resulting in higher LAI due to increased enzymatic activity in crop. Similar result was also reported by Zayed *et. al.* (2011).

Root length (22.4 cm), root weight (4.3 g $hill^{-1}$) and root volume (8.9 cc) at MT observed in T_2 (ZnSO4 @ 20 kg ha^{-1} basal) was significantly superior to all the other treatments. However, root length (22.0 cm), root weight (23.1 g $hill^{-1}$) and root volume (39.8 cc) at harvest observed in T_7 (ZnSO4 @ 0.1% foliar at MT and M stages) was comparable to T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal), T_3 ($ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages) and T_4 ($ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages). Zn either soil applied basally immediately after

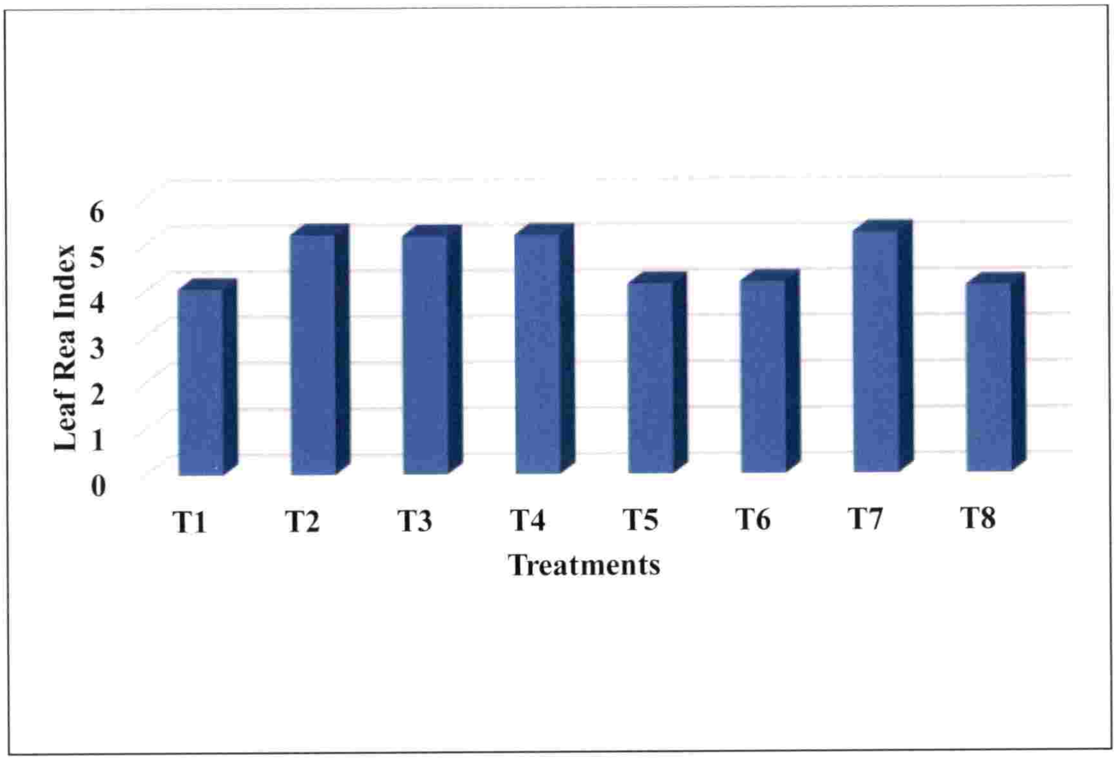


Fig. 3. Effect of time and method of zinc application on Leaf Area Index

transplanting or foliar sprayed at MT were effective in stimulating increased root growth for better absorption and translocation of nutrients in order to meet the nutritional requirement of crop. Chen *et al* (2009) reported that Zn application in Zn deficient soils can enhance root length, root weight and root biomass. This is in conformity with findings of Dhanya (2014) in yard long bean and Sakthidharan (2013) in amaranthus.

Dry matter production (20.4 g per hill) at MT was significantly higher in T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal) than all other treatments. However, dry mater production (28.2 and 37.8 g per hill) at PI and harvest respectively in T_7 ($ZnSO_4$ @ 0.1% foliar at MT and M stages) were on par with T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal), T_3 ($ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages) and T_4 ($ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages). Zn either soil applied basally immediately after transplanting or foliar sprayed at MT were effective in increasing dry matter production due to better Zn availability to crop during vegetative phase. Daivakrupa (2012) reported that Zn application either through foliar or soil had increased total dry matter production due to high Zn availability, when Zn is a limiting factor. Dry matter production depends on potential ability of plant for photosynthesis, which in turn depends on leaf area, nutrient uptake and favorable environmental conditions (De Datta, 1981). Application of Zn either soil applied basally immediately after transplanting or foliar sprayed at MT in the present study had increased LAI, proliferation of roots resulting in enhanced nutrient uptake (Tables 20 to 26) and higher dry matter production. These results are in accordance with findings of Shivay *et al* (2016).

5.1.2 Effect on Yield and Yield Attributes

Critical analysis of results indicated that Zn applied either in soil basally immediately after transplanting or foliar sprayed at MT had ensured better Zn availability to crop during vegetative phase and played a positive role in determining yield attributes and yield. Days to 50 percent flowering and productive tillers m^{-2} were not significantly influenced by treatments (Table 13). However, length of the panicle, grain weight per panicle, filled grains per panicle, per cent filled grains per panicle, thousand grain weight, grain yield and straw yield were significantly influenced by the treatments (Tables 12 to 14).

Length of panicle (20.9 cm), grain weight per panicle (1.88 g), filled grains per panicle (127 nos.), per cent filled grains per panicle (83.9%) and thousand grain weight (26 g) in T_7

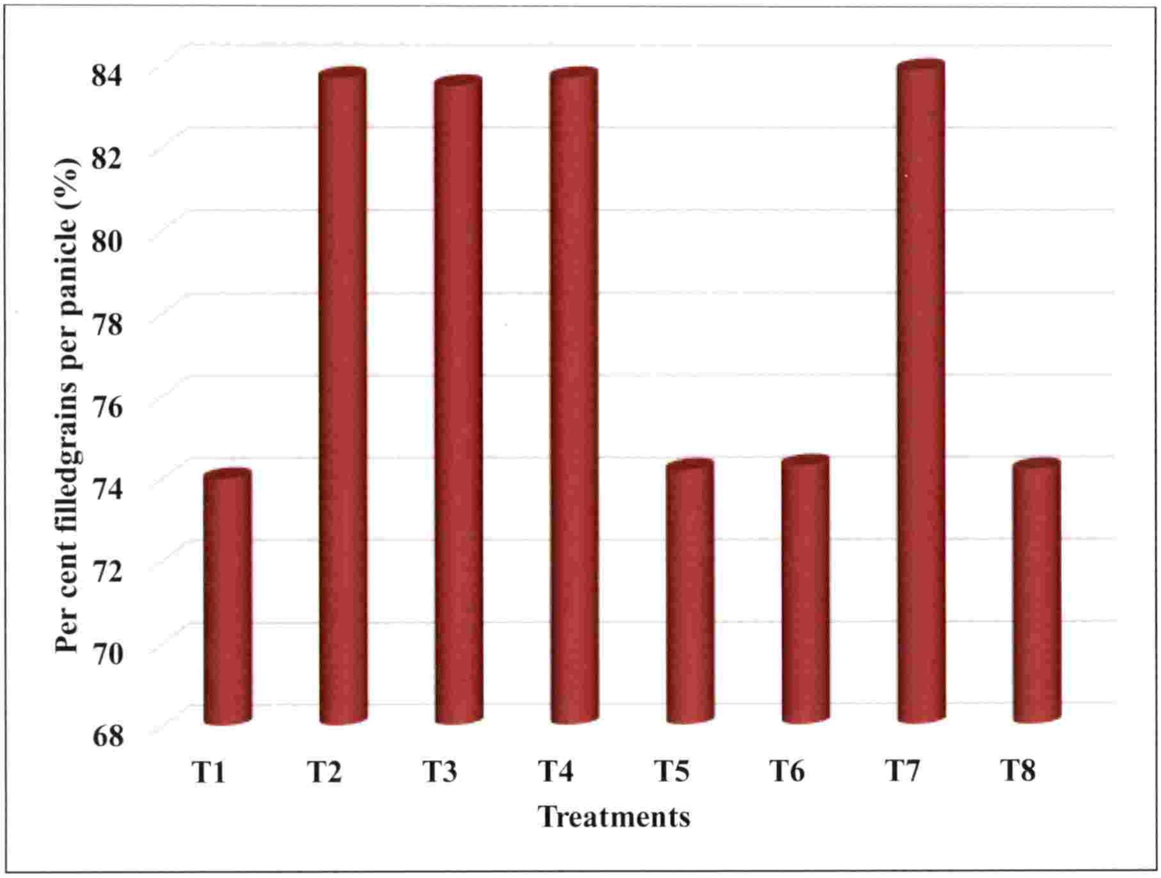


Fig. 4. Effect of time and method of zinc application on per cent filled grains per panicle

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($ZnSO_4$ @ 0.1% foliar at MT and M stages) were on par with T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal), T_3 ($ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages) and T_4 ($ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages). Fig.4 indicates the data on per cent filled grains per panicle. Further, the treatment T_7 registered significantly higher yield attributes compared to the other treatments. Hussain (2015) reported that formation of higher number of spikelets during spikelet initiation process results in longer panicle. Rani (2013) observed that application of Zn sulphate increased Zn uptake and thus higher number of filled grains per panicle. Zn application during vegetative phase of crop had enhanced nutrient uptake (Tables 21 to 27), photosynthesis, translocation of carbohydrates to grains, which ultimately increased grain weight per panicle, per cent filled grains per panicle and thousand grain weight. Corroborative findings were reported by Naik and Das (2008), Daivakrupa (2012), Hussain (2015) and Shivay *et al* (2016).

The result of the study indicated the variation in yield of transplanted rice due to the time and method of Zn application along with the recommended dose of NPK @ 90:45:45 kg ha^{-1} (KAU, 2016), in the control plot (T_1) only the recommended dose of NPK was applied. Grain (6605 kg ha^{-1}) and straw (7024 kg ha^{-1}) yield recorded in T_7 ($ZnSO_4$ @ 0.1% foliar at MT and M stages) were on par with T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal), T_3 ($ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages), T_4 ($ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages) and significantly higher than all other treatments. Fageria *et al.* (2009) reported that crops respond faster to foliar sprayed fertilizers within 2 days, while crop response to soil applied fertilizers took 5 to 6 days. It was observed from the present study that in Zn deficient soil with initial soil Zn status (0.79 mg kg^{-1}), Zn either soil applied basally immediately after transplanting or foliar sprayed at MT during vegetative phase of crop were effective in increasing grain and straw yield. Lowest grain (5093 kg ha^{-1}) and straw (5536 kg ha^{-1}) yield observed in Zn unfertilized treatment T_1 (Control-without Zn) were comparable with T_5 ($ZnSO_4$ @ 0.1% foliar at PI, B and M stages), T_6 ($ZnSO_4$ @ 0.1% foliar at B and M stages) and T_8 ($ZnSO_4$ @ 0.1% foliar at M stage). The grain and straw yield the treatment T_7 recorded 29 and 26.6 per cent increase in grain and straw yield respectively over the control treatment (Fig.5). It was observed from the present study that treatments foliar sprayed with Zn after vegetative phase of crop viz. panicle initiation, booting and milking resulted in lower grain and straw yield.

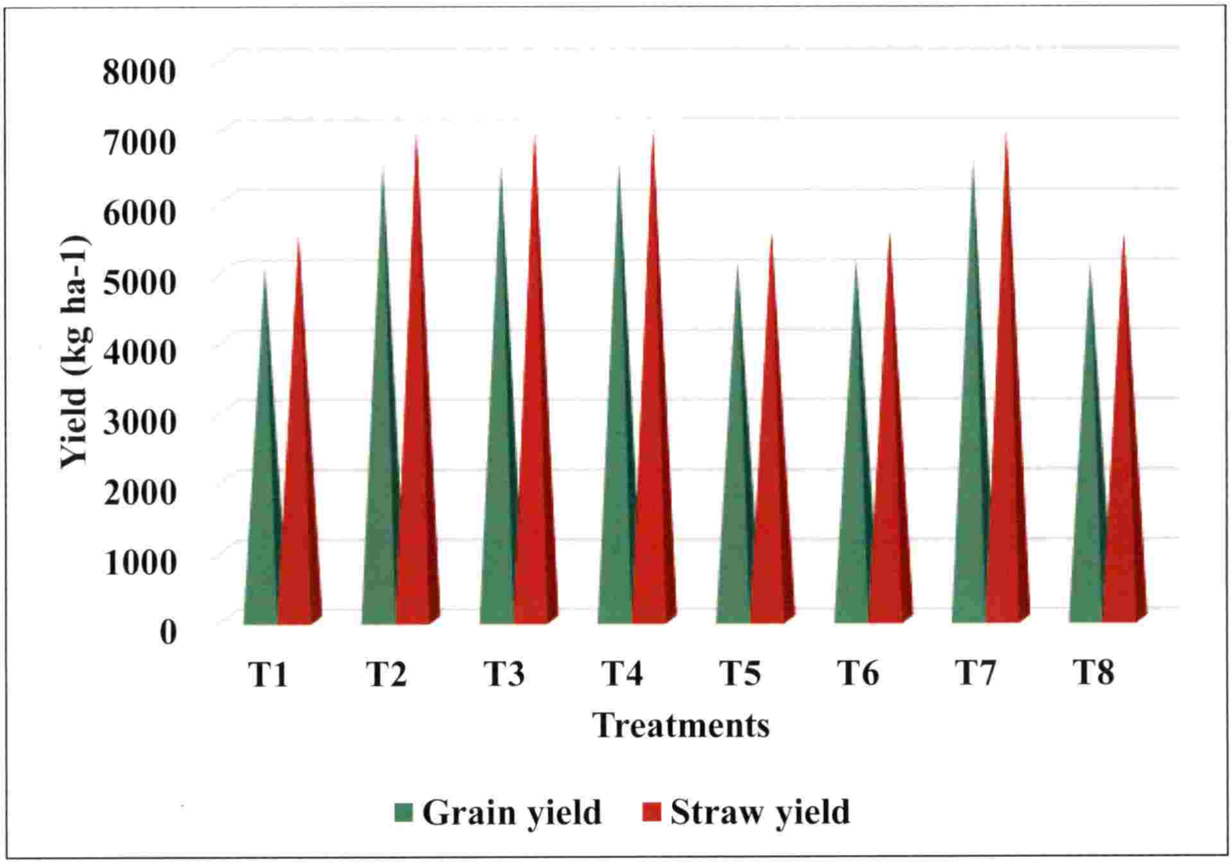

Fig. 5. Effect of time and method of zinc application on grain yield and straw yield

Zn foliar sprayed at MT during vegetative phase of crop T_7 ($ZnSO_4$ @ 0.1% foliar at MT and M stages) increased grain and straw yield by 30% and 27% respectively over Zn unfertilized treatment T_1 (Control-without Zn). In the present study, better crop response to Zn applied during vegetative phase of crop could be attributed to initial soil Zn deficient situation. The increase in grain yield could be credited to increased filled grains per panicle, per cent filled grains per panicle and thousand grain weight. Prasad *et al* (2014) reported that Zn enrichment of urea @ 2% Zn as Zn sulphate increased grain yield of rice by 29.4%. The increase in straw yield could be attributed to the fact that Zn plays a vital role in photosynthesis thus stimulating vegetative growth leading to increased LAI. Zn is a cofactor of enzymes within the plant concerned with carbohydrate metabolism, both in photosynthesis and in the conversion of sugars to starch, protein metabolism, auxin metabolism, pollen formation, maintenance of the integrity of biological membranes and resistance to infection (Das and Green, 2016). Significant improvement in yield with Zn fertilization was reported by Stalin *et al*, (2011), Phattarakul *et al*, (2012), Daivakrupa (2012), Rani (2013) and Shivay *et al,* (2016).

5.1.3 Effect on Quality Attributes of Whole Grain and its Milled Fractions

5.1.3.1 Zn Biofortification

Zn concentrations in whole grain, brown rice, polished white rice, rice husk and rice bran were significantly influenced by the treatments (Tables 16 to 20).

Zn concentration in whole grain, brown rice and polished rice is illustrated in Fig.6. Zn unfertilized treatment T_1 (Control-without Zn) recorded Zn concentrations 23, 13.7, 6.8, 4 and 57.8 mg kg^{-1} in whole grain, brown rice, polished white rice, rice husk and rice bran respectively. Shivay *et al* (2008) reported whole grain Zn concentrations of 27 to 42 mg kg^{-1}. Welch and Graham (2004) reported polished white contains Zn concentrations of 13 to 15 mg kg^{-1}.

Gogoi *et al.* (2016) reported that Zn application either as soil or foliar at critical growth stages of crop enhanced absorption of Zn and increased grain Zn concentration. With Zn foliar at milking stage alone T_8 ($ZnSO_4$ @ 0.1% foliar at M stage), Zn concentrations 23.6, 14.1, 7, 4.1 and 58.6 mg kg^{-1} were recorded in whole grain, brown rice, polished white rice, rice husk and rice bran respectively and were on par with Zn unfertilized treatment T_1 (Control-without Zn). Treatments, Zn foliar sprayed at booting stage in addition to milking stage T_6 ($ZnSO_4$ @ 0.1% foliar at B and M stages) and Zn foliar sprayed at PI and booting stages in addition to milking

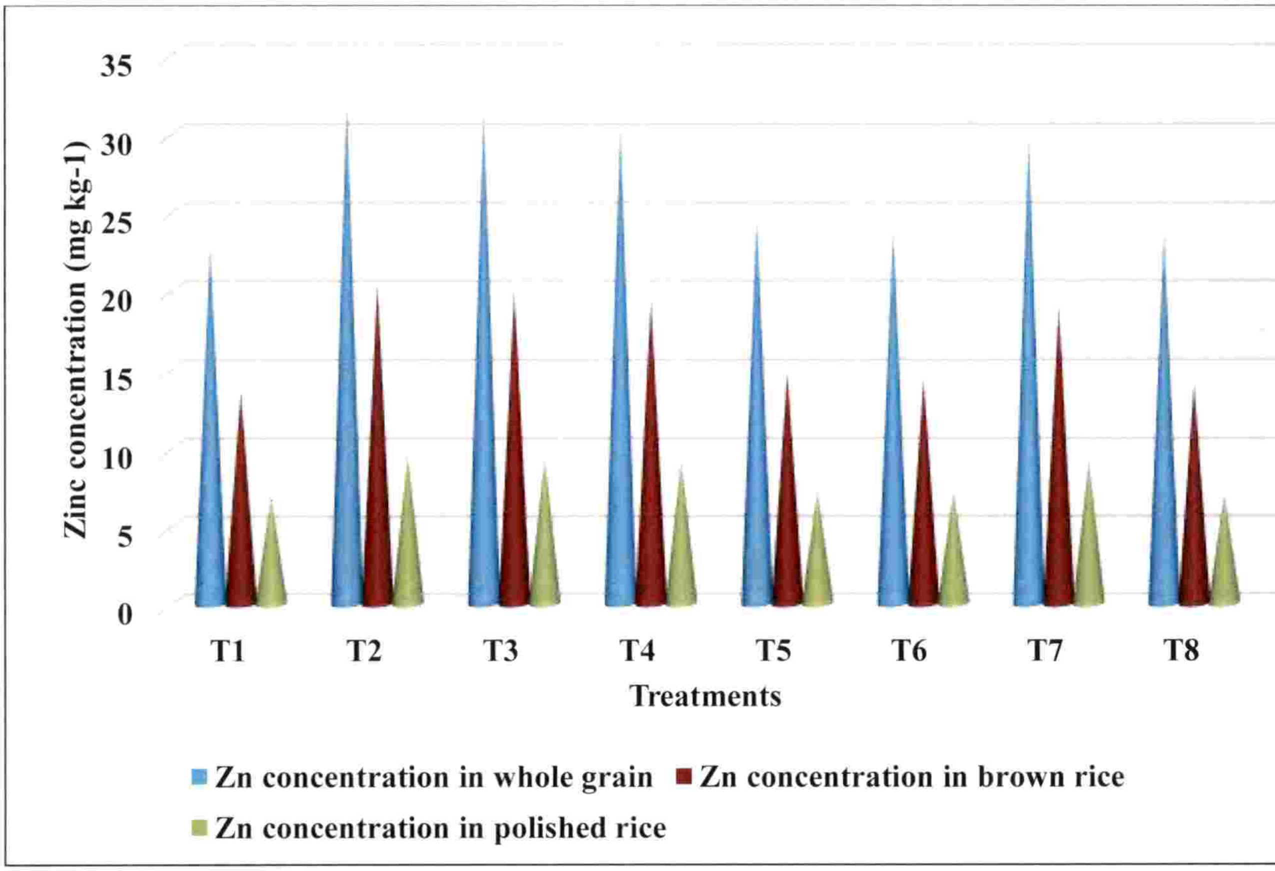

Fig. 6. Effect of time and method of zinc application on zinc concentration of whole grain, brown rice and polished rice

stage T_5 ($ZnSO_4$ @ 0.1% foliar at PI, B and M stages) had Zn concentrations comparable with Zn foliar spray at milking stage alone T_8 ($ZnSO_4$ @ 0.1% foliar at M stage). Lone (2015) reported that Zn foliar sprayed at PI stage had no effect on grain Zn concentration.

Zn foliar sprayed at MT stage in addition to milking stage T_7 (ZnSO4 @ 0.1% foliar at MT and M stages) had Zn concentrations 29.8, 19.1, 9.11, 5.4 and 75.8 mg kg^{-1} in whole grain, brown rice, polished white rice, rice husk and rice bran respectively and increased Zn concentrations by 30, 39, 33, 35 and 31 percent respectively over Zn unfertilized treatment T_1 (NPK 90:45:45 kg ha^{-1} SA). Prasad *et al* (2014) reported that Zn enrichment of urea @ 2% Zn as $ZnSO_4$ increased grain Zn concentration by 61.8 %. Zn concentrations in whole grain and its milled fractions in treatment T_7 (NPK 90:45:45 kg ha^{-1} SA) were comparable with treatments where Zn was either soil applied basally immediately after transplanting T_2 (ZnSO4 @ 20 kg ha^{-1} basal) or foliar sprayed at MT T_3 ($ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages) and T_4 ($ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages). Shivay *et al.* (2016) reported that Zn foliar sprayed at tillering, booting and grain filling stages recorded higher grain Zn concentration. The present study contrasts with previous finding, where Zn foliar sprayed at milking stage was more effective than soil application in increasing grain Zn concentration (Lone, 2015). This discrepancy may be attributed to higher concentration of 0.5% Zn sulphate foliar sprayed at milking stage. In the present study, a low concentration of 0.1% Zn sulphate was foliar sprayed at milking stage T_8 ($ZnSO_4$ @ 0.1% foliar at M stage).

Foliar Zn application has the advantage of avoiding Zn losses through soil fixation (Nasri *et al.*, 2011). Studies using radioactive Zn (^{65}Zn) had revealed that most of the Zn present in grain is remobilized from vegetative tissues such as stems and old leaves and translocated to grains during reproductive phase (Jiang *et al.*, 2007). Foliar applied radioactive Zn (^{65}Zn) was found to be remobilized and translocated into the grains through the phloem (Wu *et al*, 2010). More than half of the Zn in rice grain at harvest had been taken up by crop during vegetative phase (Wu *et al*, 2011). Zn foliar sprayed during late maturity phase of crop will not translocate through aleurone layer in rice grain so that concentration of Zn will be more at aleurone layer or bran (Ozturk *et al.*, 2006).

5.1.3.2 Zn bioavailability

Data on phytate concentrations and phytate: Zn ratios in whole grain and its milled fractions are presented in Tables 16 to 20. Perusal of data revealed that treatments did not significantly decrease phytate concentrations in whole grain, brown rice, polished white rice, rice husk and rice bran. However treatments significantly influenced phytate: Zn ratios in whole grain and its milled fractions.

Phytate, technically called as myo-inositol hexaphosphate is the primary storage form of phosphorous in seed accounting for 60 to 90% of total seed phosphorous. Anti-nutrient factor phytate is a powerful chelator and forms complexes with divalent metal ions, such as Zn^{2+}, Fe^{2+}, Ca^{2+} and Cu^{2+}, which are not absorbed in the gastrointestinal tract and reduce the bioavailability of essential elements leading to a deficiency in humans (Wahab *et al.*, 2004; Bohn *et al.*, 2008). Zn is very tightly bound to phytate and the formation of additional protein-Zn-phytate complexes increases the resistance to hydrolysis. Phytate is salt of phytate found in seeds of grains where most of Zn and other minerals are localised. The present study with treatments having no significant effect on phytate concentrations in whole grain and its milled fractions contrasts with previous finding where it was observed that soil and foliar Zn application significantly reduced phytate concentrations in rice grain (Mabesa *et. al.*, 2013; Imran *et al.*, 2015; Hussain, 2015; Lone, 2015). Possible explanation could be that increased Zn concentrations in whole grain and its milled fractions due to Zn application either as soil or foliar was not sufficiently high enough to inhibit conversion of inorganic phosphorous to phytate.

Phytate: Zn ratio is considered as a qualitative estimate of bioavailability of Zn in human diet (Graham, 1984; Brown *et. al.*, 2001; Weaver and Kannan, 2002). Ratios of phytate:Zn >10:1 is associated with Zn deficiency in humans (Morris and Ellis, 1980). Fig.7 represents the phytate: Zn ratio of whole grain, brown rice and polished rice. Phytate: Zn ratio of $<5:1$, 5-15:1 and >15:1 are considered as having high, medium and low bioavailability of Zn respectively (Graham, 1984). Zn unfertilized treatment T_1 (Control - without Zn) recorded phytate: Zn ratios 39.5, 84.3, 29.7,0.13 and 51.3 mg kg^{-1} in whole grain, brown rice, polished white rice, rice husk and rice bran respectively and were at par with T_5 ($ZnSO_4$ @ 0.1% foliar at PI, B and M stages), T_6 ($ZnSO_4$ @ 0.1% foliar at B and M stages) and T_8 ($ZnSO_4$ @ 0.1% foliar at M stage).

Zn foliar sprayed at MT stage in addition to milking stage T_7 (ZnSO4 @ 0.1% foliar at MT and M stages) recorded phytate: Zn ratios 30.6, 67.3, 18.8, 0.10 and 38.5 mg kg^{-1} in whole

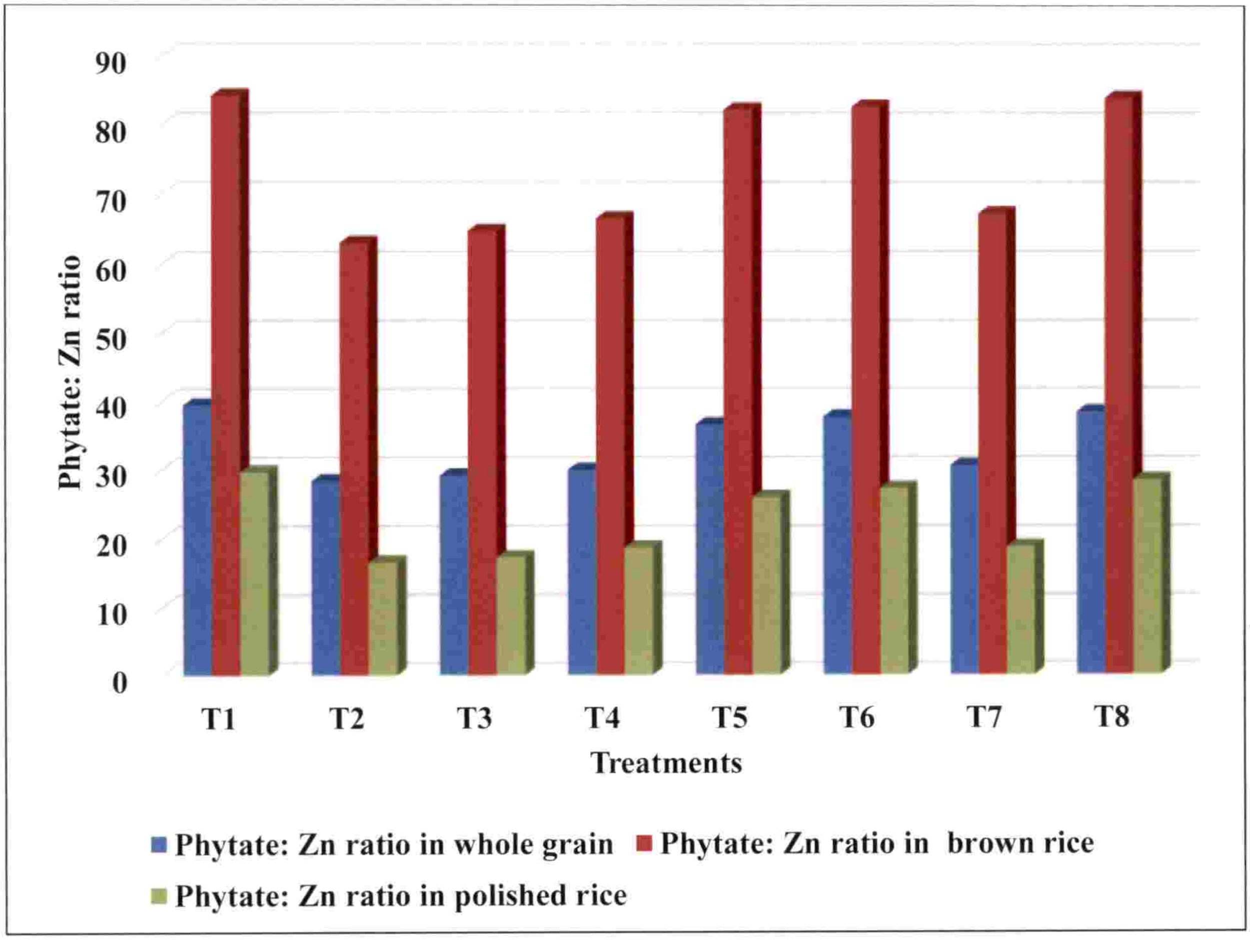

Fig. 7 Effect of time and method of zinc application on Phytate: zinc ratio of whole grain, brown rice and polished rice

grain, brown rice, polished white rice, rice husk and rice bran respectively and decreased Zn concentrations by 23, 20, 37, 26 and 25 percent respectively over Zn unfertilized treatment T_1 (Control- without Zn). Phytate: Zn ratios in whole grain and its milled fractions in treatment T_7 ($ZnSO_4$ @ 0.1% foliar at MT and M stages) were comparable with T_2 (ZnSO4 @ 20 kg ha^{-1} basal), T_3 ($ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages) and T_4 ($ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages). Treatments T_2, T_3, T_4 and T_7 had significantly higher Zn concentrations in whole grain and its milled fractions resulting in significantly lower phytate: Zn ratios, which brought down phytate: Zn ratios near to desired reference levels for better Zn bioavailability.

As most of the phytate in cereals is located in the aleurone layers or bran, milling of cereals and subsequent separation of bran results in a significant reduction of phytate in flours (Singh and Reddy, 1977). This explains lower phytate: Zn ratios and thus better Zn bioavailability recorded in polished white rice compared to whole grain and its milled fractions namely brown rice and rice bran, irrespective of treatments in the present study.

5.1.3.3 Starch and Protein

Present study revealed that starch and protein content of rice grain were significantly affected by time and method of Zn application (Tables 15 to 19).

Zn foliar sprayed at MT stage in addition to milking stage T_7 ($ZnSO_4$ @ 0.1% foliar at MT and M stages) had showed starch contents 74.3, 84.3, 90.9 and 46.3 percent in whole grain, brown rice, polished white rice and rice bran respectively which were on par with T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal), T_3 ($ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages), T_4 ($ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages) and were significantly higher than all the other treatments. However, treatments did not have any significant influence on starch content of rice husk. Zn is a cofactor of carbonic anhydrase enzyme. Carbonic anhydrase functions in fixation of photosynthetic carbon dioxide by elevating carbon dioxide concentration in chloroplast, catalyses conversion of carbon dioxide and water into bicarbonates and helps in transport and distribution of carbon dioxide through plasma membrane and chloroplast (Gonzalez *et al.*, 2018)

Protein contents of 6.78, 8.65 and 14.1 recorded by T_7 ($ZnSO_4$ @ 0.1% foliar at MT and M stages) in whole grain, brown rice and rice bran respectively were on par with T_2 ($ZnSO_4$ @

20 kg ha^{-1} basal), T_3 ($ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages), T_4 ($ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages) and were significantly higher than all other treatments (Table 15 to 19). However treatments did not have any significant influence on protein contents of polished white rice and rice husk. Zn is a constituent of RNA polymerase enzyme associated with N metabolism. Low Zn levels lowers activity of RNA polymerase leading to loss of structural integrity of ribosomes and degradation of RNA. This lower activity of RNA polymerase inhibits protein synthesis leading to accumulation of amino acid (Gonzalez *et al.*, 2018).

Alloway (2009) reported that Zn is involved in carbohydrate and protein metabolism. It was observed from the present study that Zn application during vegetative phase of crop had enhanced Zn uptake (Table 27). This enhanced Zn uptake might have improved starch and protein synthesis leading to their increased concentrations in rice grain. Nalini *et al.* (2013) reported that Zn foliar applied had enhanced seed Zn density, seed carbohydrate (sugar and starch) and storage proteins (albumins, globulins and prolamines) in black gram. Increased protein content due to Zn fertilization had been reported by Radhari *et al.* (2013) in soyabean, Sakthidharan (2013) in amaranthus and Dhanya (2014) in yard long bean.

5.1.4 Effect on Nutrient Content and Uptake by Crop

Nutrient content and uptake by crop was significantly influenced by time and method of Zn application (Tables 20 to 26).

Grain contents of significantly higher N, S and Zn recorded in Zn foliar sprayed at MT stage in addition to milking stage T_7 ($ZnSO_4$ @ 0.1% foliar at MT and M stages) were 1.18, 0.12 and 29.8 percent respectively and were comparable to T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal), T_3 ($ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages) and T_4 ($ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages). However treatments did not have any significant influence on P, K, Ca and Mg contents of grain.

Similar trend was observed in respect of straw contents of N, S and Zn. The treatment T_7 ($ZnSO_4$ @ 0.1% foliar at MT and M stages) recorded higher straw N, S and Zn contents of 0.83, 0.08 and 43.6 per cent respectively and were comparable to T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal), T_3 (ZnSO4 @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages) and T_4 (ZnSO4 @ 0.1%

foliar at MT, PI, B and M stages). Straw contents of P, K, Ca and Mg were not significantly affected by treatments.

Crop uptake of significantly higher N, P, K, Ca, Mg, S and Zn in Zn foliar sprayed at MT stage in addition to milking stage T_7 (ZnSO4 @ 0.1% foliar at MT and M stages) were 136, 24.2, 140, 49.3, 27.8, 13.8 and 0.5 kg ha^{-1} respectively and were comparable to T_2 (ZnSO4 @ 20 kg ha^{-1} basal), T_3 (ZnSO4 @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages) and T_4 (ZnSO4 @ 0.1% foliar at MT, PI, B and M stages).

Foliar or soil + foliar application of Zn fertilizers under field conditions are highly effective practical ways to maximize uptake and accumulation of Zn in rice grain (Wissuwa *et al.*, 2008). Rani (2013) reported that higher available Zn during initial growth stages of crop favored more utilization of N, P and K by crop in producing higher dry matter and nutrient uptake. However, Lone (2015) based on omission plot studies reported that increase in Zn concentration in rice grain is associated with higher soil available N and lower soil available P thus suggesting a synergistic relationship between N and Zn and an antagonistic relationship between P and Zn. In the present study, it was observed that ensuring better availability of Zn in addition to N and P during vegetative phase of crop through fertilizer application significantly increased Zn concentration in rice grain suggesting that synergistic relationship between N and Zn helped to overcome antagonistic relationship between P and Zn.

Biofortification recovery efficiency (BRE_{Zn}) (Fig.8) which denotes the increase in Zn uptake in edible portion of fertilized plant over unfertilized plant per quantity of Zn applied is a major tool for comparing Zn use efficiency (Shivay *et al.*, 2008). Higher biofortification recovery efficiency (43.8%) recorded in Zn foliar sprayed at MT stage in addition to milking stage T_7 (ZnSO4 @ 0.1% foliar at MT and M stages) was superior to all the other treatments. Increased BRE due to Zn fertilization had been reported by Dhanya (2014) in yard long bean and (Shivay *et al.*, 2016) in rice.

5.1.5 Effect on Soil Available Nutrients Status after Experiment

Soil available nutrients after experiment showed significantly lower N, P, K and S in Zn foliar sprayed at MT stage in addition to milking stage T_7 ($ZnSO_4$ @ 0.1% foliar at MT and M stages) and were comparable to T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal), T_3 ($ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B and M stages) and T_4 (ZnSO4 @ 0.1% foliar at MT, PI, B and M

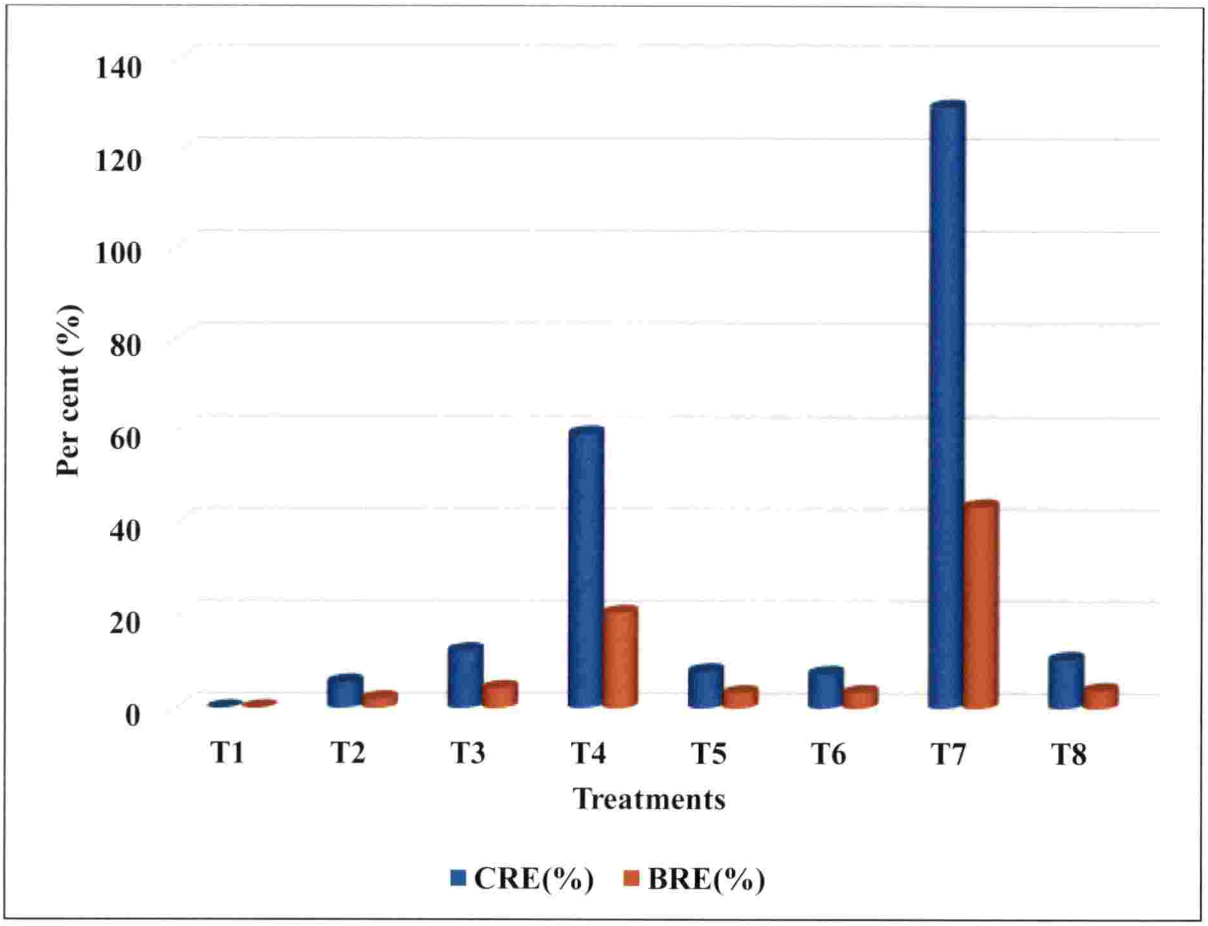

Fig. 8. Effect of time and method of zinc application on Biofortification Recovery efficiency (BRE_{Zn}) and Crop Recovery Efficiency (CRE_{Zn}) of whole grain, brown rice and polished rice

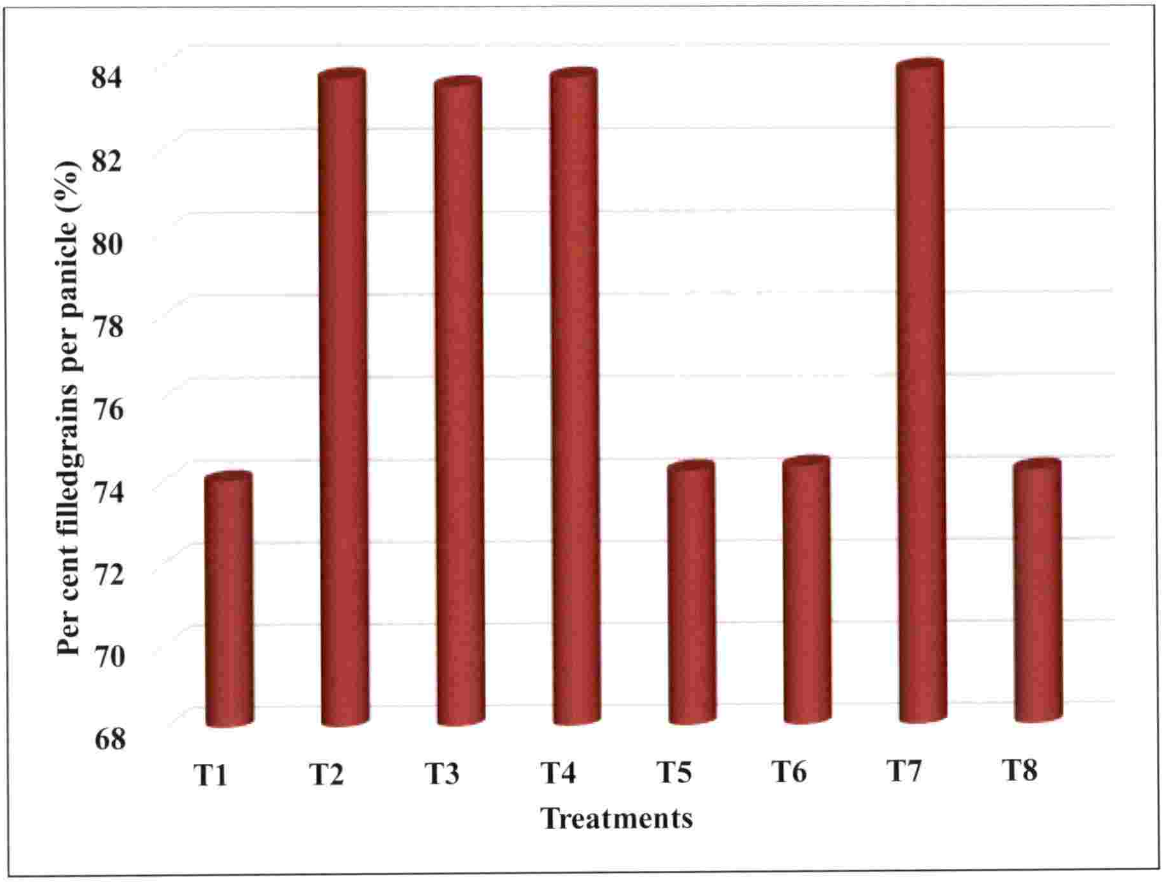

Fig. 4. Effect of time and method of zinc application on per cent filled grains per panicle

stages). However treatments did not have any significant influence on available Ca and Mg in soil (Table 29 and table 30)

Lower available Zn in soil after experiment (0.82 mg kg^{-1}) was recorded in Zn foliar sprayed at MT stage in addition to milking stage T_7 ($ZnSO_4$ @ 0.1% foliar at MT and M stages). Available Zn in soil after experiment in Zn soil applied basally immediately after transplanting T_2 ($ZnSO_4$ @ 20 kg ha^{-1} basal) was significantly higher (2.83 mg kg^{-1}) than all other treatments. This indicated that Zn soil applied basally at early vegetative phase of crop far exceeds the Zn requirement of crop leading to excess available Zn in soil after experiment, whereas Zn foliar sprayed at MT stage was effectively utilized by crop.

5.2 ECONOMICS OF CULTIVATION

Data on economics of time and method of Zn application on yield are presented in Table 31.

Net income was highest (₹ 91213 ha^{-1}) in Zn foliar sprayed at MT stage in addition to milking stage T_7 ($ZnSO_4$ @ 0.1% foliar at MT and M stages). This might be due to increased yield and low cost of cultivation as foliar spray involves less quantity of fertilizers. Zn unfertilized treatment T_1 (Control-without Zn) and Zn foliar sprayed at milking stage alone T_8 ($ZnSO_4$ @ 0.1% foliar at M stage) resulted lower net income of ₹ 11412 ha^{-1} and ₹ 10166 ha^{-1} respectively. This is due to unavailability of Zn to crop during critical growth phases leading to lower yield attributes and lower yield. Foliar sprayed at milking stage alone T_8 ($ZnSO_4$ @ 0.1% foliar at M stage) resulted in lower net income due to additional cost incurred for foliar spaying of Zn at milking stage, which did not result in increased yield.

Similar trend was observed in respect of benefit: cost ratio (Fig.9) T_7 (ZnSO4 @ 0.1% foliar at MT and M stages) had highest benefit: cost ratio of 1.88. T_1 (Control-without Zn) and T_8 ($ZnSO_4$ @ 0.1% foliar at M stage) had lower benefit: cost ratio of 1.11 and 1.10 respectively.

Summary

6. SUMMARY

An investigation entitled "Agronomic biofortification of Zn in rice (*Oryza sativa* L.)" was conducted during 2017-2019 at College of Agriculture, Vellayani. The main objectives of the study were to assess the effect of time and method of Zn application on growth, yield, Zn biofortification and Zn bioavailability in transplanted rice. The field experiment was conducted in the farmer's field at Chirayinkeezhu, Thiruvananthapuram during May to September, 2018 (*Virippu* 2018) using the rice variety Uma (MO 16).

The experiment comprised eight treatments laid out in randomized block design and replicated thrice. Treatments included two basal dressing [T_1: Control (without Zn), T_2: $ZnSO_4$ @ 20 kg ha^{-1} basal (*adhoc* recommendation) and six foliar Zn spray [T_3 $ZnSO_4$ @ 0.5% + Lime @ 0.25% foliar at MT, PI, B (Booting) and M (Milking) stages *(adhoc* recommendation), T_4 $ZnSO_4$ @ 0.1% foliar at MT, PI, B and M stages, T_5 $ZnSO_4$ @ 0.1% foliar at PI, B and M stages, T_6: $ZnSO_4$ @ 0.1% foliar at B and M stages, T_7: $ZnSO_4$ @ 0.1% foliar at MT and M stages and T_8: $ZnSO_4$ @ 0.1% foliar at M stage].

Growth characters such as LAI, root length, root weight, root volume, dry matter production were significantly influenced by treatments, whereas the treatments failed to exhibit significant effects on plant height and number of tillers m^{-2}. At MT stage, application of $ZnSO_4$ @ 20 kg ha^{-1} as basal (T_2) resulted in greater root length, root weight, root volume (20 cm, 4.5 g, 8.9 cc) and higher dry matter production (20.4 g per hill). At PI stage, maximum LAI (5.26) and dry matter production (28 g per hill) observed in foliar spray of ZnSO4 @ 0.1% at MT+ M stages (T_7) were comparable with basal dressing of ZnSO4 @ 20 kg ha^{-1}(T_2) and foliar spray of either $ZnSO_4$ @ 0.5% + lime @ 0.25% or $ZnSO_4$ @ 0.1% at MT + PI + B + M stages (T_3, T_4). Root length (22.4 cm) and root volume (40.1 cc) at harvest stage was recorded the highest with foliar spray of ZnSO4 @ 0.1% at MT + PI + B + M stages (T_4) which was on par with soil application of $ZnSO_4$ @ 20 kg ha^{-1} (T_2) and treatments T_3 and T_7. At harvest stage, higher root weight (23.2 g per hill) recorded for soil application of $ZnSO_4$ @ 20 kg ha^{-1}(T_2) was comparable to foliar spray of $ZnSO_4$ @ 0.1% at MT+ M stages (T_7) and foliar spray of either $ZnSO_4$ @ 0.5% + lime @ 0.25% or $ZnSO_4$ @ 0.1% at MT + PI + B + M stages (T_3, T_4) and significantly superior to all other treatments. Maximum dry matter production (40.9 g per hill) at harvest stage recorded with foliar spray of $ZnSO_4$ @ 0.1% at MT+ M stages (T_7) was comparable to basal dressing of $ZnSO_4$ @ 20 kg ha^{-1}(T_2) and foliar spray of either $ZnSO_4$ @ 0.5% + lime @ 0.25% or $ZnSO_4$ @ 0.1% at MT + PI + B + M stages (T_3, T_4).

Yield attributes namely days to 50 percent flowering, productive tillers m^{-2} were not affected by any of the treatments whereas length of the panicle, grain weight per panicle, filled grains per panicle, per cent filled grains per panicle and thousand grain weight were significantly influenced by treatments. Harvest index did not exhibit significant variation among the treatments. Numerically higher length of the panicle (21 cm), grain weight per panicle (1.88 g), filled grains per panicle (127nos.) and per cent filled grains $panicle^{-1}$ (84%) recorded with foliar spray of $ZnSO_4$ 0.1% at MT + M (T_7) were comparable with basal application of $ZnSO_4$ @ 20 kg ha^{-1}(T_2) and foliar spray of either ZnSO4 @ 0.5% + lime @ 0.25% or $ZnSO_4$ @ 0.1% at MT + PI + B + M stages (T_3, T_4). Higher thousand grain weight (26 g) recorded in $ZnSO_4$ @ 20 kg ha^{-1} as basal (T_2) was comparable with foliar spray of $ZnSO_4$ @ 0.1% at MT+ M stages (T_7) and foliar spray of either $ZnSO_4$ @ 0.5% + lime @ 0.25% or $ZnSO_4$ @ 0.1% at MT + PI + B + M (T_3, T_4).

Grain yield (6605 kg ha^{-1}) and straw yield (7024 kg ha^{-1}) recorded with foliar spray of $ZnSO_4$ 0.1% at MT + M was comparable with soil application of $ZnSO_4$ @ 20 kg ha^{-1} (T_2) and foliar spray of either $ZnSO_4$ @ 0.5% + lime @ 0.25% or $ZnSO_4$ @ 0.1% at MT + PI + B + M stages (T_3,T_4) and was significantly higher than all other treatments.

The Zn content in whole grain (32.1 mg kg^{-1}) and milled fractions namely brown rice (20.4 mg kg^{-1}), white rice (9.5 mg kg^{-1}), husk (5.94 mg kg^{-1}) and bran (80.5 mg kg^{-1}) increased significantly with foliar spray of $ZnSO_4$ @ 0.1% at MT + M stages (T_7) and the magnitude of increase was comparable with soil application of $ZnSO_4$ 20 kg ha^{-1}(T_2) and foliar spray of either $ZnSO_4$ @ 0.5% + lime @ 0.25% or $ZnSO_4$ @ 0.1% at MT + PI + B + M stages (T_3,T_4).

Phytate, which is an anti-nutritional factor in whole grain and whole grain milled fractions namely brown rice, white rice, rice husk and rice bran did not show significant variation due to treatments. Higher Zn concentration lead to lower phytate: Zn ratio which is an indicator of increased bioavailability. Lower phytate: Zn ratio was recorded in whole grain (28.4), brown rice (63.1), white rice (16.6), husk (0.1) and bran (34.4) with soil application of $ZnSO_4$ @ 20 kg ha^{-1}(T_2) was comparable to foliar spray of $ZnSO_4$ @ 0.1% at MT + M stages (T_7) and foliar spray of either $ZnSO_4$ @ 0.5% + lime @ 0.25% or $ZnSO_4$ @ 0.1% at MT + PI + B + M stages (T_3, T_4) and was significantly lower than the other treatments.

Among other quality attributes, higher starch was observed in soil application of $ZnSO_4$ 20 kg ha^{-1} (T_2) in whole grain (75%), brown rice (86%), white rice (92%) and rice bran (50%) was comparable with foliar spray of $ZnSO_4$ @ 0.1% at MT+ M stages (T_7) and foliar spray of either $ZnSO_4$ @ 0.5% + lime @ 0.25% or $ZnSO_4$ @ 0.1% at MT + PI + B + M stages (T_3, T_4). Starch in rice husk did not exhibit significant variation due to treatments. Higher crude protein recorded in whole grain (6.5%), brown rice (8.6%) and rice bran (14%) with foliar spray of 0.1% $ZnSO_4$ at MT + M stages (T_7) and it was comparable with soil application of $ZnSO_4$ @ 20 kg ha^{-1}(T_2) and foliar spray of either $ZnSO_4$ @ 0.5% + lime@ 0.25% or $ZnSO_4$ @ 0.1% at MT + PI + B + M stages (T_3, T_4). Crude protein in white rice and rice husk did not exhibit significant variation due to treatments.

Total uptake of N, P, K, Ca, Mg, S and Zn improved significantly with soil application of $ZnSO_4$ 20 kg ha^{-1}(T_2), foliar spray of either $ZnSO_4$ @ 0.5% + lime @ 0.25 % or $ZnSO_4$ @ 0.1% at MT + PI + B + M (T_3,T_4) and foliar spray of $ZnSO_4$ @ 0.1% at MT + M (T_7). Plant uptake of N (136 kg ha^{-1}), P (24.2 kg ha^{-1}), K (140 kg ha^{-1}), Ca (49.3 kg ha^{-1}), Mg (27.8 kg ha^{-1}), S (13.8 kg ha^{-1}) and Zn (0.50 kg ha^{-1}) were recorded in foliar spray of $ZnSO_4$ @ 0.1% at MT + M (T_7). Total plant uptake of N (138 kg ha^{-1}), P (23.6 kg ha^{-1}), K (140 kg ha^{-1}), Ca (49.6 kg ha^{-1}), Mg (27.8 kg ha^{-1}), S (14.9 kg ha^{-1}) and Zn (0.52 kg ha^{-1}) were observed with soil application of $ZnSO_4$ @ 20 kg ha^{-1}(T_2). The highest CRE (130%) and BRE (43.7%) of Zn was recorded in foliar spray of $ZnSO_4$ @ 0.1% at MT + M stages (T_7) which was superior to all other treatments

Soil at experimental site irrespective of treatments showed increased pH, electrical conductivity, available P, Ca and S and decreased organic carbon, available N, K and Mg after experiment compared to initial pre-treatment soil status. Final soil status after experiment in the treatments $ZnSO_4$ @ 20 kg ha^{-1} (T_2), foliar spray of either $ZnSO_4$ @ 0.5% + lime @ 0.25 % or $ZnSO_4$ @ 0.1 % at MT + PI + B + M stages (T_3, T_4) and foliar spray of $ZnSO_4$ @ 0.1 % at MT + M stages (T_7) indicated significantly lower available N, P, K and S due to higher uptake of these nutrients leading to increased dry matter production with these treatments. Available Zn recorded the highest (2.83 mg kg^{-1}) in $ZnSO_4$ 20 kg ha^{-1} (T_2) was superior to all other treatments.

Economic analysis showed that the highest net income (₹.91213 ha^{-1}) was obtained with foliar spray of $ZnSO_4$ @ 0.1% at MT + M stages (T_7) followed by net income ₹ 87125 ha^{-1} with soil application of $ZnSO_4$ @ 20 kg ha^{-1} (T_2). The highest benefit: cost ratio of 1.88 was recorded with foliar spray of $ZnSO_4$ @ 0.1% at MT + M stages (T_7) followed by benefit: cost ratio of 1.85 in soil application of $ZnSO_4$ @ 20 kg ha^{-1} (T_2).

Based on results of present investigation, it can be concluded that in very strongly acidic Zn deficient soil, $ZnSO_4$ 0.1% foliar spray at maximum tillering and milking stages (T_7) could be recommended for enhanced productivity, profitability, Zn biofortification and Zn bioavailability in transplanted rice.

FUTURE LINE OF WORK

- Exploration on feasibility of applying higher levels of $ZnSO_4$ foliar spray without damaging foliage to enhance Zn content of whole grain rice.
- Study on effect of lime addition to $ZnSO_4$ spray solution on Zn availability in spray solution.
- Study on the effect of basal soil application of $ZnSO_4$ in strongly acidic Zn deficient soil on yield and Zn biofortification of subsequent rice crop in rice-rice cropping system.
- Study on the effect of $ZnSO_4$ foliar spray on yield and Zn biofortification of rice crop in strongly acidic Zn sufficient soil.

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Appendices

APPENDIX- I

Standard week	Temperature (°C)		Relative humidity (%)		Rainfall (mm)
	Maximum	Minimum	Maximum	Minimum	
19	33.2	25.7	89.6	75.0	46.4
20	32.3	24.8	89.6	75.0	109.2
21	33.2	25.7	89.6	75.0	64.1
22	32.8	25.3	89.6	75.0	98.0
23	30.6	24.7	96.7	85.7	126.6
24	31.2	25.1	92.9	81.6	63.5
25	31.0	24.6	92.4	83.7	57.0
26	31.5	24.4	89.7	80.7	25.2
27	31.6	24.7	86.6	75.4	10.2
28	29.6	23.0	93.9	85.4	69.3
29	30.4	23.5	91.1	79.1	56.3
30	31.4	23.6	89.3	73.3	13.1
31	29.5	23.9	90.4	80.9	136.2
32	30.3	23.3	91.0	85.1	107.3
33	29.1	22.6	94.9	89.9	205.2
34	31.0	24.0	89.4	76.6	0.5
35	32.0	24.5	89.1	71.9	0.0

AGRONOMIC BIOFORTIFICATION OF ZINC IN RICE
(*Oryza sativa* L.)

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ABSTRACT

AGRONOMIC BIOFORTIFICATION OF ZN IN RICE (*Oryza sativa* L.)

An investigation entitled "Agronomic biofortification of Zn in rice (*Oryza sativa* L.)" was conducted during 2017-2019 at College of Agriculture, Vellayani and farmer's field at Chirayinkeezhu, Thiruvananthapuram during May to September, 2018 (*Virippu* 2018) to assess the effect of time and method of Zn application on growth, yield, Zn biofortification and Zn bioavailability in transplanted rice variety Uma (MO 16).

The experiment comprised eight treatments laid out in randomized block design and replicated thrice. Treatments included two basal dressing [Control-without Zn, $ZnSO_4$ @ 20 kg ha^{-1} as basal-*Adhoc* recommendation] and six foliar Zn spray [$ZnSO_4$ 0.5% + lime 0.25% foliar spray at maximum tillering (MT) + panicle initiation (PI) + booting (B) + milking (M), $ZnSO_4$ @ 0.1% foliar spray at MT + PI + B + M stages, $ZnSO_4$ @ 0.1% foliar spray at PI + B + M stages, $ZnSO_4$ @ 0.1% foliar spray at B + M stages, $ZnSO_4$ @ 0.1% foliar spray at MT + M stages and $ZnSO_4$ @ 0.1% foliar spray at M stage]. The recommended NPK dose of 90:45:45 kg ha^{-1} and FYM 10 t ha^{-1} as per KAU, 2016 was applied uniformly to all treatments.

Soil application of $ZnSO_4$ @ 20 kg ha^{-1} recorded higher length, weight and volume of root and dry matter production at maximum tillering. Foliar spray of $ZnSO_4$ @ 0.1% at MT + M stages resulted in higher leaf area index and dry matter production at panicle initiation and was comparable with soil application of $ZnSO_4$ @ 20 kg ha^{-1} and foliar spray of either $ZnSO_4$ @ 0.5% + lime @ 0.25% or $ZnSO_4$ @ 0.1% at MT + PI + B + M stages. Length, weight and volume of root, length of panicle, grain weight per panicle, filled grains per panicle, thousand grain weight and dry matter production at harvest recorded in foliar spray of $ZnSO_4$ @ 0.1% at MT + M stages was comparable

to soil application of $ZnSO_4$ @ 20 kg ha^{-1} and foliar spray of either $ZnSO_4$ @ 0.5% + lime @ 0.25% or $ZnSO_4$ 0.1% at MT + PI + B + M stages

Grain yield (6605 kg ha^{-1}) and straw yield (7024 kg ha^{-1}) recorded with foliar spray of $ZnSO_4$ @ 0.1% at MT + M stages was comparable to soil application of $ZnSO_4$ 20 kg ha^{-1} and foliar spray of either $ZnSO_4$ @ 0.5% + lime @ 0.25 or $ZnSO_4$ @ 0.1% at MT + PI + B + M stages and was significantly higher than other treatments.

Zn content in whole grain (30 mg kg^{-1}) and milled fractions namely brown rice (19 mg kg^{-1}), white rice (9.1 mg kg^{-1}), husk (5.4 mg kg^{-1}) and bran (76 mg kg^{-1}) increased significantly higher with foliar spray of $ZnSO_4$ @ 0.1% at MT + M and the magnitude of increase was comparable to soil application of $ZnSO_4$ @ 20 kg ha^{-1} and foliar spray of either $ZnSO_4$ @ 0.5% + lime @ 0.25% or $ZnSO_4$ @ 0.1% at MT + PI + B + M stages.

Anti-nutritional factor phytate in whole grain (9.2 g kg^{-1}), brown rice (13 g kg^{-1}), white rice (1.7 g kg^{-1}), husk (5.3 mg kg^{-1}) and bran (29 g kg^{-1}) did not show significant variation due to treatments. Lower phytate: Zn molar ratio, which is an indicator of increased bioavailability, recorded in whole grain (31), brown rice (67), white rice (19), husk (0.1) and bran (39) with foliar spray of $ZnSO_4$ @ 0.1% at MT + M stages was comparable to soil application of $ZnSO_4$ @ 20 kg ha^{-1} and foliar spray of either $ZnSO_4$ @ 0.5% + lime @ 0.25% or $ZnSO_4$ @ 0.1% at MT + PI + B + M stages and was significantly lower than other treatments.

Starch content of whole grain, brown rice, white rice, bran and protein content of whole grain, brown rice and bran was recorded with foliar spray of $ZnSO_4$ @ 0.1% at MT + M was comparable with soil application of $ZnSO_4$ @ 20 kg ha^{-1} and foliar spray of either $ZnSO_4$ @ 0.5% + lime 0.25% or $ZnSO_4$ @ 0.1% at MT + PI + B + M stages and was significantly higher than other treatments.

Total uptake of N, P, K, Ca, Mg, S and Zn improved significantly with soil application of $ZnSO_4$ @ 20 kg ha^{-1}, foliar spray of either $ZnSO_4$ @ 0.5% + lime @ 0.25 % or $ZnSO_4$ @ 0.1% at MT + PI + B + M stages and foliar spray of $ZnSO_4$ @ 0.1% at MT + M stages. The highest biofortification recovery efficiency of Zn (43.7%) was recorded in foliar spray of $ZnSO_4$ @ 0.1% at MT + M stages.

Foliar spray of $ZnSO_4$ @ 0.1% at MT + M stages fetched higher net return ₹ 91213 ha^{-1} and B:C ratio 1.88 and was followed by soil application of $ZnSO_4$ @ 20 kg ha^{-1} with net return ₹ 87125 ha^{-1} and B:C ratio 1.85.

The present study indicated that in very strongly acidic Zn deficient soil, foliar spray of $ZnSO_4$ @ 0.1% at maximum tillering and milking stages could be recommended for enhanced productivity, profitability, Zn biofortification and Zn bioavailability in transplanted rice.

സംഗ്രഹം

നെല്ലിൽ സിങ്ക് ഉപയോഗിച്ചുള്ള അഗ്രോണോമിക് ബയോഫോർട്ടിഫിക്കേഷൻ എന്ന വിഷയത്തെ സംബന്ധിച്ച ഒരു പഠനം രണ്ടായിരത്തിപതിനെട്ട് മെയ് - സെപ്റ്റംബർ വരെയുള്ള കാലയളവിൽ തിരുവനന്തപുരത്തെ ചിറയിൻകീഴിലെ ഒരു കർഷകന്റെ കൃഷിയിടത്തിൽ വെച്ച് നടത്തുകയുണ്ടായി. സിങ്കിന്റെ പ്രയോഗരീതി, സമയം എന്നിവയ്ക്ക് നെല്ലിന്റെ വളർച്ച, വിളവ്, നെല്ലിലെ സിങ്കിന്റെ അളവ്, ലഭ്യത തുടങ്ങിയവയിലുള്ള സ്വാധീനം വിലയിരുത്തുകയായിരുന്നു പഠന ലക്ഷ്യം. റാൻഡമൈസിഡ് ബ്ലോക്ക് ഡിസൈൻ എന്ന രീതി അവലംബിച്ചു നടത്തിയ പരീക്ഷണത്തിൽ എട്ട് വിവിധ തരം സിങ്ക് സൾഫേറ്റിന്റെ തലങ്ങൾ പഠന വിധേയമാക്കി മൻകൊമ്പ് നെല്ല് ഗവേഷണ കേന്ദ്രത്തിൽ നിന്നും വികസിപ്പിച്ചെടുത്ത ഉമ എന്നയിനമാണ് പഠനത്തിന് ഉപയോഗിച്ചത്.

സിങ്ക് സൾഫേറ്റിന്റെ എട്ട് വിവിധ തലങ്ങളിൽ, ആദ്യത്തേത് സിങ്ക് കൂടാതെ കേരള കാർഷിക സർവകലാശാല ശുപാർശ ചെയ്ത വള പ്രയോഗം ടി-1 (90:45:45 കി.ഗ്രാം/ഹെ. എൻ പി കെ) ആണ്. രണ്ടാമത്തേത് കേരള കാർഷിക സർവകലാശാലയുടെ അഡ്ഹോക് ശുപാർശ പ്രകാരം 20 കി.ഗ്രാം/ഹെ. സിങ്ക് സൾഫേറ്റ് അടിവളമായി നൽകി. ശേഷിക്കുന്ന ആറ് തലങ്ങളിൽ സിങ്ക് സൾഫേറ്റ് ലായനി നെല്ലിന്റെ വളർച്ചയുടെ വിവിധ ഘട്ടങ്ങളിലായി ഇലകളിൽ തളിച്ചു. ടി-3 യിൽ 0.5 % സിങ്ക് സൾഫേറ്റ് ലായിനിയുടെയും 0.25 % കുമ്മായത്തിന്റെ ലയിനിയുടെയും മിശ്രിതം നെല്ലിന്റെ പരമാവധി ചിനപ്പുകൾ ഉണ്ടാകുന്ന സമയം, കതിര് രൂപപ്പെടുന്ന സമയം, ബൂട്ടിങ് സമയം, കതിരിലെ പാലുറയ്ക്കുന്ന സമയം എന്നി ഘട്ടങ്ങളിൽ തളിച്ചു. ടി-4 ൽ സിങ്ക് സൾഫേറ്റിന്റെ 0.1 % ലായനി നെല്ലിന്റെ പരമാവധി ചിനപ്പുകൾ ഉണ്ടാകുന്ന സമയം, കതിര് രൂപപ്പെടുന്ന സമയം, ബൂട്ടിങ് സമയം, കതിരിലെ പാലുറയ്ക്കുന്ന സമയം എന്നി ഘട്ടങ്ങളിലായി ഇലകളിൽ തളിച്ചു. ടി-5 ൽ സിങ്ക് സൾഫേറ്റിന്റെ 0.1 % ലായനി കതിര് രൂപപ്പെടുന്ന സമയം, ബൂട്ടിങ് സമയം, കതിരിലെ പാലുറക്കുന്ന സമയം എന്നി ഘട്ടങ്ങളിലായി ഇലകളിൽ തളിച്ചു.

ടി-6 ൽ ബൂട്ടിങ് സമയത്തും കതിരിലെ പാലുറയ്ക്കുന്ന സമയത്തും സിങ്ക് സൾഫേറ്റ്ന്റെ 0.1 % ലായനി ഇലകളിൽ തളിച്ചു. ടി-7 ൽ നെല്ലിന്റെ പരമാവധി ചിനപ്പുകൾ ഉണ്ടാകുന്ന സമയത്തും, കതിരിലെ പാലുറയ്ക്കുന്ന സമയത്തും ഇലകളിൽ തളിച്ചു. ടി-8 ൽ സിങ്ക് സൾഫേറ്റ്ന്റെ 0.1 % ലായനി കതിരിലെ പാലുറയ്ക്കുന്ന സമയത്തിൽ മാത്രം ഇലകളിൽ തളിച്ചു.

ഈ പഠനത്തിൽ നിന്നും നെല്ലിന്റെ വളർച്ച, വിളവ്, കതിരിലെ സിങ്കിന്റെ അളവ്, ലഭ്യത, വരുമാനം എന്നിവ സിങ്ക് സൾഫേറ്റ്ന്റെ 0.1 % ലായനി പരമാവധി പരമാവധി ചിനപ്പുകൾ ഉണ്ടാകുന്ന സമയത്തും, കതിരിലെ പാലുറയ്ക്കുന്ന സമയത്തും ഇലകളിൽ തളിച്ചപ്പോൾ കൂടുന്നതായി കാണപ്പെട്ടു.