Zinc Biofortification for Enhancing Yield and Quality of Yard Long Bean (*Vigna unguiculata* subsp. *Sesquipedalis* (L.) Verdcourt) in Ferralitic Soils

by

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"Zinc Biofortification for Enhancing Yield and QualityofYard Long Bean(*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt) in Ferralitic Soils"** 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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CERTIFICATE

Certified that this thesis entitled **"Zinc Biofortification for Enhancing Yield and Quality of Yard Long Bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt) in Ferralitic Soils"** is a record of research work done independently by Ms. Dhanya G. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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LIST OF ABREVIATIONS

%	per cent
^{0}C	degree Celsius
µg	microgram
B: C	Benefit: Cost
Ca	Calcium
CD	Critical Difference
cm	centimeter
Cu	Copper
DALY	Disability-Adjusted Life Years
DAS	Days After Sowing
dS	desi Siemen
et al	and others
Fe	Iron
Fig.	Figure
FYM	Farm Yard Manure
g	gram
g plant $^{-1}$	gram per plant
g plot $^{-1}$	gram per plot
ha^{-1}	per hectare
I	Iodine
IAA	Indole 3-acetic acid
K	Potassium
KAU	Kerala Agricultural University
kg	Kilogram
kg plant^{-1}	kilogram per plant
kg ha^{-1}	kilogram per hectare
L^{-1}	per litre
Mg	Magnesium
Mn	Manganese

m	metre
mg	milligram
min	minutes
ml	millilitre
MOP	Muriate of Potash
MSL	Mean Sea Level
mm	millimeter
N	Nitrogen
nm	nanometer
No.	number
P	Phosphorus
P_2O_5	Phosphate
POP	Package of Practices
ppm	parts per million
RBD	Randomized Block Design
RH	Relative Humidity
RF	Rain fall
RP	Rock phosphate
S	Sulphur
s	seconds
Se	Selenium
SOC	Soil Organic Carbon
STBR	Soil Test Based Recommendation
t	tonnes
Var.	variety
viz.	namely
Zn	Zinc
$ZnSO_4$	Zinc sulphate

Introduction

1.INTRODUCTION

Green revolution has made self-sufficiency in food grain production, but the increased production and productivity has greatly enhanced the demand for soil nutrition in India. The continuous cultivation in our soils with high yielding varieties and application of fertilizers mainly for nitrogen, phosphorus and potassium had depleted most of the secondary and micronutrients from soil. Eventually micronutrient deficiency has become a limiting factor for crop production. Cultivation in micronutrient deficient soils resulted crop produces with low micronutrient content and consumption of such produces leads to micronutrient malnutrition in humans and animals. Among the micronutrient deficiencies in human beings, zinc deficiency ranks second (Prasad, 2010).

Zinc is an exceptional micronutrient regarding its relevance in biological systems because it is the only trace metal represented in all classes of enzymes (Broadley *et al.*, 2007). It is involved in a number of physiological processes of plant growth and metabolism including enzyme activation, protein synthesis, metabolism of carbohydrates, lipids, auxins and nucleic acids, gene expression and regulation, reproductive development, structural and functional integrity of biological membranes and protection against reactive oxygen species (Cakmak, 2000). In humans, Zn is required for the activity of more than hundred enzymes involved in most of the major metabolic pathways and consequently is necessary for a wide range of biochemical, immunological and clinical functions (Hotz and Brown, 2004). The Zn required by humans are mainly derived from food which in turn derive nutrients from the soil–plant system and any deficiency in that system will reflect on human health as malnutrition. Low dietary Zn intake and/or poor availability and lack of functional reserve or body store of available zinc are considered to be the major reasons for widespread occurrence of Zn deficiency in human populations. In the world population, 2.7 billion people are estimated to be Zn deficient. A large percentage of global population is affected by mild to moderate zinc deficiency, particularly among low income group (Krebs *et al.*, 2012).

Zinc deficiency has been estimated to be responsible for approximately 4 per cent of the worldwide burden of morbidity and mortality in children less than five years of age, and a loss of nearly 16 million global disability-adjusted life years (DALYs) (Black *et al.*, 2008). WHO (2005) recommended 12-15 mg Zn as daily dietary intake for an adult. Clearly, there is an urgent need to implement strategies to combat zinc deficiency in low income countries. Potential strategies include zinc supplementation, fortification, dietary diversification / modification and biofortification, the later being easily accessible and provide immediate effect. Hence biofortification is considered as an ideal technology to address mineral malnutrition along with yield enhancement of crops (White and Broadley, 2009). Biofortification is the process of enriching the nutrient content of crops through various approaches which could increase root growth and result in a high transfer of minerals from soil to plants. Improving the processes by which zinc moves from soil into plant and eventually into the edible part of the plant has the potential to mitigate problems associated with zinc deficiency in crops and human beings. It can be accomplished either through mineral fertilization or by conventional breeding / transgenic approaches of which former is more economic and faster. Agronomic biofortification through fertilizers, which is also known as fertifortification has proved to be sustainable, can be implemented at relatively with low cost, highly efficient and has a large coverage, especially in the financially poorer regions of the world.

India has Zn deficient soils in some regions. As regards to Kerala, ferralitic soils are the most extensive group of soils which occupies the mid land and mid upland region of the state covering an area of 22,370 sq. km. Nearly 34 per cent of these soils are low in plant available Zn (Singh, 2009). Consequently, the crops grown on such Zn deficient soils contribute only marginally to daily Zn intake. Zn biofortification (agronomic) through soil application increased grain yield up to 29 per cent, grain Zn concentration up to 95 per cent and estimated Zn availability up to 74 percent. While absorption of Zn from nutrient solution was more efficient than from soil, foliar application of Zn was even more effective than application to the root environment in providing Zn for transport to grains

(White and Broadley, 2011) indicating that foliar spraying with Zn in field grown crops can be effective in increasing Zn concentration in grains.

Legumes have better Zn accumulation capacity than cereals (Oseni, 2009). Therefore Zn enrichment of pulse grains which forms a part of the diet of low and marginal income group can address Zn malnutrition in a better way than its enrichment in cereals. The availabilityof Zn is also more for legumes compared to cereals. The agronomic biofortification approaches are highly suitable for soils with severe Zn deficiency. Even in soils that contains adequate zinc, application of zinc either to soil or to foliage was found to enhance crop yield and zinc content of plant parts (Singh, 2009). So agronomic biofortification of zinc through mineral fertilization is considered as the most economical and immediate solution to address zinc deficiency in plants and human Zn malnutrition. Hence the present study was formulated with the following objective.

- To study the effect of zinc biofortification on pod yield and quality of yard long bean in ferralitic soils.

Review of literature

1. REVIEW OF LITERATURE

2.1. IMPORTANCE OF ZINC IN NUTRITION

Zinc (Zn) has a unique place in the biology of planet earth. Across all phyla, from bacteria to humans, more proteins bind or require Zn for their function than those binding all other biologically essential cations combined (Gladeshev *et al.*, 2004). It is a relatively rare element with a surprisingly central role in life on earth. It is an essential micronutrient for normal growth and development of all living organisms including humans and plants and is exceptional micronutrients regarding its relevance in biological systems because it is the only trace metal represented in all classes of enzymes (Broadley *et al.*, 2007).

Essentiality of Zn as a micronutrient for higher plants was established for the first time by Sommer and Lipman (1926). Jyung*et al.* (1975) reported that, Zn was involved in the carbohydrate and protein metabolism. Cakmak *et al.* (1989) stated that, Zn is required for the synthesis of tryptophan, a precursor for the synthesis of IAA. Zn plays multiple roles in basic biochemical processes such as enzyme catalysis or activation, protein synthesis, carbohydrate and auxin metabolism, chlorophyll production, pollen formation, cytochrome and nucleotide synthesis, maintenance of membrane integrity and energy dissipation (Alloway, 2009).

Cakmak (2000) proved that Zn has a role in protecting cells by both controlling regeneration as well as detoxification of reactive oxygen species. A large diversity of essential cellular functions and metabolic pathways are directly influenced by Zn including function and structural stability of proteins, integrity of biological membranes and protection against reactive oxygen species. Nearly 2,800 proteins in biological systems require Zn for their activity and structural stability (Andreini*et al.*, 2009).

The role of Zn in higher plants is as a divalent cation (Zn^{++}) which acts either as a functional, structural and a regulatory co- factor of a large number of enzymes (Brown *et al.*, 1993). Shuman *et al.* (1995) indicated the involvement of Zn in stomatal opening, possibly as a constituent of carbonic anhydrase needed to maintain adequate bicarbonate in the guard cell and also Zn affected in the influx of K^+ uptake into guard cells.

Fox and Guerinot (1998) asserted that, Zn is required for functioning of more than 300 enzymes. Zn is a structural part of carbonic anhydrase, alcohol dehydrogenase, Cu / Zn-superoxide dismutase and RNA polymerase (Marschner, 1986) and serves as a cofactor for all 6 classes of enzymes *viz.* oxidoreductases, transferases, hydrolases, lyases, isomerase and ligases (Broadley *et al.*, 2007).

In humans, Zn acts as a co factor for the activity of more than 200 enzymes and is required for many biological processes such as normal development and function of the immune system, neuro sensory functions, reproductive health and brain function (Coleman, 1998; Huddle *et al.*, 1998; Meunier *et al.*, 2005). WHO (2005) recommended 12-15 mg Zn as daily dietary intake for an adult. Zn is also an essential element regulating human intestinal Fe absorption and sufficient quantity of Zn along with Fe in human body is crucial for treating iron deficiency anemia (Graham *et al.*, 2012).

2.2. BEHAVIOUR AND PHYTOAVAILABILITY OF ZINC

The concentration of Zn in different soils mainly depends upon the parent material, atmospheric depositions and human activities *viz.*, addition of farm yard manure, fertilizers, sewage sludge and industrial waste products (Alloway, 2003).

Zinc is present in soil in a number of chemical forms with varying solubilities. Adsorbed Zn is in equilibrium with solution Zn, controlling Zn availability by adsorption and desorption reactions (Takkar and Sidhu, 1977). Soil Zn occurs in three primary fractions: (i) water-soluble Zn (including Zn^{2+} and soluble organic fractions), (ii) adsorbed and exchangeable Zn in the colloidal fraction (associated with clay particles, humic compounds and Al and Fe hydroxides) and (iii) insoluble Zn complexes and minerals of which the first two fractions are easily available to the plants (Alloway, 1995; Barber, 1995). Zinc present in the soil solution is readily available for plant uptake (Marschner, 1995). These forms include soluble Zn present in soil solution (water soluble), adsorbed on exchange sites (exchangeable), associated with organic matter, co-precipitated as secondary minerals or associated with sesquioxides and as structural part of primary minerals. These different forms control solubility and availability of Zn to plants (Almendros *et al.*, 2008). However, soil chemical properties *viz.* pH, redox potential, organic matter and soil sulfur contents have strong influence on these adsorption-desorption reactions and play a critical role in regulating Zn solubility and fractionation in soils (Alloway, 2009).

The decrease in available Zn concentration in soils is usually associated with several factors like high P availability, precipitation of $Zn(OH)_2$ with an increase in pH, formation of insoluble franklinite ($ZnFe_2O_4$) (Sajwan and Lindsay, 1986) and ZnS (Kittrick, 1976) in acidic soils and $ZnCO_3$ in calcareous soils (Bostick *et al.*, 2001).

Soil sulphur content and redox potential can also influence soil Zn availability. Low redox potential favors the precipitation of Zn as ZnS due to reduced soil conditions and thus decreases the Zn availability to plants in calcareous soils (Beebout *et al.*, 2009).

Information on the forms of mineral elements acquired by plant roots and the limitations to the supply and phytoavailability of mineral elements in the rhizosphere solution are essential for the success of an agronomic biofortification strategy. The supply and phytoavailability of mineral elements in the rhizosphere solution ultimately limit the accumulation of mineral elements by crops, unless foliar fertilizers are applied. Roots of all plant species can take up Fe, Zn, Cu, Ca and Mg in their cationic forms and graminaceous species can also take up Fe, Zn and Cu as metal-chelates (Marschner, 1995; White, 2003).

Mineral elements can present in the soil as free ions or as ions adsorbed onto the mineral or organic surfaces, as dissolved compounds or precipitates, as part of lattice structures or contained within the soil biota. The most important soil properties governing mineral availability are soil pH, redox conditions, cation exchange capacity, activity of microbes, soil structure, organic matter and water content. The phytoavailability of Zn is often restricted by soil properties which predetermine both genetic and agricultural strategies for its effective utilization (Shuman, 1998; Frossard *et al.*, 2000).

Concentration of Zn in the rhizosphere solution is determined by soil-specific precipitation, complexation and adsorption reactions and high pH is often the major factor limiting its phytoavailability. It is estimated that, about half the agricultural soils in India lack sufficient phytoavailable Zn. In non-polluted areas, typical Zn^{2+} concentrations in the soil solution range from 10^{-8} to 10^{-6} M (Frossard *et al.*, 2000; Broadley *et al.*, 2007). Because of its low concentration in the soil solution and small diffusion coefficient, Zn^{2+} have limited mobility in the soil and plant roots must forage through the soil to acquire sufficient Zn for plant nutrition (Broadley *et al.*, 2007; Cakmak, 2008).

Processes that increase Fe and Zn phytoavailability in the rhizosphere, such as the exudation of protons, phytosiderophores and organic acids by roots, generally increase the concentrations of these elements in crops (Abadia *et al.*, 2002; Degryse *et al.*, 2008).

Many strategies for the biofortification of crops with essential mineral elements rely on increasing the acquisition of these elements from the soil. However, it is obvious that, if the soil contains insufficient amounts of these elements then they must be added to the agricultural system as fertilizers. If sufficient amounts of these elements are present in the soil, then the focus turns to increasing the supply and phytoavailability of these elements in the rhizosphere, and their uptake by plant roots and redistribution to edible portions, such that agronomic biofortification is effective (White and Broadley, 2009). As the quantity of Zn added decreases, phytoavailability of Zn also decreased (White and Broadley, 2011).

2.3. EXTRACTION OF ZINC BY PLANTS

Transpiration and diffusion had a significant role in Zn uptake and transport in plants. Under aerobic conditions, a decrease in soil water content may restrict Zn transport towards roots (Yoshida, 1981) because Zn movement in soil is mainly controlled by diffusion (Marschner, 1995). A decrease in transpiration rate influences the mass flow, resulting in reduced Zn transport towards plants and loading into grains as well (Gao*et al.*, 2005; Gao*et al.*, 2006).

Zn is transported symplastically across the root to the xylem, although a substantial fraction may traverse the root and reach the xylem via the apoplast (Broadley *et al.*, 2007). Zinc can be taken up across the plasma membrane of root cells as Zn^{2+} or as a Zn-phytosiderophore complex (Broadley *et al.*, 2007).

Although some plasma membrane Ca^{2+} channels are permeable to Zn^{2+} (White *et al.*, 2002), it is thought that, most Zn^{2+} influx to the cytoplasm is mediated by ZIPs (ZIP1, ZIP3 and ZIP4) (Broadley *et al.*, 2007; Palmgren*et al.*, 2008). As the cytoplasm of plant cells contains an abundance of proteins that bind Zn^{2+}, cytoplasmic Zn^{2+} concentrations are likely to be vanishingly small (Broadley *et al.*, 2007). Zn is sequestered in the vacuole as an organic acid complex (Broadley *et al.*, 2007). Within the xylem, Zn may be transported as Zn^{2+} or complexed with organic acids, histidine or nicotinamine (Broadley *et al.*, 2007; Palmgren *et al.*, 2008). Although Zn mobility in the phloem is generally considered to be low, this may not always be the case (Haslett *et al.*, 2001).

2.4. REMOBILIZATION OF ZINC FROM VEGETATIVE PARTS TO SEEDS / EDIBLE PARTS

In many soils, which are even sufficient to support mineral dense crops, Zn uptake is limited by its phytoavailability and acquisition by roots. Zn taken up by roots continues its movement through xylem, finally reaches the leaves and when leaf concentrations are sufficient, it is translocated to fruits / seeds (White and Broadley, 2009). Soil or foliar applied Zn fertilisers increased the Zn concentration in phloem fed tissues such as fruits, seeds, tubers (White *et al.*, 2009). Soluble Zn applied to foliage enter leaf apoplast and then taken up by plant cells (Cakmak, 2008; Brown, 2009). Retranslocation of micronutrients deposited in shoot tissues plays a critical role in the grain accumulation of micronutrients. In wheat, ≤ 70 per cent of the amount of Zn in vegetative plant parts is remobilized to seed (Zubaidi *et al.*, 1999).

Remobilization and retranslocation of Zn from vegetative tissues into seeds through the phloem may be affected by the level of N nutrition. Zinc transporter proteins located on root cell membranes and the plasma membranes of phloem cells are possibly involved in phloem transport of Zn and Fe into seeds (Curie *et al.*, 2009).

Beneficial effect of the use of Zn fertilizer has been reported in tomato (Jyolsana, 2005), and fodder maize (Thankamoni, 2010). Better nutrition provided to the POP treatment through bioaugmentation and additional foliar application of zinc had favourably influenced the growth, yield and Zn content of amaranthus (Sakthidharan, 2013).

2.5. AVAILABILITY OF ZINC TO HUMAN BEINGS

The researchon availability of zinc was mainly limited to grain crops. Pulses and millets are rich source of micronutrients. The availability of zinc was decreased by the antinutritional factors like phytate and poly phenols (Nieto *et al.*, 2007). In the case of grain crops, seed coats were confirmed to be exclusive tissue containing polyphenols and the removal of seed coat can improve the availability of zinc.

Phytate, present in pulses, binds with Zn and other metal cations to form insoluble complexes that hinder Zn absorption in the human intestine. Therefore, the [phytate] : [Zn] ratio has been generally employed to categorize the Zn availability of food (Brown *et al.,* 2001).

Agronomic zinc biofortification through Zn application is generally suggested to increase grain Zn concentration (Rengel *et al.,* 1999; Cakmak, 2008) and Zn availability (Hussain*et al.,* 2012 a, b). Various studies have also reported differential localization of Zn and phytate in various grain parts (Ozturk *et al.,* 2006; Cakmak *et al.,* 2010) and their removal with various milling streams (Liang *et al.,* 2008; Peng *et al.,* 2010).

2.6. ZINC STATUS AND AVAILABILITY IN FERRALITIC SOILS

2.6.1. India

Indian soils are generally low in zinc and as much as half of the country soils are categorized to be zinc deficient. Total and available zinc content in Indian soils ranged from 7 to 2,960 mg kg^{-1} and 0.1 to 24.6 mg kg^{-1}, respectively with an average deficiency of 12 to 87 per cent. Crops grown in these soils have low Zn content in shoot and seed. Zinc soil fertility is a good index of high zinc content in fodders and grains as significant correlation is found between available zinc content in soil and zinc content in rice grains (Singh, 2009).

Absence of sufficient quantities of Zn in soil or its unavailability due to some antagonistic factors / transformation to less soluble forms are the major reasons for low Zn content in crops. Continuous cultivation of high yielding varieties have led to depletion of native micronutrient soil fertility and now most of the soils are showing signs of fatigue for sustaining higher crop production. As much as 48, 12, 5, 4, 33 and 41 per cent soils in India are affected with deficiency of Zn, Fe, Mn, Cu, B and S respectively (Singh, 2001). This situation is attributed mainly to crop production in areas with low mineral phytoavailability and/or consumption of crops with low tissue mineral concentration. Besides this, hidden hunger of micronutrients is widely noticed leading to even entire failure of crops and reduced content of micronutrients in plant parts (Singh, 2009).

Increased cropping intensity in marginal lands, lesser use of micronutrients in the states like Tamil Nadu, Karnataka, Kerala, Chattisgarh and Maharashtra has further escalated the magnitude of zinc deficiency. In many areas, hidden deficiency has surfaced. Singh (2009) reported the overall zinc deficiency is expected to increase from 48 per cent found in the year 1970 to 63 per cent by the year 2025, because more and more marginal areas are brought under intensive cultivation without adequate micronutrient supplementation. The states of Punjab and Haryana have however, shown a build up of zinc and decline in deficiency. It is estimated that to correct zinc deficiency, India need 324 t ha^{-1} per year of fertilizer zinc by the year 2025 (Singh, 2009).

2.6.2. Kerala

Ferralitic soils are the most extensive soils of Kerala occupying the mid land and mid–upland region of the state covering an area of 22370 sq. km. Crop production in these soils has remained low due to inherent low soil fertility and aberrant weather conditions. Since green revolution era, main thrust was given for enhancing yield by fertilizer application with primary nutrients. The continuous cultivation in these soils with high yielding varieties has depleted most of the secondary and micronutrients from soil. About 34 per cent of Kerala soils are deficient in Zn, 31 per cent in Cu and less than 1 per cent in Fe and no deficiency for Mn (Singh, 2009). He also reported that, Zn deficiency is expected to increase from 49 to 63 per cent by 2025. Deficiency of zinc ranged from 2.3 to 50 per cent in ten districts of Kerala (Mathew and Aparna, 2012).Total Zn status of Kerala soils ranged from 25-55 mg kg^{-1}. Zn deficiency was wide spread in Kerala and this deficiency was due to excessive quantity of phosphatic fertilizers applied and excessive levels of Fe, Mn and Al due to ion competition (Kumar *et al.*, 2013).

2.7. METHODS FOR ENHANCING ZINC AVAILABILITY

Agriculture is the vital tool for ameliorating micronutrient malnutrition as it is primary source of all micronutrients consumed by humans and animals worldwide. Logically, agricultural farming systems are the root cause of hidden hunger (Cakmak, 2002). The agricultural practices to enhance nutrient density include agronomic practices like cultivation of high density seed, advanced fertilization and organic manuring, cultivation of micronutrient efficient varieties, fertifortification etc.

Increasing micronutrient density of edible parts of crop plants is an important issue as it helps in providing more micronutrient nutrition from crop produces. Agronomic biofortification or fitting plants to the soil is a good approach, rather than ameliorating soil to support normal plant growth. Pulses and vegetables are better sources of zinc; their enrichment with zinc will be more helpful in addressing zinc malnutrition (Singh, 2009).

Absorption of Zn from nutrient solution was more efficient than from soil. Foliar application of Zn was even more effective than application to the root environment in providing Zn for transport to soya bean grains (Khan and Weaver, 1989), indicating that foliar spraying with Zn in field-grown crops can be effective in increasing Zn concentration in grains. Soil or foliar application of Zn-containing fertilizers improved grain Zn concentrations in both durum and bread wheat (Cakmak, 2008).

2.8. AGRONOMICBIOFORTIFICATIONTHROUGH MINERAL FERTILIZATION FOR ENHANCING ZINC DENSITY AND CROP YIELD

Agronomic biofortification of staple grains / pulses with Zn is considered as the most economical solution to address human Zn deficiency (Bouis*et al.*, 2011).

The biofortification approaches such as fertilizer application, conventional breeding and genetic engineering were found to enhance Zn content of crop plants, of which Zn fertifortification is the easiest and fastest one (Bouis and Welch, 2010; Cakmak, 2008; Rengel *et al.*, 1999; Welch and Graham, 2004; White and Broadley, 2009).

Application of Zn fertilizers to soils is a general strategy to cope with Zn deficiency (Rengel *et al.*, 1999) and to increase grain Zn concentration (Yilmaz *et al.*, 1997; Jiang *et al.*, 2008; Hussain *et al.*, 2012 a, b). Therefore, Zn fertilization of crop plants is a rapid solution to on-going human deficiency, with an added benefit of increased grain yield (Cakmak, 2009). Zinc application is important in situations where plant-available Zn is low in soils correlates with human Zn deficiency (Alloway, 2009).

Extensive research has been completed on the role of Zn fertilizers in increasing the Zn density of grain, suggesting that where fertilizers are available, making full use of Zn fertilizers can provide an immediate and effective option to increase grain Zn concentration and productivity in particular, under soil conditions with severe Zn deficiency.

Soil or foliar application of Zn-containing fertilizers greatly improved grain Zn concentrations in both durum and bread wheat (Cakmak, 2008). In field trials in Central Anatolia, a well-known highly Zn-deficient region of Turkey (Cakmak *et al.*, 1996), applying $ZnSO_4$ to soil enhanced both grain yield (Yilmaz *et al.*, 1997; Ekiz *et al.*, 1998) and grain Zn concentration of durum wheat. An increase in grain Zn concentration by soil Zn application was almost two fold, whereas combined application of Zn through soil and foliar was more effective and resulted in a more than threefold increase in Zn concentration in durum wheat grain. Similar increases in grain concentrations of Zn in wheat following soil Zn application were also seen in Australia (Graham *et al.*, 1992) and in India (Shivay *et al.*, 2008 a, b) under field conditions.

In two-year field experiments, Khanda and Dixit (1996) compared $ZnSO_4$ and Zn-EDTA via soil and foliar application in combination with four N levels (0, 30, 60, 90 kg ha^{-1}) in sandy loam soil with suboptimal soil Zn (0.84 mg kg^{-1}) under lowland rice conditions. However, maximum gain in yield, nutrient uptake and economic returns was observed with combined application of N and Zn, particularly with 90 kg N ha^{-1}. Among the Zn application methods, soil application was considered superior (Khanda and Dixit, 1996).

Zn use efficiency is often determined based on the ratio of shoot dry matter or grain yield produced under Zn deficiency to that produced with Zn fertilization (Graham, 1984). Zinc application methods and sources are aimed at improving Zn availability for plant uptake. Zn can be applied to soil, seed and leaves (Johnson *et al.*, 2005) and by dipping seedlings into a fertilizer solution.

The efficiency of applied Zn fertilizer is reduced under continuous flooding due to formation of insoluble ZnS and zinc franklinite ($ZnFe_2O_4$) (Ponnamperuma, 1972), $ZnCO_3$ formation due to organic matter decomposition (Bostick *et al.*, 2001) and $Zn(OH)_2$ formation in alkaline soils (Brar and Sekhon, 1976). Most common method of Zn fertilization is through soil application. Zinc can be applied to soil by broadcasting, banding in vicinity of seed, or via irrigation.

Selection of appropriate Zn sources for soil application can also be an alternative strategy to improve plant availability of Zn. Zinc fertilizers with good solubility (such as Zn-EDTA and $ZnSO_4$) generally results in greater Zn transport to the roots compared with insoluble ZnO or fritted Zn (Giordano and Mortvedt, 1972; Kang and Okoro, 1976). In a field experiment, Naik and Das (2007) found that split application of $ZnSO_4$ was better than just basal application.

Zinc can be absorbed by leaf stomata when applied as foliar spray and then transported via the vascular system to where it is needed (Marschner, 1995). A number of Zn sources [$ZnSO_4$, $Zn(NO_3)_2$, Zn-EDTA] have been used as foliar fertilizers in a number of crops (Yoshida *et al.*, 1970). Foliar application of $ZnSO_4$ is effective in correcting Zn deficiency and improving grain Zn concentration (Jiang *et al.*, 2008; Stomph *et al.*, 2011). Significant increases in grain yield and grain Zn contents were observed with foliar application of Zn as Zn-EDTA and $ZnSO_4$ (Karak and Das, 2006).

Although foliar application is effective in increasing seed Zn content (Welch, 2002; Yang *et al.*, 2007; Jiang *et al.*, 2008; Cakmak, 2009), time of foliar Zn application is an important factor in this regard (Jiang *et al.*, 2008; Stomph *et al.*, 2011). Generally, large increases in grain Zn occur when it is foliarly applied at later stages of plant development. This increase in grain Zn concentration was attributed to improved leaf remobilization of Zn during grain filling. Foliar application can avoid the problems of Zn binding in soil, but the time of Zn application should be around flowering for increasing grain Zn concentration.

Locations differed in their native soil Zn status. The critical soil Zn level for the occurrence of Zn deficiency established for the standard DTPA method is 0.8 mg Zn kg^{-1} (Dobermann and Fairhurst, 2000). Applications of Zn fertilizers, most typically as $ZnSO_4$ at rates of 5-10 kg Zn ha^{-1} is suitable to correct soil Zn deficiency (Dobermann and Fairhurst, 2000; Qadar, 2002) but higher rates of 14 - 15 kg Zn ha^{-1} are not uncommon in parts of Northern India (Singh *et al.*, 2005).

When Zn fertilizers are applied to foliage, it enters leaf apoplast and can be taken up by plant cells (Cakmak, 2009). When it is applied to soil, Zn uptake is limited by its phytoavailability and acquisition by roots (White and Broadley, 2009). This might be the reason for better Zn content under foliar application. However, soil or foliar application of Zn fertilizers increased Zn concentration in phloem fed tissues such as seeds and fruits (White and Broadley, 2009; Bouis and Welch, 2010; Cakmak *et al.*, 2010).

2.9. IMPACT OF BIOFORTIFICATION (MINERAL) ON YIELD AND NUTRIENT UPTAKE

Shivay and co-workers (2008 a, b) have reported that Zn application to soil as zinc sulphate or zinc enriched / coated urea not only increased yield but also zinc concentration in rice and wheat grain. Thus adequate fertilization of food crops can partly help in Zn intake by humans.

Zn fertilization of crops on Zn-deficient soils helps attaining both food security and overcoming Zn malnutrition. Based on the estimates by Takkar *et al.* (1997) and considering the fact that 49 per cent of Indian soils are deficient in Zn, India will need about 6 lakh tonnes of Zn per year by 2025 (Singh, 2009).

Enrichment of commonly applied compound fertilizers with Zn is a further fertilizer practice useful for increasing Zn concentration of plants. In India, application of Zn-coated urea fertilizer significantly improved both grain yield and grain Zn concentrations (Shivay*et al.*, 2008 a, b).

Decreases in grain P concentration by Zn applications are associated with a corresponding decrease in grain phytate concentration and the phytate to Zn molar ratios. Phytate is the major P storage compound in cereal grains and has a high potential for binding Zn and Fe, making them less soluble and less available for humans (Wise, 1995; Lott *et al.*, 2000). Formation of insoluble phytate complexes of Zn and Fe are suggested to be a major reason for a high incidence of micronutrient deficiency in countries with diets high in phytate (Gibson, 2006; Rimbach*et al.*, 2008). The phytate to Zn molar ratio is commonly used to estimate Zn bioavailability in food (Oberleas and Harland, 2005; Hambidge *et al.*, 2008).

In field trials in Central Anatolia, soil Zn application combined with foliar application significantly decreased phytate to Zn molar ratios in grain of both durum and bread wheat (Cakmak *et al.*, 1999) and these effects may result in significant effects on Zn nutritional status of populations relying on cereals as a micronutrient source.

Such important decreases in grain P and phytic acid after Zn fertilization might be a consequence of increasing grain yield (and thus a dilution effect). However, the decreasing effect of Zn fertilization on grain P and phytic acid was also found in rye that is highly tolerant to Zn deficiency and its yield was very slightly affected by Zn deficiency (Cakmak *et al.*, 1997; Erdal, 1998). The effect of Zn on grain P under Zn-deficient conditions seems to be specific and most probably related to Zn-deficiency-induced root uptake of P (Loneragan *et al.*, 1982; Cakmak and Marschner, 1986).

The impact of biofortified produce on the nutritional status of humans has rarely been tested. The biofortification of edible produce can improve the nutritional status of humans. It is evident that, the application of mineral fertilizers containing Se, I or Zn can have a significant impact on the nutritional status of a vulnerable population (Cakmak, 2008).

Khan and Weaver (1989) had reported that, foliar application of Zn was more effective than soil application in providing Zn for transport to soya bean grains and thus enhancing Zn concentration in grains. Foliar spray of zinc improved the grain yield appreciably in pulses (Savithri, 2001).

Although direct uptake of Zn by leaves is possible through foliar Zn application, the primary source of Zn for plants is through root uptake from soil. Plant root uptake of Zn is influenced by several root-related processes such as release of phytosiderophores, proton exudation, rhizosphere oxidation, mychorrizal colonization and root architecture (Graham and Rengel, 1993). Foliar Zn application has the conceptual advantage of avoiding soil chemistry problems that make Zn less available to crops and the crop performance was better at lower concentrations of Zn compared to higher concentrations (Slaton *et al.*, 2005). Zn foliar application is the simplest way for increasing nutrient density in maize (Grzebisz*et al.*, 2008) and green gram (Pathak and Pandey, 2010). It is the ideal technique for making quick correction of plant nutritional status and enhancement of Zn content in seeds for improved dietary intake by humans (Pathak*et al.*, 2012).

Foliar application of Zn has a positive effect on plant growth and its reproductive development. Zn deficiency leads to loss of pollen function, impairment in fertilization, and poor development of the seed, which contribute to poor seed yield of legumes grown on low Zn soils. This can be alleviated through foliar Zn fertilization of crops at the onset of reproductive phase, especially in Zn deficient areas. Foliar fertilization not only enhances productivity, but it is an important strategy for increasing Zn density in seeds improved for human consumption (Pathak *et al.*, 2012).

Enhanced root growth has been reported under moderate Zn availability, since under such conditions, more energy is expended by the plant for root growth to facilitate nutrient extraction from more volume of soil (Chen *et al.*, 2009). Though not significant, application of Zn either to soil or foliar increased the vegetative growth and in response to that, the plants need more nutrients and hence better exhibition of root characteristics. Zinc application resulted in more vegetative growth for legumes in acid soils (Singh *et al.*, 1992; Khan *et al.*, 2000) and thereby produced more dry matter, derived mainly from increase in the number of pods (Brennen*et al.*, 2001; Valenciano *et al.*, 2007).

Increasing seed concentration of Zn by soil and / or foliar application of zinc also brings several agronomic benefits for crop production. Applying zinc to plants grown under potentially zinc-deficient soils is effective in reducing uptake and accumulation of phosphorus (and thus phytate) in plants (Mirvat *et al.*, 2006; Cakmak, 2008). Application of Zn reduced the accumulation of P as phytate and this agronomic side effect of zinc fertilization resulted in better availability of zinc in the human digestive system (Mirvat *et al*., 2006; Cakmak, 2008).

The inhibitory effect of phytate on the availability of zinc was determined by measuring their molar ratios. The high phytate content may impair the availability of the minerals in the body. Phytic acid is an essential food component that has crucial negative impact on the absorption of Zn (Oberleas *et al*., 1961). Zinc concentration in grains increased linearly with increasing Zn application rate in the soil and P concentration as phytate decreased directly with increased Zn levels (Singh *et al*., 2005).

The zinc availability is significantly reduced due to a high intake of phytate which significantly affect the absorption of Zn in the body (Alloway, 2008). Phytate: zinc ratio of <5:1, 5-15:1 and >15:1 are considered an index of bioavailability high, medium and low Zn respectively (Graham, 1984). If phytate: zinc ratio exceeds 15:1 the absorption is low.

Application of Zn either to soil or foliar have facilitated better removal of nutrients from soil and their transportation and to plant parts and its accumulation in leaves, fruits and seeds (Cakmak, 2008; Samreen *et al*., 2013).

Slaton *et al*. (2001) indicated that, the agronomic efficiency, recovery efficiency and partial factor productivity can usually be further improved by applying Zn at their active growing stages, when the plants can take up more Zn, rather than basally. Effect of zinc biofortification on zinc utilization efficiency can be evaluated by computing Biofortification Recovery Efficiency (BRE_{Zn}) index (Shivay *et al*., 2008 a, b).The same fertilization techniques that improve recovery efficiency for soil-applied Zn fertilizers are expected to be more effective in biofortifying the edible parts with Zn. When the plant is able to access Zn at a time when it is able to take it up (*i.e*. later than basal), it is more likely to be taken up in higher quantities and therefore may possibly result in higher Zn concentration (White and Broadley, 2011). Foliar Zn fertilization would be expected to affect grain Zn concentration only in genotypes with high remobilization capability (movement of Zn from leaves to grain) (Rozane *et al.*, 2008; Slaton *et al.*, 2001).

By supplying plants with Zn, either through soil application, foliar spray, or seed treatment, increased yield, quality, and Zn use efficiency. In consideration of the important role of Zn in promoting and maintaining human health, more research is needed to determine the advantages of using the optimum level of Zn (Malakouti, 2008).

2.10. ECONOMICS OF THE ZINC BIOFORTIFICATION

Zinc fertilizer strategies can also provide an immediate and effective option to increase grain Zn concentration and productivity in wheat, particularly with severe soil Zn deficiency. However, fertilizer strategies must be practical and economically feasible. In low-income countries where resource- poor farmers do not have access to or cannot afford fertilizer, breeding for mineral density may remain the sole agricultural intervention to improve the nutritional content of staple crops (White and Broadley, 2011).

Agronomic biofortification of edible produce through mineral supplementation is potentially cost effective and will deliver most benefits to the 40 per cent of the world's population who rely primarily on their own food for sustenance. Most economic analyses suggest that biofortification through mineral supplementation was more cost effective than genetic biofortification, dietary diversification, supplementation or food fortification programmes (Bouis, 1999; Bouis*et al.,* 2000; Horton, 2006; Stein *et al.*, 2007; Ma *et al.,* 2008).

Early economic analyses for Zn biofortification was mainly done for rice and wheat only and such data on other crops are not available. Early economic analyses for Zn biofortification of wheat in Turkey suggested a cost-to-benefit quotient of greater than 20 over two decades (Bouis, 1999; Bouis*et al.*, 2003). More recently, the potential impact of biofortification has been quantified as the saving of disability-adjusted life years (DALYs) (Stein *et al.*, 2005). It has been estimated that, the annual burden of Zn deficiency in India is 2.8 million lost DALYs and Zn biofortification of rice and wheat may reduce this burden by 20-51 per cent (Stein *et al.*, 2007). The cost of saving 1 DALY from Zn biofortification of rice and wheat in India was estimated as $US 0.73–7.31 (Stein *et al.*, 2007).

2.11. ENVIRONMENTAL IMPACT ON ACCEPTABILITY

It is thought that, consumers in both developed and developing countries will accept foods prepared from biofortified crops provided that, they are not appreciably more expensive than the alternatives and that biofortification does not alter the appearance, taste, texture or cooking quality of foods (Bouis *et al.*, 2003). It is thought unlikely that, small quantities of mineral elements will alter these properties of foods, but manipulating the concentrations of promoters and anti-nutrients might affect both taste and colour. If it can be demonstrated that, foods prepared using biofortified produce are more beneficial to human health, this will, of course, influence consumer choice in both developed and developing countries.

In application of Zn fertilizers or Zn-containing NPK fertilizers a special attention should be paid to environmental aspects regarding the possibility of Zn toxicity in soils. After application of Zn fertilizers to Zn-deficient soils, Zn is rapidly fixed by soil. Application of high rates of Zn fertilizers over many years may be required before a Zn toxicity problem may occur. Nevertheless, in areas where Zn fertilizers will be applied regularly, concentrations of Zn in soils and plants should be periodically monitored to avoid possible development of Zn toxicity problem. Zinc fertilizers are effective up to 3 - 4 years in correcting Zn deficiency (Martens and Westermann, 1991), and should not be re-applied every year in case of soil applications.

Materials and Methods

3. MATERIALS AND METHODS

The present study titled 'Zinc biofortification for enhancing yield and quality of yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt) in ferralitic soils' has been carried out at the College of Agriculture, Vellayani during October, 2013 to January, 2014. The study was envisaged to assess the effect of zinc biofortification on zinc bioavailability, pod yield and quality of yard long bean in ferralitic soils. The details of the experimental site, season and weather conditions, materials used and methods adopted are presented in this chapter.

3.1. DETAILS OF THE EXPERIMENTAL SITE

3.1.1. Location

The experiment was conducted in the D block of the Instructional Farm at the College of Agriculture, Vellayani. The site is situated at 8^0 30’ N latitude and 76^0 54’ E longitude at an altitude of 29 m above MSL.

3.1.2. Soil

The soil of the experimental site belongs to the family of Loamy Kaolinitic Isohypothermic Typic Haplustalf of Vellayani series. Initial soil samples were collected from the experimental area before application of treatments, air dried and sieved through 2 mm sieve and analysed for various physicochemical properties. The initial data on soil physico-chemical properties as per standard procedures are presented in Table 1.

3.1.3. Season

The experiment was conducted during October, 2013 to January, 2014.

Table 1.Physico-chemical properties of the soil of experimental site

Sl No.	Physicochemical properties	Status
Physical properties		
1	Texture	Sandy clay loam
2	Coarse sand	48.99 %
3	Fine sand	14.78 %
4	Silt	6.39 %
5	Clay	28.10 %
6	Particle density	2.39 Mg m^{-3}
7	Bulk density	1.24 Mg m^{-3}
8	Water holding capacity	23.40 %
9	Porosity	48.11%
Chemical properties		
1	pH	4.94
2	EC	0.18 dS m^{-1}
3	Organic Carbon	0.95%
4	Available N	192.34 kg ha^{-1}
5	Available P	57.79 kg ha^{-1}
6	Available K	365.42 kg ha^{-1}
7	Exchangeable Ca	1.29 meq 100 g^{-1}
8	Exchangeable Mg	2.36 meq 100 g^{-1}
9	Available S	9.33ppm
10	Available Fe	11 mg kg^{-1}
11	Available Mn	11.6 mg kg^{-1}
12	Available Zn	2.3 mg kg^{-1}
13	Available Cu	0.5 mg kg^{-1}

3.1.4. Weather Conditions

Data on weekly averages of the weather parameters *viz.* maximum and minimum temperature, relative humidity and rainfall received during the cropping period were collected from the Agro - Meteorological Observatory attached to NARP, Southern Region, at College of Agriculture, Vellayani and are presented in Fig.1.

3.2. EXPERIMENTAL MATERIALS

3.2.1. Planting Material and Variety

The experiment was carried out with yard long bean variety Vellayani Jyothika. It is a trailing vegetable type variety. The seeds of the variety Vellayani Jyothika were purchased from the Instructional Farm, Vellayani.

3.2.2. Manures and Fertilizers

Urea (46 per cent N), Rajphos (20 per cent P_2O_5) and MOP (60 per cent K_2O) were used as sources of N, P and K respectively. Zn was given to the crop through fertilizer grade zinc sulphate ($ZnSO_4.7H_2O$) containing 22 per cent Zn. FYM @ 20 t ha^{-1} as per Package of Practices Recommendations of Kerala Agricultural University (KAU, 2011) was applied to all treatments.

3.3. DESIGN AND LAYOUT OF THE EXPERIMENT

3.3.1. Experiment Details

Crop	: Yard long bean
Variety	: Vellayani Jyothika
Design	: Randomized Block Design
Spacing	: 1.5 m × 45 cm
Plot size	: 4.5 m × 1.8 m
Treatments	: 10
Replication	: 3

Fig 1. Weather parameters at weekly intervals at Vellayani during October, 2013 – January, 2014

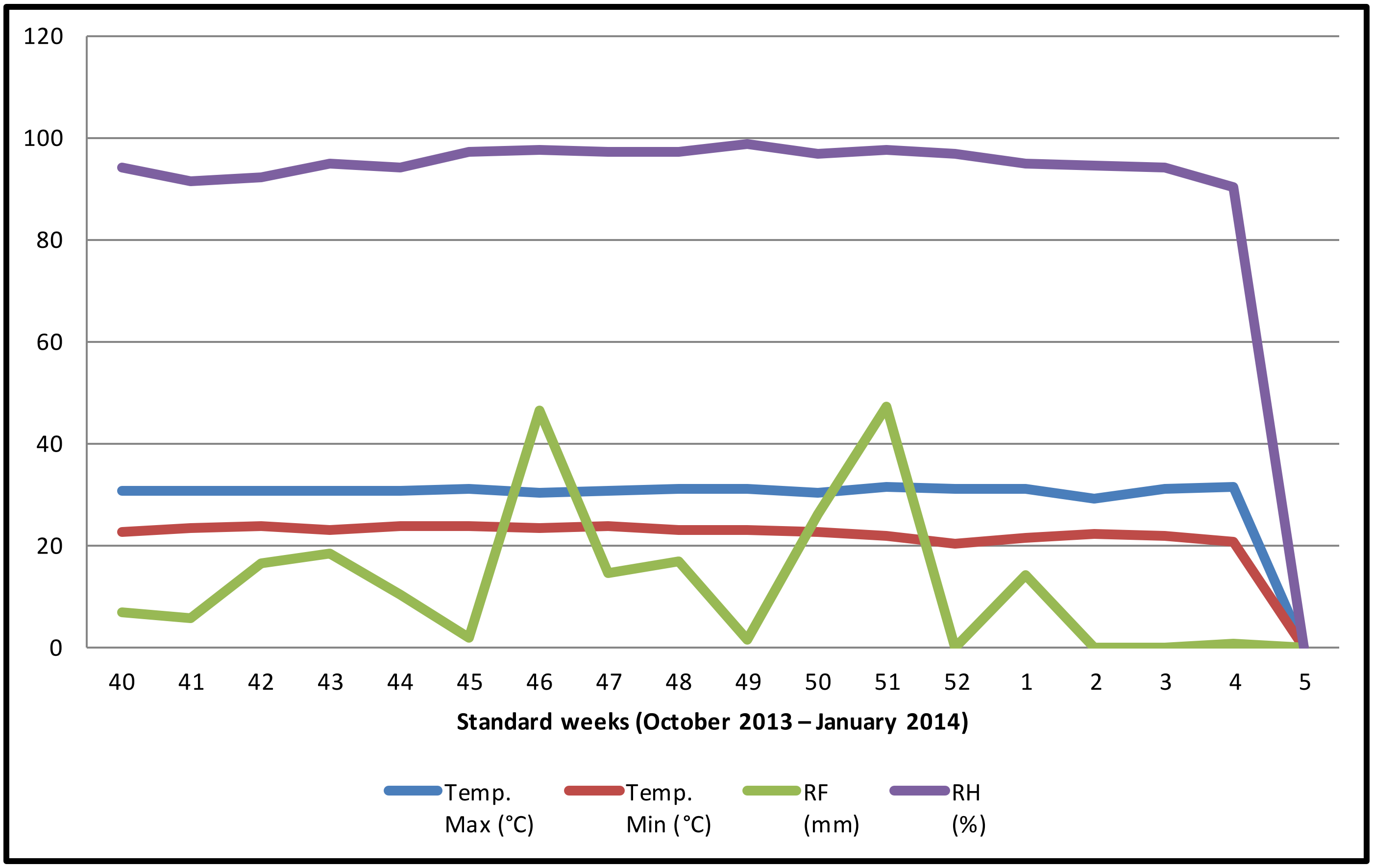
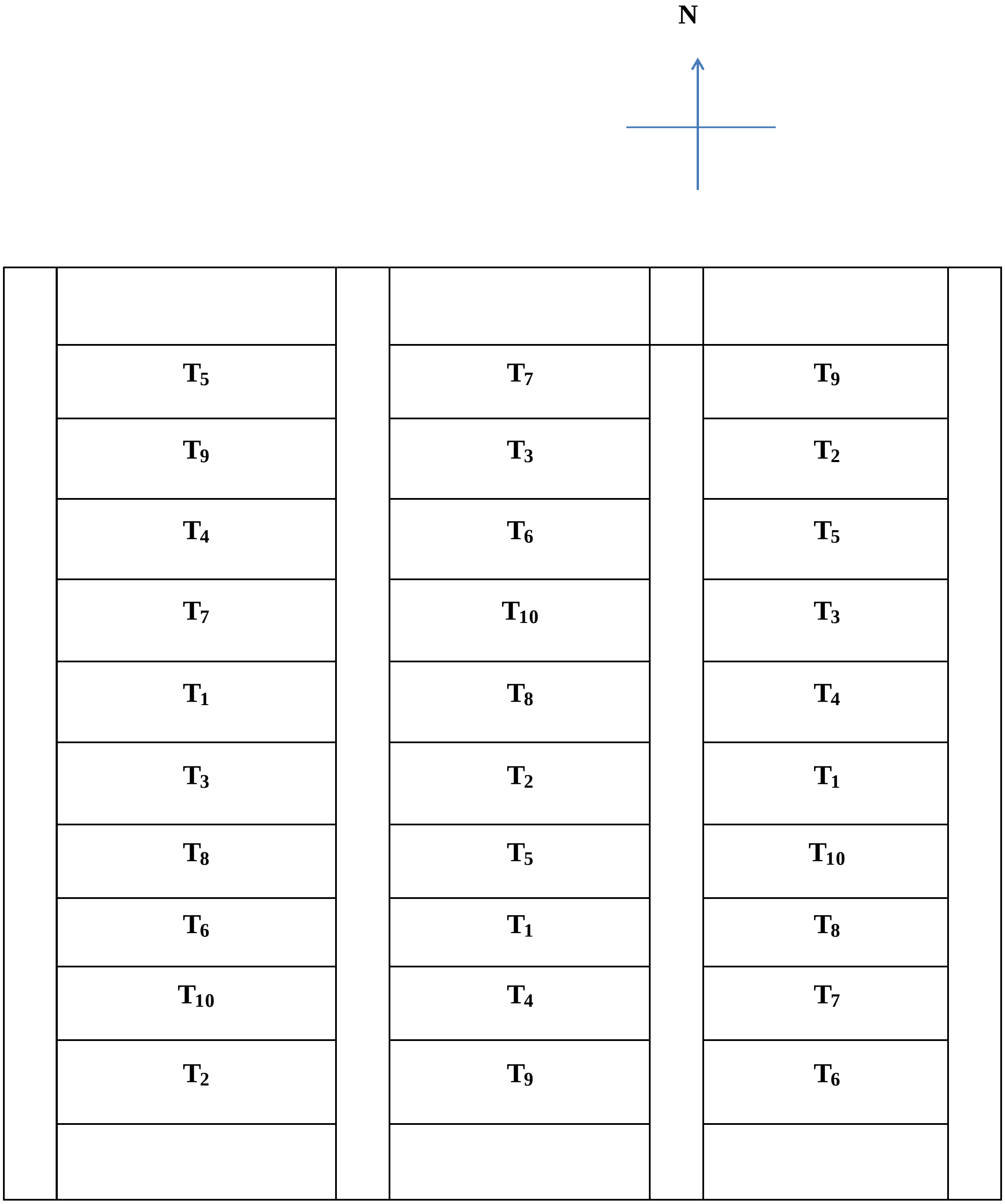


Fig 2. Layout of the experimental field



T_5	T_7	T_9
T_9	T_3	T_2
T_4	T_6	T_5
T_7	T_{10}	T_3
T_1	T_8	T_4
T_3	T_2	T_1
T_8	T_5	T_{10}
T_6	T_1	T_8
T_{10}	T_4	T_7
T_2	T_9	T_6

Plate 1. A general view of the field experiment before the application of zinc

Plate 2. A general view of the field experiment after treatments

3.3.2. Treatments

1. T_1 - Package of Practices Recommendations of KAU–NPK @ 20:30:10 kg ha^{-1} + FYM @ 20 t ha^{-1}
2. T_2 - Soil Test Based Recommendations for N, P and K (STBR) – NPK @ 18.2: 7.5: 2.5 kg ha^{-1}
3. T_3 - POP + Soil applicationof 2.5 kg Zn ha^{-1} as $ZnSO_4$
4. T_4 - POP + Soil applicationof 5 kg Zn ha^{-1} as $ZnSO_4$
5. T_5 - POP + Foliar application of 0.025% $ZnSO_4$at branching and flowering stages
6. T_6 - POP + Foliar application of 0.05% $ZnSO_4$at branching and flowering stages
7. T_7 - STBR + Soil applicationof 2.5 kg Zn ha^{-1} as $ZnSO_4$
8. T_8 - STBR + Soil applicationof 5.0 kg Zn ha^{-1} as $ZnSO_4$
9. T_9 - STBR + Foliar application of Zn at branching and flowering stage with 0.025% $ZnSO_4$
10. T_{10} - STBR + Foliar application of Zn at branching and flowering stage with 0.05% $ZnSO_4$

All cultural practices as per Package of Practices Recommendations of KAU were followed.

3.4. DETAILS OF OPERATIONS DURING FIELD EXPERIMENT

3.4.1. Land Preparation

The experimental site was leveled and ploughed thoroughly. Weeds were removed. The field was laid out into three blocks each with ten plots according to the orientation of the land.

3.4.2. Manure and Fertilizer Application

The entire quantity of farm yard manure, phosphorous (as Rajphos) and potassium (as muriate of potash) and half the quantity of nitrogen (as urea) were applied as basal dose. The remaining quantity of nitrogen was applied 20 days after sowing as first split application.

3.4.3. Sowing

Yard long bean seeds were sown on 18 October 2013, in the respective plots containing farm yard manure and required quantity of basal dose of fertilizers as per treatments with a spacing 1.5 m × 45 cm. Sprinkling of water was carried out at regular intervals.

3.4.4. Application of Treatments

Zinc treatments were applied to the soil at 20 days after sowing. Foliar application of treatments was done to the seedlings at branching stage (30 days after sowing) and at flowering stages (45 days after sowing).

3.4.5. After Cultivation and Irrigation

The crop received timely management practices as per the Package of Practices of KAU. Uniform germination was observed and gap filling was done 5 days after sowing. The crop was thinned to one plant per pit one week after emergence. Regular weeding was done throughout the cropping period. Irrigation was provided to the crop as and when required.

3.4.6. Plant Protection

Oxuron @ 5 ml L^{-1} were applied against stem borer attack when the pest incidence was noticed.

3.4.7. Harvesting

Pods were harvested for vegetable purpose from 40 days after sowing onwards. Subsequent harvests of green immature pods were done in alternate days from all the treatments up to 100 days after sowing and fresh weights were recorded. After the crop period, when the vegetable yield had fallen well below the economic level, the plants were pulled out, and bhusa yield was recorded. The same plants were oven dried and dry weight was recorded.

Plate 3. Foliar application of zinc

Plate 4. Soil application of zinc

3.5. BIOMETRIC OBSERVATIONS RECORDED

3.5.1. Vine Length

Vine length was measured from the base of the two observation plants from each plot to the terminal leaf bud and average was taken and expressed in centimeter.

3.5.2. Branches per Plant

The total number of branches arising from the main stem of each observation plants was counted at the peak harvest stage and average was found out.

3.5.3. Root Length

Observation plants from each plot was uprooted, separated the root portion, washed well and root length was taken from the base of the root to the tip of the longest root and the average was expressed in centimeters.

3.5.4. Root Weight

The fresh weights of the washed roots from two observation plants were noted and the average was expressed in g per plant.

3.5.5. Root Volume

Root volume per plant was found out by water displacement method. The roots of the two observation plants were washed free of adhering soil with water. The roots were immersed in 1000 ml measuring cylinder containing water and the rise of water level was recorded. Displacement of volume of water was taken as the volume of the root and the average was expressed in cubic centimeter.

3.5.6. Nodules per Plant

The observation plants were uprooted from each plot without disturbing the root system and nodules were separated at flowering stage. The nodules with pink or reddish colour were counted to get the number of effective nodules and average was taken.

3.5.7. Days to Fifty per cent Flowering

The number of days taken for flowering by fifty percent of the plant population in each treatment and the period taken was recorded as number of days by visual observation.

3.6. YIELD CHARACTERISTICS

3.6.1. Pod Yield

Total weight of pods from the two observation plants from each plot at each harvest was taken and the average was expressed as pod yield per plant.

3.6.2. Bhusa Yield

After the pods were picked from observation plants from each plot, the plants were uprooted and weighed and the average weight was expressed in kg ha^{-1}.

3.6.3. Pod Length

Length of the pods was measured as the distance from pedicel attachment of the pod to the apex using twine and scale. Average of the pod length from two observation plants at peak harvesting time was taken and expressed in centimeters.

3.6.4. Pod Weight

Average weight of single pod from two observation plants was measured and expressed in grams.

3.6.5. Pods per Plant

The total number of pods harvested from the two observation plants from each plot was noted and the average was recorded.

3.6.6. Seeds per Pod

Seeds of each pod from two observation plants at peak harvesting time were removed, counted and average was recorded.

3.6.7. Total Dry matter Production

Total dry matter production was recorded at final harvest stage. The two observation plants were uprooted without damaging the roots and separated into leaves, stem and roots. These were dried under shade and then oven dried at 65°C for 10 hours till two consecutive weights coincided. The total dry weight of pods and bhusa were added to get the total dry matter production and expressed in gram per plant.

3.7. CHEMICAL ANALYSIS

3.7.1. Soil Analysis

Soil samples for chemical analysis were drawn before sowing the seeds and at the time of final harvest of pods. The samples were air dried under shade, sieved through 2 mm sieve and used for the analysis of pH, EC, organic carbon and available N, P, K, Ca, Mg, S, Fe, Mn, Zn and Cu using standard analytical procedures as presented in Table 2.

3.7.2. Plant Analysis (Pod and Bhusa)

Pod samples were taken at the time of each harvest, mixed together and a lot was taken for further drying and powdering. Bhusa was collected at the time of final harvest. The collected samples were washed, air dried, powdered and subjected to chemical analysis to find out their chemical composition. The methods used for each analysis are presented in Table 3.

Table 2. Standard analytical methods followed for soil analysis

SI No.	**Parameters**	**Method**	**Reference**
1	Mechanical analysis	International pipette method	Piper (1967)
2	Bulk density	Core sampling method	Gupta and Dakshinamurthy (1980)
3	Particle density	Pycnometer method	Gupta and Dakshinamurthy (1980)
4	Water holding capacity	Core sampling method	Black (1965)
6	pH	Potentiometric method with pH meter	Jackson (1973)
7	EC	Conductometric method using electrical conductivity meter	Jackson (1973)
8	Organic carbon	Walkley and Black rapid titration method	Walkley and Black (1934)
9	Available nitrogen	Alkaline potassium permanganate method	Subbiah and Asija (1956)
10	Bray No.1 extractable phosphorus	Bray and Kurtz extraction method, chlorostannous – reduced molybdo phosphoric blue colour method in HCl system and estimation by spectrophotometry	Jackson (1973)
11	Neutral normal NH_4OAC extractable K	Flame photometry	Jackson (1973)
12	Neutral normal NH_4OAC extractable Ca and Mg	Versanate titration method	Hesse (1971)
13	0.01 N $Ca(PO_4)_2$ extract able sulphur	Turbidimetry	Chesnin and Yien (1950)
14	0.5 NHCl extractable Fe, Mn, Zn and Cu	Atomic Absorption Spectrophotometer	O'Connor (1988)

Table 3. Standard analytical methods followed for plant analysis

Sl. No	**Parameters**	**Method**	**Reference**
Elemental Composition			
1	Nitrogen	Micro Kjedahl method	Jackson (1973)
2	Phosphorus	Nitric- perchloric acid (9:4) digestion and spectrophotometry using vanadomolybdo phosphoric yellow colour method	Jackson (1973)
3	Potassium	Nitric - perchloric acid (9:4) digestion and flame photometry	Jackson (1973)
4	Calcium and magnesium	Nitric - perchloric acid (9:4) digestion and versanate titration	Piper (1967)
5	Sulphur	Nitric - perchloric acid (9:4) digestion and turbidimetry	Chesnin and Yien (1950)
6	Micro nutrients – Fe, Mn, Zn and Cu	Nitric- perchloric acid (9:4) digestion and Absorption Spectrophotometry	Jackson (1973)
7	Crude protein	Multiplication of N content by a factor of 6.25	Simpson (1965)
8	Phytate	Phytate is extracted with trichloroacetic acid and precipitated as ferric salt. Fe content is determined colorimetrically and phytate P calculated using 4Fe : 6P molecular ratio in the precipitate	Sadasivam and Manickam (1992)

3.8. COMPUTED INDICES

3.8.1. Harvest Index

Harvet index of each treatment was calculated by using the formula,

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

3.8.2. Nutrient Uptake

Uptake of nutrients calculated by using the formula,

$$\text{Nutrient uptake} = \frac{\text{Concentration of nutrient} \times \text{Total dry matter production} \atop (\%)}{100}$$

3.8.3. Biofortification Recovery Efficiency

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

$\text{BRE}_{Zn}(\%)$ =

$$\frac{\text{Increase in uptake in edible plant part of } +\text{Zn treatment over } -\text{Zn treatment} \times 100}{\text{Quantity of Zn applied}}$$

3.9. SCORING OF PEST (%)

Stem borer incidence was calculated using the formula

$$\text{Stem borer incidence (\%)} = \frac{\text{Number of affected branches} \times 100}{\text{Total number of branches}}$$

3.10. BENEFIT – COST RATIO

Benefit – cost ratio was computed using the formula

$$\text{B : C ratio} = \frac{\text{Gross income}}{\text{Total expenditure}}$$

3.11. STATISTICAL ANALYSIS

The experimental data generated from the study were subjected to statistical analysis as described by Cochran and Cox(1965).

Results

4. RESULTS

An experiment titled ‘Zinc biofortification for enhancing yield and quality of yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt) in ferralitic soils’ has been carried out at the Department of Soil Science and Agricultural Chemistry, College of Agriculture, Vellayani during 2013-14.The above investigation was undertaken to study the effect of zinc biofortification on pod yield and quality of yard long bean and bioavailability of zinc. Results based on statistically analysed data pertaining to the experiment conducted during the course of investigation are presented in this chapter.

4.1. SOIL ANALYSIS

The data on soil chemical parameters *viz*. pH, EC, organic carbon, available N, P, K, Ca, Mg, S, Fe, Mn, Zn and Cu content at the time of final harvest are presented in Tables 4 to 7.

4.1.1. Soil pH

The data on soil pH at the time of final harvest are presented in Table 4. The mean values ranged from 5.98 to 6.47. The Treatment T_8 (STBR + Zn @ 5 kg ha^{-1}) recorded the highest value which was on par with T_7 (STBR + Zn @ 2.5 kg ha^{-1}) and T_6 (POP + $ZnSO_4$ foliar @ 0.05 %) and were significantly superior to all other treatments. Treatment T_1 (POP) registered the lowest value of 5.98.

4.1.2. Electrical Conductivity

Critical appraisal of the data presented in Table 4 revealed that the treatments did not vary significantly with respect to electrical conductivity of the soil at the time of final harvest. The mean values ranged from 0.19 to 0.30 dS m^{-1}. Here also, the treatment T_8 (STBR + Zn @ 5 kg ha^{-1}) registered the highest mean value of 0.30 dS m^{-1} and the lowest value was registered by the treatment T_2 (STBR).

Table 4.Effect of treatments on soil properties at final harvest

Treatments	pH	EC (dS m^{-1})	Organic C (%)
T_1 – POP - N: P: K @ 20:30:10 kg ha^{-1}	5.98	0.20	0.80
T_2 – STBR – N: P: K @ 18.2: 7.5: 2.5 kg ha^{-1}	5.99	0.19	0.85
T_3 - POP + Zn_{soil}@ 2.5 kg ha^{-1}	6.20	0.22	1.18
T_4 - POP + Zn_{soil} @ 5 kg ha^{-1}	6.12	0.22	1.25
T_5 - POP + $ZnSO_4$ foliar @ 0.025 %	6.12	0.23	1.32
T_6 - POP + $ZnSO_4$ foliar @ 0.05 %	6.24	0.24	1.41
T_7 - STBR + Zn_{soil} @ 2.5 kg ha^{-1}	6.31	0.27	1.13
T_8 - STBR + Zn_{soil} @ 5 kg ha^{-1}	6.47	0.30	1.21
T_9 - STBR + $ZnSO_4$ foliar @ 0.025 %	6.11	0.22	1.28
T_{10} - STBR + $ZnSO_4$ foliar @ 0.05 %	6.18	0.22	1.25
CD (0.05)	0.241	NS	0.07

4.1.3. Organic Carbon

The organic carbon content of soil was significantly influenced by the different treatments. It is inferred from Table 4 that, the mean values ranged from 0.80 to 1.41 per cent. The highest value was recorded by T_6 (POP + $ZnSO_4$ foliar @ 0.05%) which was significantly superior to all other treatments. The lowest value was observed for the treatment POP (0.80%)which was on par with T_2 STBR(0.85%) also.

4.1.4. Available Nitrogen

The available N content was significantly influenced by the various treatments (Table 5). The mean values ranged from 204.88 to 272.33 kg ha^{-1}. Treatment T_{10} (STBR + $ZnSO_4$ foliar @ 0.05 %) registered the highest value of 272.33 kg ha^{-1} which on par with T_6 (POP + $ZnSO_4$ foliar @ 0.05 %), T_9 (STBR + $ZnSO_4$ foliar @ 0.025 %), T_5 (POP + $ZnSO_4$ foliar @ 0.025 %), T_8 (STBR + Zn @ 5 kg ha^{-1}),T_4 (POP + Zn @ 5 kg ha^{-1}) and T_7 (STBR + Zn @ 2.5 kg ha^{-1}).Treatment T_1 (POP) registered the lowest value of 204.88 kg ha^{-1}, which was found to be on par with T_2 (STBR).

4.1.5. Available Phosphorus

The treatments had significantly influenced the available P content in the soil at final harvest (Table 5). The mean values ranged from 58.87 to 86.66 kg ha^{-1}. Treatment T_6 (POP + $ZnSO_4$ foliar @ 0.05 %) has recorded the highest value for available P and was found to be on par with treatment T_5 (POP + $ZnSO_4$ foliar @ 0.025 %) and T_4 (POP + Zn @ 5 kg ha^{-1}). Lowest value of available P was recorded by the treatment T_2 (STBR).

4.1.6. Available Potassium

The available K content of soil was significantly influenced by the different treatments (Table 5) and the mean values ranged from 370.96 to 574.72 kg ha^{-1}. The treatment T_{10} (STBR+$ZnSO_4$ foliar @ 0.05%) registered the highest value which was on par with T_9 (STBR+$ZnSO_4$ foliar @ 0.025%), T_8 (STBR + Zn @ 5 kg ha^{-1}), T_7 (STBR + Zn @ 2.5 kg ha-1), T_6 (POP + $ZnSO_4$ foliar @ 0.05 %) and T_5 (POP + $ZnSO_4$ foliar @ 0.025 %). Lowest value of 370.96 kg ha^{-1} was recordedby T_1 (POP) which was on par with T_3 (POP + Zn @ 2.5 kg ha^{-1}), T_4 (POP + Zn @ 5 kg ha^{-1}) and T_2 (STBR).

4.1.7. Exchangeable Calcium

The treatments had significantly influenced the exchangeable Ca content in the soil at final harvest as observed from Table 6. The mean values ranged from

3.16 to 4.85 cmol kg^{-1}. Treatment T_8 (STBR + Zn @ 5 kg ha^{-1}) recorded the highest value which was on par with T_7 (STBR + Zn @ 2.5 kg ha^{-1}), T_4 (POP + Zn @ 5 kg ha^{-1}) andT_6 (POP + $ZnSO_4$ foliar @ 0.05 %). The lowest value was observed for the treatment T_1 (POP).

Table 5.Effect of treatments on soil primary nutrients at final harvest

Treatments	Available N	Available P	Available K
	kg ha^{-1}		
T_1 – POP - N: P: K @ 20:30:10 kg ha^{-1}	204.88	66.97	370.96
T_2 – STBR – N: P: K @ 18.2: 7.5: 2.5 kg ha^{-1}	220.76	58.87	407.60
T_3 - POP + Zn_{soil} @ 2.5 kg ha^{-1}	238.34	70.39	383.64
T_4 - POP + Zn_{soil} @ 5 kg ha^{-1}	250.88	75.11	400.02
T_5 - POP + $ZnSO_4$ foliar @ 0.025 %	251.17	76.09	486.55
T_6 - POP + $ZnSO_4$ foliar @ 0.05 %	256.76	86.66	522.60
T_7 - STBR + Zn_{soil} @ 2.5 kg ha^{-1}	250.70	59.91	549.64
T_8 - STBR + Zn_{soil} @ 5 kg ha^{-1}	250.88	60.33	563.17
T_9 - STBR + $ZnSO_4$ foliar @ 0.025 %	254.21	66.13	568.72
T_{10} - STBR + $ZnSO_4$ foliar @ 0.05 %	272.33	68.51	574.72
CD (0.05)	26.885	12.982	94.055

4.1.8. Exchangeable Magnesium

Exchangeable magnesium content in soil at the time of final harvest was significantly influenced by different treatments (Table 6). The mean values ranged from 0.99 to 2.80 cmol kg^{-1}. The highest value of 2.80 cmol kg^{-1} was recorded by T_6 (POP + $ZnSO_4$ foliar @ 0.05 %) and was observed to be on par with T_4 (POP + Zn @ 5 kg ha^{-1}), T_{10} (STBR + $ZnSO_4$ foliar @ 0.05 %), T_5 (POP + $ZnSO_4$ foliar @ 0.025 %) and T_9 (STBR + $ZnSO_4$ foliar @ 0.025 %). Lowest mean was recorded by the treatment T_2 (STBR) and was on par with T_1 (POP), T_7 (STBR + Zn @ 2.5 kg ha^{-1}) and T_3 (POP + Zn @ 2.5 kg ha^{-1}).

Table 6.Effect of treatments on soil secondary nutrients at final harvest

Treatments	Exchangeable Ca (cmol kg^{-1})	Exchangeable Mg (cmol kg^{-1})	Available S (ppm)
T_1 – POP - N: P: K @ 20:30:10 kg ha^{-1}	3.16	1.04	4.72
T_2 – STBR – N: P: K @ 18.2: 7.5: 2.5 kg ha^{-1}	3.54	0.99	4.88
T_3 - POP + Zn_{soil} @ 2.5 kg ha^{-1}	3.78	1.62	7.07
T_4 - POP + Zn_{soil} @ 5 kg ha^{-1}	4.12	2.34	8.16
T_5 - POP + $ZnSO_4$ foliar @ 0.025 %	3.73	2.22	6.25
T_6 - POP + $ZnSO_4$ foliar @ 0.05 %	4.00	2.80	7.11
T_7 - STBR + Zn_{soil} @ 2.5 kg ha^{-1}	4.61	1.24	7.14
T_8 - STBR + Zn_{soil} @ 5 kg ha^{-1}	4.85	1.89	9.53
T_9 - STBR + $ZnSO_4$ foliar @ 0.025 %	3.30	2.10	6.60
T_{10} - STBR + $ZnSO_4$ foliar @ 0.05 %	3.60	2.34	6.86
CD (0.05)	0.918	0.740	2.44

4.1.9. Available Sulphur

Table 6 presents the available S content in the soil at the time of final harvest. The mean values ranged from 4.72 to 9.53 ppm. Highest value of 9.53 ppm was registered by T_8 (STBR + Zn @ 5 kg ha^{-1}) and was on par with treatments T_4 with the application of POP + Zn @ 5 kg ha^{-1} with the mean value of 8.16ppm,T_7 (STBR + Zn @ 2.5 kg ha^{-1}) and T_6 (POP + $ZnSO_4$ foliar @ 0.05 %). The lowest mean was observed in treatment T_1 (POP).

4.1.10. Available Iron, Manganese, Zinc and Copper

Treatments significantly influenced the available Fe content of the soil at the time of final harvest. The mean values ranged from 16.13 to 35.73 mg kg^{-1}. Treatment T_{10} (STBR + $ZnSO_4$ foliar @ 0.05 %) recorded the highest value 35.73 mg kg^{-1}which was on par with T_2 (STBR), T_8 (STBR + Zn @ 5 kg ha^{-1}) and T_9 (STBR + $ZnSO_4$ foliar @ 0.025 %). The lowest value was observed for the treatment T_1 (POP) which was significantly inferior to all other treatments (Table 7).

Available Mn content in the soil was also significantly influenced by the different treatments (Table 7). The mean values ranged from 20.31 to 32.47 mg kg^{-1}. Treatment T_3 (POP + Zn @ 2.5 kg ha^{-1}) recorded the highest value which was on par with T_8 (STBR + Zn @ 5 kg ha^{-1}) and T_9 (STBR + $ZnSO_4$ foliar @ 0.025 %). The lowest value was observed for the treatment T_4 (POP + Zn @ 5 kg ha^{-1}) which was found to be on par with T_7 (STBR + Zn @ 2.5 kg ha^{-1}), T_{10} (STBR + $ZnSO_4$ foliar @ 0.05 %), T_5 (POP + $ZnSO_4$ foliar @ 0.025 %), T_2 (STBR) and T_1 (POP).

Available zinc content in the soil at the time of final harvest was significantly influenced by the treatments. The mean values ranged from 2.06 to 5.43 mg kg^{-1} (Table 7).Highest value for available Zn content in the soil was recorded by T_4 (POP + Zn @ 5 kg ha^{-1}) and was found to be on par with T_3 (POP + Zn @ 2.5 kg ha^{-1}), T_7 (STBR + Zn @ 2.5 kg ha^{-1}) andT_8 (STBR + Zn @ 5 kg ha^{-1}). The lowest value was observed for the treatment T_1 (POP).

In the case of available Cu, the mean values ranged from 2.24 to 3.93 mg kg^{-1}. Highest value of 3.93 mg kg^{-1} was registered by $T_4$with the application of

POP + Zn @ 5 kg ha^{-1} and the lowest mean was observed in treatment T$_1$(POP) (Table 7).

Table 7.Effect of treatments on available Fe, Mn, Zn and Cu at final harvest

Treatments	Fe	Mn	Zn	Cu
	mg kg^{-1}			
T$_1$ – POP - N: P: K @ 20:30:10 kg ha^{-1}	16.13	24.4	2.06	2.24
T$_2$ – STBR – N: P: K @ 18.2: 7.5: 2.5 kg ha^{-1}	32.87	23.62	2.56	2.63
T$_3$ - POP + Zn$_{soil}$ @ 2.5 kg ha^{-1}	26.12	32.47	4.54	2.39
T$_4$ - POP + Zn$_{soil}$ @ 5 kg ha^{-1}	29.99	20.31	5.43	3.93
T$_5$ - POP + ZnSO$_4$ foliar @ 0.025 %	27.09	23.44	2.94	3.55
T$_6$ - POP + ZnSO$_4$ foliar @ 0.05 %	22.28	26.80	3.02	2.75
T$_7$ - STBR + Zn$_{soil}$ @ 2.5 kg ha^{-1}	21.83	20.86	4.53	2.48
T$_8$ - STBR + Zn$_{soil}$ @ 5 kg ha^{-1}	31.82	30.30	5.12	2.99
T$_9$ - STBR + ZnSO$_4$ foliar @ 0.025 %	31.55	27.43	3.02	3.21
T$_{10}$ - STBR + ZnSO$_4$ foliar @ 0.05 %	35.73	23.07	3.26	3.76
CD (0.05)	4.550	5.505	1.022	NS

4.2. EFFECT OF TREATMENTS ON SHOOT BIOMETRIC CHARACTERISTICS

The biometric characteristics of shoot *viz.*, vine length and branches per plant were not significantly influenced by the treatments. Days to fifty per cent flowering was also not significantly influenced by the treatments.

4.2.1. Vine length

The data presented in Table 8 revealed that, the mean values for vine length ranged from 4.22 to 5.23 m. The treatment T_5 with the application of POP + $ZnSO_4$ foliar @ 0.025 % recorded the highest value of 5.23 m and lowest value of 4.22 m was observed for the treatment T_{10} (STBR + $ZnSO_4$ foliar @ 0.05 %).

Table 8.Effect of treatments on biometric characters of yard long bean

Treatments	Vine length (m)	Branches per plant	Days to fifty percent flowering
T_1 – POP - N: P: K @ 20:30:10 kg ha^{-1}	4.93	6.63	45
T_2 – STBR – N: P: K @ 18.2: 7.5: 2.5 kg ha^{-1}	4.43	6.63	44
T_3 - POP + Zn_{soil} @ 2.5 kg ha^{-1}	5.00	6.67	44
T_4 - POP + Zn_{soil} @ 5 kg ha^{-1}	5.13	7.00	44
T_5 - POP + $ZnSO_4$ foliar @ 0.025 %	5.23	6.33	43
T_6 - POP + $ZnSO_4$ foliar @ 0.05 %	5.03	7.67	43
T_7 - STBR + Zn_{soil} @ 2.5 kg ha^{-1}	4.44	6.63	44
T_8 - STBR + Zn_{soil} @ 5 kg ha^{-1}	4.41	6.63	43
T_9 - STBR + $ZnSO_4$ foliar @ 0.025 %	4.77	6.67	44
T_{10} - STBR + $ZnSO_4$ foliar @ 0.05 %	4.22	7.00	43
CD(0.05)	NS	NS	NS

4.2.2. Branches per plant

Critical appraisal of the data presented in Table 8 revealed that, the treatments did not vary significantly with respect to number of branches. The highest value of 7.67 was reported for the treatment T_6 (POP + $ZnSO_4$ foliar @

0.05 %) and the lowest value of 6.33 was observed for T_5 (POP + $ZnSO_4$ foliar @ 0.025 %).

4.2.3. Days to fifty percent flowering

A perusal of the data presented in Table 8 revealed that the mean values for the days to fifty percent flowering ranged from 43 days to 45 days. The smallest duration was noticed for treatments T_{10} (STBR + $ZnSO_4$ foliar @ 0.05 %), T_5 (POP + $ZnSO_4$ foliar @ 0.025 %), T_6 (POP + $ZnSO_4$ foliar @ 0.05 %) and T_8 (STBR + Zn @ 5 kg ha^{-1}) and the longest duration of 45 days for 50 per cent flowering was noticed for the treatment T_1 (POP).

4.3. EFFECT OF TREATMENTS ON ROOT BIOMETRIC CHARACTERISTICS

The biometric characteristics of root viz., root length, root weight, root volume and number of active nodule per plant are presented in Table 9. The treatments had significantly influenced the above characteristics.

4.3.1. Root Length

The mean values for root length ranged from 49.33 to 65.33 cm. The highest value was recorded for the treatment T_6 (65.33 cm) with the application of POP + $ZnSO_4$ foliar @ 0.05% which was found to be significantly superior to other treatments. However, the treatment T_1 (POP) recorded the lowest value for the root length with a value of 49.33 cm. The treatments T_4 (POP + Zn @ 5 kg ha^{-1}), T_2 (STBR), T_3 (POP + Zn @ 2.5 kg ha^{-1}), T_8 (STBR + Zn @ 5 kg ha^{-1}), T_7 (STBR + Zn @ 2.5 kg ha^{-1}) and T_9 (STBR + $ZnSO_4$ foliar @ 0.025%) were statistically on par with T_1 (POP) (Table 9).

4.3.2. Root Weight

The mean values for root weight ranged from 34.10 to 43.93 g per plant. The highest root weight was noticed for T_6 (POP + $ZnSO_4$ foliar @ 0.05%) and was found to be statistically on par with T_5 (POP + $ZnSO_4$ foliar @ 0.025 %), T_{10}

(STBR + $ZnSO_4$ foliar @ 0.05%),T_9 (STBR + $ZnSO_4$ foliar @ 0.025%) andT_7 (STBR + Zn @ 2.5 kg ha^{-1}). The root weight was lowest for the treatment T_1 (POP). The treatments T_2 (STBR),T_4 (POP + Zn @ 5 kg ha^{-1}) and T_3 (POP + Zn @ 2.5 kg ha^{-1}) did not differ significantly from the above treatment (Table 9).

Table 9.Effect of treatments on root characters of yard long bean

Treatments	Root length (cm)	Root weight (g $plant^{-1}$)	Root volume (cm^3 $plant^{-1}$)	Nodules $plant^{-1}$
T_1 – POP - N: P: K @ 20:30:10 kg ha^{-1}	49.33	34.10	73.96	19.00
T_2 – STBR – N: P: K @ 18.2: 7.5: 2.5 kg ha^{-1}	51.07	36.05	76.66	20.33
T_3 - POP + Zn_{soil} @ 2.5 kg ha^{-1}	51.43	36.70	78.27	20.67
T_4 - POP + Zn_{soil} @ 5 kg ha^{-1}	51.03	36.67	91.26	23.67
T_5 - POP + $ZnSO_4$ foliar @ 0.025 %	57.33	43.80	99.36	23.66
T_6 - POP + $ZnSO_4$ foliar @ 0.05 %	65.33	43.93	99.44	21.67
T_7 - STBR + Zn_{soil} @ 2.5 kg ha^{-1}	52.43	40.17	91.05	26.33
T_8 - STBR + Zn_{soil} @ 5 kg ha^{-1}	52.13	39.45	85.21	28.67
T_9 - STBR + $ZnSO_4$ foliar @ 0.025 %	53.67	41.07	85.53	25.67
T_{10} - STBR + $ZnSO_4$ foliar @ 0.05 %	56.03	43.79	97.75	30.00
CD (0.05)	6.576	4.357	12.524	4.092

4.3.3. Root Volume

The mean value for root volume ranged from 73.96 to 99.44cm^3plant^{-1}. The treatments imposed a significant effect with respect to the root volume. The highest root volume was recorded by T_6 (POP + $ZnSO_4$ foliar @ 0.05 %) which was on par with T_5 (POP + $ZnSO_4$ foliar

@ 0.025 %), T_{10} (STBR + $ZnSO_4$ foliar @ 0.05%),T_4 (POP + Zn @ 5 kg ha^{-1}) andT_7 (STBR + Zn @ 2.5 kg ha^{-1}) and T_1 (POP) recorded the lowest value (Table 9).

4.3.4. Number of Active Nodule per Plant

Observations revealed that, the treatments were found to impose significant effects with respect to number of nodules per plant (Table 9). The mean value for number of nodules per plant ranged from 19.0 to 30.0. The highest value for nodules $plant^{-1}$ was noticed for T_{10} (STBR + $ZnSO_4$ foliar @ 0.05%) followed by T_8 (STBR + Zn @ 5 kg ha^{-1}). The lowest value was recorded by T_1 (POP).

4.4. EFFECT OF TREATMENTS ON YIELD CHARACTERISTICS

4.4.1. Pod Yield per Plant

Observations revealed that, the treatments had significantly influenced the pod yield per plant. The mean values ranged from 609.67 to 978.67 g $plant^{-1}$ (Table 10). The highest value for pod yield was noticed for the treatment T_{10}(STBR + $ZnSO_4$ foliar @ 0.05 %) which was found to be statistically on par with T_9 (STBR + $ZnSO_4$ foliar @ 0.025 %), T_8 (STBR + Zn @ 5 kg ha^{-1}), T_6 (POP + $ZnSO_4$ foliar @ 0.05 %), T_5 (POP + $ZnSO_4$ foliar @ 0.025 %) and T_7 (STBR + Zn @ 2.5 kg ha^{-1}). The lowest value was recorded for the treatment T_1 (POP).

4.4.2. Pod Length

Pod length was not significantly influenced by the treatments and the mean values ranged from 47.82 to 50.46 cm (Table 10). T_{10} (STBR + $ZnSO_4$ foliar @ 0.05 %) got the highest value of 50.46 cm and the lowest value was recorded by the treatment T_1 (POP).

4.4.3. Pod Weight

There was significant difference among treatments with respect to the pod weight (Table 10). The mean values ranged from 19.21 to 24.00 g per pod. The highest value was recorded by the treatment T_{10} (STBR + $ZnSO_4$ foliar @ 0.05 %) and was found to be on par with T_8 (STBR + Zn @ 5 kg ha^{-1}), T_9 (STBR + $ZnSO_4$

foliar @ 0.025 %), T_6 (POP + $ZnSO_4$ foliar @ 0.05 %) and T_4 (POP + Zn @ 5 kg ha^{-1}). Treatment T_1 (POP) registered the lowest pod weight. The treatments T_2(STBR), T_3 (POP + Zn @ 2.5 kg ha^{-1}) and T_7 (STBR + Zn @ 2.5 kg ha^{-1}) did not significantly differ from T_1 (POP).

Table 10.Effect of treatments on pod yield and yield attributes of yard long bean

Treatments	Pod yield		Pod length (cm)	Weight pod^{-1} (g)	Pods $plant^{-1}$	Seeds pod^{-1}
	g $plant^{-1}$	kg ha^{-1}				
T_1 – POP - N: P: K @ 20:30:10 kg ha^{-1}	609.67	9023	47.82	19.21	32.3	16.3
T_2 – STBR – N: P: K @ 18.2: 7.5: 2.5 kg ha^{-1}	774.00	11455	47.94	19.32	40.6	16.3
T_3 - POP + Zn_{soil} @ 2.5 kg ha^{-1}	763.33	11297	48.71	20.13	38.6	17.3
T_4 - POP + Zn_{soil} @ 5 kg ha^{-1}	801.00	11854	48.78	22.02	36.3	17.6
T_5 - POP + $ZnSO_4$ foliar @ 0.025 %	901.67	13344	48.45	21.82	43.0	17.0
T_6 - POP + $ZnSO_4$ foliar @ 0.05 %	936.00	13852	48.72	22.26	41.6	17.6
T_7 - STBR + Zn_{soil} @ 2.5 kg ha^{-1}	894.33	13236	49.64	20.93	45.6	18.0
T_8 - STBR + Zn_{soil} @ 5 kg ha^{-1}	953.00	14104	49.51	23.30	41.3	18.3
T_9 - STBR + $ZnSO_4$ foliar @ 0.025 %	964.33	14272	50.07	22.64	44.6	19.3
T_{10} - STBR + $ZnSO_4$ foliar @ 0.05 %	978.67	14484	50.46	24.00	42.6	20.0
CD(0.05)	122.86	1818	NS	2.083	5.367	1.148

4.4.4. Pods per Plant

It was observed from Table 10 that, the treatments imposed significant effects with respect to pods per plant. The mean values ranged from 32.30 to 45.60. The highest value was recorded for the treatment T_7 (45.60) with the application of STBR + Zn @ 2.5 kg ha^{-1} which was found to be on par with T_9

(STBR + $ZnSO_4$ foliar @ 0.025 %), T_5 (POP + $ZnSO_4$ foliar @ 0.025 %), T_{10} (STBR + $ZnSO_4$ foliar @ 0.05 %), T_6 (POP + $ZnSO_4$ foliar @ 0.05 %), T_8 (STBR + Zn @ 5 kg ha^{-1}) and T_2 (STBR). The treatment T_1 (POP) recorded the lowest value for the pods per plant**.**

4.4.5. Number of Seeds per Pod

The treatment effect was significant on the number of seeds per pod (Table 10). The mean values ranged from 16.30 to 20.00. The highest value was recorded by the treatment T_{10} (STBR + $ZnSO_4$ foliar @ 0.05 %) which was found to be on par with T_9 (STBR + $ZnSO_4$ foliar @ 0.025 %). The lowest value was recorded by T_2 (STBR) which did not significantly differ from the treatments T_1 (POP), T_5 (POP + $ZnSO_4$ foliar @ 0.025 %) and T_3 (POP + Zn @ 2.5 kg ha^{-1}).

4.5. EFFECT OF TREATMENTS ON BHUSA YIELD, TOTAL DRY MATTER PRODUCTION AND HARVEST INDEX

4.5.1. Bhusa Yield

The data on bhusa yield of yard long bean (Table 11) showed that the treatment effect was not significant. The treatment T_6 (STBR + $ZnSO_4$ foliar @ 0.05 %) recorded the highest value and T_1 (POP) recorded the lowest value.

4.5.2. Total Dry matter Production

Statistical analysis of the data on total dry matter production indicated a significant effect due to application of treatments (Table 11). The mean value ranged from 3009.33 to 4474.53 kg ha^{-1} with the highest value recorded for T_{10} (STBR + $ZnSO_4$ foliar @ 0.05 %) which was found to be on par with T_9 (STBR + $ZnSO_4$ foliar @ 0.025 %), T_6 (POP + $ZnSO_4$ foliar @ 0.05 %), T_8 (STBR + Zn @ 5 kg ha^{-1}) and T_7 (STBR + Zn @ 2.5 kg ha^{-1}). Lowest value was registered by the treatment T_1 (POP) which do not significantly differ from treatments T_3 (POP + Zn @ 2.5 kg ha^{-1}), T_2 (STBR) and T_4 (POP + Zn @ 5 kg ha^{-1}).

Table 11.Effect of treatments on bhusa yield, total dry matter production and harvest index of yard long bean

Treatments	Bhusa yield (kg ha^{-1})	Total dry matter production (kg ha^{-1})	Harvest index
T_1 – POP - 20:30:10 kg ha^{-1} N: P: K.	5895.33	3009.33	0.578
T_2 – STBR – 18.2: 7.5: 2.5 kg ha^{-1} N: P: K.	6043.33	3522.40	0.653
T_3 - POP + Zn @ 2.5 kg ha^{-1}	5920.00	3487.87	0.654
T_4 - POP + Zn @ 5 kg ha^{-1}	6265.33	3626.00	0.654
T_5 - POP + $ZnSO_4$ foliar @ 0.025 %	6068.00	3700.00	0.686
T_6 - POP + $ZnSO_4$ foliar @ 0.05 %	6704.40	4292.00	0.674
T_7 - STBR + Zn @ 2.5 kg ha^{-1}	6413.33	3922.00	0.673
T_8 - STBR + Zn @ 5 kg ha^{-1}	6438.00	4272.27	0.681
T_9 - STBR + $ZnSO_4$ foliar @ 0.025 %	6512.00	4336.40	0.680
T_{10} - STBR + $ZnSO_4$ foliar @ 0.05 %	6684.67	4474.53	0.683
CD (0.05)	NS	652.42	0.055

4.5.3. Harvest Index

Critical appraisal of the HI data revealed that, treatments had significantly influenced the harvest index (Table 11). The mean value ranged from 0.578 to 0.686. Treatment T_5 (POP + $ZnSO_4$ foliar @ 0.025 %) registered the highest mean value of 0.686 which on par with all other treatments except the lowest mean value of 0.578 with the treatment T_1 (POP).

4.6. EFFECT OF TREATMENTS ON NUTRIENT COMPOSITION OF YARD LONG BEAN – POD

4.6.1. Effect of Treatments on N, P and K Contents of Yard Long Bean Pod

The data on N, P and K content of yard long bean pod are presented in Table 12. The treatment T_{10} (STBR + $ZnSO_4$ foliar @ 0.05 %) had the highest N content (6.62 per cent) and the treatment T_1 (POP) recorded the lowest value of 5.76 per cent N.

Table 12.Effect of treatments on N, P and K contents of pods of yard long bean

Treatments	N (%)	P (%)	K (%)
T_1 – POP - N: P: K @ 20:30:10 kg ha^{-1}	5.76	0.55	2.76
T_2 – STBR – N: P: K @ 18.2: 7.5: 2.5 kg ha^{-1}	5.78	0.53	2.98
T_3 - POP + Zn_{soil} @ 2.5 kg ha^{-1}	5.82	0.55	3.21
T_4 - POP + Zn_{soil} @ 5 kg ha^{-1}	5.97	0.63	3.44
T_5 - POP + $ZnSO_4$ foliar @ 0.025 %	6.07	0.49	3.33
T_6 - POP + $ZnSO_4$ foliar @ 0.05 %	6.31	0.57	3.38
T_7 - STBR + Zn_{soil} @ 2.5 kg ha^{-1}	5.87	0.44	2.85
T_8 - STBR + Zn_{soil} @ 5 kg ha^{-1}	6.16	0.53	2.86
T_9 - STBR + $ZnSO_4$ foliar @ 0.025 %	6.26	0.48	3.33
T_{10} - STBR + $ZnSO_4$ foliar @ 0.05 %	6.62	0.45	3.40
CD (0.05)	NS	0.069	NS

The P content of the pods under different treatments varied significantly. The mean values ranged from 0.44 to 0.63 per cent P (Table 12). The treatment T_4 (POP + Zn @ 5 kg ha^{-1}) recorded the highest value of 0.63 per cent and T_6 (POP + $ZnSO_4$ foliar @ 0.05 %) was the second best one, which were found to be statistically on par with each other. The lowest value for pod P content was reported for the treatment T_7 (STBR + Zn @ 2.5 kg ha^{-1}) *i.e.* 0.44per cent which was found to be on par with T_{10} (STBR + $ZnSO_4$ foliar @ 0.05 %), T_9 (STBR + $ZnSO_4$ foliar @ 0.025 %) and T_5 (POP + $ZnSO_4$ foliar @ 0.025 %).

On scrutinizing the results of pod K content, it was noted that, the mean values ranged from 2.76 to 3.44 per cent. The treatment with POP + Zn @ 5 kg ha^{-1} (T_4) recorded the highest value of 3.44 per cent and T_1 (POP) recorded the lowest value of 2.76 per cent. However, the treatment effect on pod K content was not significant (Table 12).

4.6.2. Effect of Treatments on Ca, Mg and S Contents of Yard Long Bean Pod

Treatment application significantly influenced the Ca content in pods as inferred from Table 13. The mean values ranged from 0.65 to 1.07 per cent. The highest value of 1.07 per cent for Ca content was noticed with treatment T_8 (STBR + Zn @ 5 kg ha^{-1}) which was on par with T_{10} (STBR + $ZnSO_4$ foliar @ 0.05 %), T_6 (POP + $ZnSO_4$ foliar @ 0.05 %) and T_4 (POP + Zn @ 5 kg ha^{-1}) and T_9 (STBR + $ZnSO_4$ foliar @ 0.025 %). The lowest value was noticed in the treatment T_1 (POP) which was on par T_3 (POP + Zn @ 2.5 kg ha^{-1}), T_2 (STBR) and T_7 (STBR + Zn @ 2.5 kg ha^{-1}).

Data on Mg content in pods (Table 13) showed that, the mean value ranges from 0.80 to 0.99 per cent. The treatment T_9 (STBR + $ZnSO_4$ foliar @ 0.025 %) got the highest value of 0.99 per cent. The lowest value was recorded by the treatment T_6 (POP + $ZnSO_4$ foliar @ 0.05 %). The treatment effect on Mg content of pod was not significant.

The effect of treatments on S content of pod was significant (Table 13). The mean value ranges from 0.17 to 0.30 per cent. Treatment T_{10} (STBR + $ZnSO_4$ foliar @ 0.05 %) recorded the highest mean value which was on par with T_6 (POP + $ZnSO_4$ foliar @ 0.05 %), T_4 (POP + Zn @ 5 kg ha^{-1}), T_8 (STBR + Zn @ 5 kg ha-

^{1}), T_5 (POP + $ZnSO_4$ foliar @ 0.025 %) and T_9 (STBR + $ZnSO_4$ foliar @ 0.025 %). Lowest mean value of 0.17 per cent was recorded by T_2 (STBR) which was found to be on par with T_1 (POP) and T_3 (POP + Zn @ 2.5 kg ha^{-1}).

Table 13.Effect of treatments on Ca, Mg and S contents of pods of yard long bean

Treatments	Ca (%)	Mg (%)	S (%)
T_1 – POP - N: P: K @ 20:30:10 kg ha^{-1}	0.65	0.84	0.20
T_2 – STBR – N: P: K @ 18.2: 7.5: 2.5 kg ha^{-1}	0.77	0.91	0.17
T_3 - POP + Zn_{soil} @ 2.5 kg ha^{-1}	0.70	0.85	0.22
T_4 - POP + Zn_{soil} @ 5 kg ha^{-1}	0.93	0.95	0.29
T_5 - POP + $ZnSO_4$ foliar @ 0.025 %	0.85	0.86	0.26
T_6 - POP + $ZnSO_4$ foliar @ 0.05 %	1.00	0.80	0.30
T_7 - STBR + Zn_{soil} @ 2.5 kg ha^{-1}	0.82	0.81	0.22
T_8 - STBR + Zn_{soil} @ 5 kg ha^{-1}	1.07	0.88	0.28
T_9 - STBR + $ZnSO_4$ foliar @ 0.025 %	0.89	0.99	0.25
T_{10} - STBR + $ZnSO_4$ foliar @ 0.05 %	1.05	0.89	0.30
CD (0.05)	0.189	NS	0.053

4.6.3. Effect of treatments on Fe, Mn, Zn and Cu contents of yard long bean pods

The data on pod Fe content (Table 14) revealed that, the treatment effect was significant. The mean values of Fe content in pods ranged from 51.81 to 104.48 mg kg^{-1}. The highest value was noticed for the treatment T_4 (POP + Zn @ 5 kg ha^{-1}) which was found to be on par with T_2 (STBR). The lowest value was recorded in treatment T_8 (STBR + Zn @ 5 kg ha^{-1}) which was found to be on par with T_6 (POP + $ZnSO_4$ foliar @ 0.05 %) and T_7 (STBR + Zn @ 2.5 kg ha^{-1}).

Table 14.Effect of treatments on Fe, Mn, Zn and Cu contents of yard long bean pods

Treatments	Fe	Mn	Zn	Cu
	(mg kg^{-1})			
T_1 – POP - N: P: K @ 20:30:10 kg ha^{-1}	83.85	43.96	18.33	5.33
T_2 – STBR – N: P: K @ 18.2: 7.5: 2.5 kg ha^{-1}	98.88	45.67	24.04	8.16
T_3 - POP + Zn_{soil} @ 2.5 kg ha^{-1}	87.59	39.55	24.58	9.17
T_4 - POP + Zn_{soil} @ 5 kg ha^{-1}	104.48	52.87	29.77	3.95
T_5 - POP + $ZnSO_4$ foliar @ 0.025 %	90.88	39.72	24.74	3.06
T_6 - POP + $ZnSO_4$ foliar @ 0.05 %	59.53	36.55	30.12	3.40
T_7 - STBR + Zn_{soil} @ 2.5 kg ha^{-1}	64.50	51.04	26.13	3.95
T_8 - STBR + Zn_{soil} @ 5 kg ha^{-1}	51.81	45.15	29.72	5.64
T_9 - STBR + $ZnSO_4$ foliar @ 0.025 %	65.21	45.63	25.33	4.29
T_{10} - STBR + $ZnSO_4$ foliar @ 0.05 %	70.92	44.99	34.01	6.56
CD (0.05)	13.08	NS	3.90	NS

Data on Mn content of pods showed that, the mean values ranged from 36.55 to 52.87 mg kg^{-1} (Table 14) and the treatment effect was not significant. T_4 (POP + Zn @ 5 kg ha^{-1}) got the highest value of 52.87 mg kg^{-1} Mn. The lowest value was recorded by the treatment T_6 (POP + $ZnSO_4$ foliar @ 0.05 %) which was found to be on par with T_3 (POP + Zn @ 2.5 kg ha^{-1}), T_5 (POP + $ZnSO_4$ foliar @ 0.025 %) and T_1 (POP).

The treatments had significantly influenced the Zn content of the pods (Table 14). The mean values ranged from 18.33 to 34.01mg kg^{-1}. Treatment T_{10} (STBR + $ZnSO_4$ foliar @ 0.05 %) recorded the highest value and was found to be on par with treatment T_6 (POP + $ZnSO_4$ foliar @ 0.05 %). Lowest value of pod Zn was recorded by the treatment T_1 (POP).

The pod Cu content ranged from 3.06 to 9.17 mg kg^{-1}. Treatment T_3 (POP + Zn @ 2.5 kg ha^{-1}) recorded the highest mean and the lowest value of 3.06 mg kg^{-1} was recorded by T_5 (POP + $ZnSO_4$ foliar @ 0.025 %). However, the treatment effects were not significant (Table 14).

4.7. QUALITY ASPECTS

4.7.1. Crude Protein

Crude protein content of pods (on fresh weight basis) was not significantly influenced by the treatments (Table 15). The treatment T_{10} (STBR + $ZnSO_4$ foliar @ 0.05 %) recorded the highest value of 6.45 per cent which was followed by T_6 (POP + $ZnSO_4$ foliar @ 0.05 %).The lowest value was recorded by the treatment T_1 (POP).

4.7.2. Phytate

The treatments had significantly influenced the phytate content (on dry weight basis) of pods (Table 15). The mean values ranged from 87.50 to 579.83mg kg^{-1}. Treatment T_{10} (STBR + $ZnSO_4$ foliar @ 0.05 %) recorded the lowest value for phytate content and was found to be on par with treatment T_6 (POP + $ZnSO_4$ foliar @ 0.05 %), T_5 (POP + $ZnSO_4$ foliar @ 0.025 %), T_9 (STBR + $ZnSO_4$ foliar @ 0.025 %), T_8 (STBR + Zn @ 5 kg ha^{-1}) and T_4 (POP + Zn @ 5 kg ha^{-1}). Highest value for phytate content was recorded by the treatment T_1 (POP) followed by T_2 (STBR) and T_7 (STBR + Zn @ 2.5 kg ha^{-1}) which differed significantly from the above treatments.

Table 15. Effect of treatments on crude protein and phytate in yard long bean pods (on dry weight basis)

Treatments	Crude protein (%)	Phytate (mg kg^{-1})	Phytate : Zn ratio
T_1 – POP - N: P: K @ 20:30:10 kg ha^{-1}	5.26	579.83	33.63
T_2 – STBR – N: P: K @ 18.2: 7.5: 2.5 kg ha^{-1}	5.80	443.75	18.54
T_3 - POP + Zn_{soil} @ 2.5 kg ha^{-1}	6.01	311.25	12.64
T_4 - POP + Zn_{soil} @ 5 kg ha^{-1}	6.06	132.50	4.45
T_5 - POP + $ZnSO_4$ foliar @ 0.025 %	6.08	96.25	3.89
T_6 - POP + $ZnSO_4$ foliar @ 0.05 %	6.41	88.75	2.94
T_7 - STBR + Zn_{soil} @ 2.5 kg ha^{-1}	6.04	321.25	12.32
T_8 - STBR + Zn_{soil} @ 5 kg ha^{-1}	6.14	125.00	4.30
T_9 - STBR + $ZnSO_4$ foliar @ 0.025 %	6.38	101.25	4.00
T_{10} - STBR + $ZnSO_4$ foliar @ 0.05 %	6.45	87.50	2.57
CD (0.05)	NS	119.368	9.02

4.7.3. Phytate : Zn Ratio

The phytate : Zn ratio (Table 15) was significantly influenced by the different treatments. The mean values ranged from 2.57 to 33.63. The lowest ratio of 2.57 was reported for T_{10} (STBR + $ZnSO_4$ foliar @ 0.05 %) which was found to be on par with T_6 (POP + $ZnSO_4$ foliar @ 0.05 %), T_5 (POP + $ZnSO_4$ foliar @ 0.025 %), T_9 (STBR + $ZnSO_4$ foliar @ 0.025 %), T_8 (STBR + Zn @ 5 kg ha^{-1}) and T_4 (POP + Zn @ 5 kg ha^{-1}). The treatment T_1 (POP) recorded the highest value of 33.63 and significantly differed from all other treatments.

4.8. EFFECT OF TREATMENTS ON NUTRIENT COMPOSITION OF YARD LONG BEAN BHUSA

4.8.1. Effect of treatments on N, P and K contents of yard long bean bhusa

It was observed from Table 16 that, the mean values of N content in bhusa ranged from 1.47 to 2.74 per cent and was significantly influenced by the treatments. The highest value was recorded inT$_6$ (POP + $ZnSO_4$ foliar @ 0.05 %) which was on par with T$_5$ (POP + $ZnSO_4$ foliar @ 0.025%). The lowest value was observed for T$_8$ (STBR + Zn @ 5 kg ha^{-1}).

Table 16. Effect of treatments on N, P and K contents of yard long bean bhusa

Treatments	N (%)	P (%)	K (%)
T$_1$ – POP - N: P: K @ 20:30:10 kg ha^{-1}	2.09	0.348	2.11
T$_2$ – STBR – N: P: K @ 18.2: 7.5: 2.5 kg ha^{-1}	2.14	0.316	2.50
T$_3$ - POP + Zn$_{soil}$ @ 2.5 kg ha^{-1}	2.09	0.496	3.31
T$_4$ - POP + Zn$_{soil}$ @ 5 kg ha^{-1}	1.86	0.502	3.47
T$_5$ - POP + $ZnSO_4$ foliar @ 0.025 %	2.57	0.478	3.18
T$_6$ - POP + $ZnSO_4$ foliar @ 0.05 %	2.74	0.503	3.49
T$_7$ - STBR + Zn$_{soil}$ @ 2.5 kg ha^{-1}	1.94	0.466	3.24
T$_8$ - STBR + Zn$_{soil}$ @ 5 kg ha^{-1}	1.47	0.509	3.39
T$_9$ - STBR + $ZnSO_4$ foliar @ 0.025 %	1.61	0.474	3.32
T$_{10}$ - STBR + $ZnSO_4$ foliar @ 0.05 %	2.08	0.482	3.40
CD (0.05)	0.559	0.107	0.691

The data revealed that, the treatment effect was significant with respect to P content. The mean values of P content in bhusa ranged from 0.316 to 0.509 % (Table 16). The highest value was noticed for T$_8$ (STBR + Zn @ 5 kg ha^{-1}) which was found to be on par with T$_6$ (POP + $ZnSO_4$ foliar @ 0.05 %), T$_4$ (POP

+ Zn @ 5 kg ha^{-1}), T$_3$ (POP + Zn @ 2.5 kg ha^{-1}), T$_{10}$ (STBR + ZnSO$_4$ foliar @ 0.05 %), T$_5$ (POP + ZnSO$_4$ foliar @ 0.025 %), T$_9$ (STBR + ZnSO$_4$ foliar @ 0.025 %) and T$_7$ (STBR + Zn @ 2.5 kg ha^{-1}). The lowest value was recorded in T$_2$ (STBR) which in turn was on par with T$_1$ (POP).

Statistical analysis of the data on bhusa K content indicated significant effect due to various treatments (Table16). The mean values ranged from 2.11 to 3.49 % with the highest value recorded by T$_6$ (POP + ZnSO$_4$ foliar @ 0.05 %) which was found to be on par with T$_4$ (POP + Zn @ 5 kg ha^{-1}), T$_{10}$ (STBR + ZnSO$_4$ foliar @ 0.05 %), T$_8$ (STBR + Zn @ 5 kg ha^{-1}), T$_9$ (STBR + ZnSO$_4$ foliar @ 0.025 %), T$_3$ (POP + Zn @ 2.5 kg ha^{-1}), T$_7$ (STBR + Zn @ 2.5 kg ha^{-1}) and T$_5$ (POP + ZnSO$_4$ foliar @ 0.025 %). Lowest value was registered by T$_1$ (POP) which was on par with T$_2$ (STBR).

4.8.2. Effect of treatments on Ca, Mg and S contents of yard long bean bhusa

The treatments imparted significant effect on the Ca content in bhusa and the mean values ranged between 0.30 and 0.85 per cent (Table 17). The highest value was recorded for T$_3$ with the application of POP + Zn @ 2.5 kg ha^{-1}and T$_5$ (POP + ZnSO$_4$ foliar @ 0.025 %) which were found to be on par with T$_2$ (STBR), T$_1$ (POP) and T$_9$ (STBR + ZnSO$_4$ foliar @ 0.025 %). Treatments T$_4$ (POP + Zn @ 5 kg ha^{-1}), T$_7$ (STBR +Zn @ 2.5 kg ha^{-1}), T$_6$ (POP + ZnSO$_4$ foliar @ 0.05 %), T$_8$ (STBR + Zn @ 5 kg ha^{-1}) and T$_{10}$ (STBR + ZnSO$_4$ foliar @ 0.05 %) were found to be on par.

There was significant difference among treatments with respect to Mg content of bhusa. The mean values ranged from 0.49 to 0.89 % (Table 17). Highest value 0.89 per cent was recorded for treatment T$_6$ (POP + ZnSO$_4$ foliar @ 0.05 %) and was found to be on par with T$_3$ (POP + Zn @ 2.5 kg ha^{-1}). Treatments T$_4$ (POP + Zn @ 5 kg ha^{-1}) and T$_8$ (STBR + Zn @ 5 kg ha^{-1}) registered the lowest mean values.

Table 17. Effect of treatments on Ca, Mg and S contents of yard long bean bhusa

Treatments	Ca (%)	Mg (%)	S (%)
T_1 – POP - N: P: K @ 20:30:10 kg ha^{-1}	0.78	0.73	0.18
T_2 – STBR – N: P: K @ 18.2: 7.5: 2.5 kg ha^{-1}	0.84	0.51	0.23
T_3 - POP + Zn_{soil} @ 2.5 kg ha^{-1}	0.85	0.77	0.23
T_4 - POP + Zn_{soil} @ 5 kg ha^{-1}	0.56	0.49	0.26
T_5 - POP + $ZnSO_4$ foliar @ 0.025 %	0.85	0.75	0.28
T_6 - POP + $ZnSO_4$ foliar @ 0.05 %	0.48	0.89	0.33
T_7 - STBR + Zn_{soil} @ 2.5 kg ha^{-1}	0.52	0.55	0.26
T_8 - STBR + Zn_{soil} @ 5 kg ha^{-1}	0.35	0.49	0.26
T_9 - STBR + $ZnSO_4$ foliar @ 0.025 %	0.68	0.67	0.27
T_{10} - STBR + $ZnSO_4$ foliar @ 0.05 %	0.30	0.51	0.34
CD (0.05)	0.266	0.131	0.068

The treatments significantly influenced the S content of bhusa as seen in Table 17. The mean values ranged from 0.18 to 0.34 %. The highest value of 0.34 % was noticed for T_{10} (STBR + $ZnSO_4$ foliar @ 0.05 %) which was on par with T_6 (POP + $ZnSO_4$ foliar @ 0.05 %) and T_5 (POP + $ZnSO_4$ foliar @ 0.025 %). The lowest value was noticed for T_1 (POP) which was on par with T_3 (POP + Zn @ 2.5 kg ha^{-1}) and T_2 (STBR).

4.8.3. Effect of treatments on Fe, Mn, Zn and Cu contents of yard long bean bhusa

The Fe content in bhusa varied significantly with different treatments (Table 18). The mean values ranged between 61.37 and 114.70 mg kg^{-1}. The highest value was recorded for T_{10} (114.70 mg kg^{-1}) having the application of STBR +

$ZnSO_4$ foliar @ 0.05 % which was significantly superior to all other treatments. The lowest value was recorded under T_9 (STBR + $ZnSO_4$ foliar @ 0.025 %).The treatments T_5 (POP + $ZnSO_4$ foliar @ 0.025 %), T_7 (STBR + Zn @ 2.5 kg ha^{-1}), T_8 (STBR + Zn @ 5 kg ha^{-1}) and T_1 (POP) were statistically on par with T_9(STBR + $ZnSO_4$ foliar @ 0.025 %).

The results of the analysis of bhusa indicated significant effect of treatments on Mn content of bhusa (Table 18). The mean values ranged from 221.37 to 462.03 mg kg^{-1}. The highest value was recorded for $T_2$with the application of STBR (462.03 mg kg^{-1}) which was on par with T_1 (POP), T_{10} (STBR + $ZnSO_4$ foliar @ 0.05 %), T_9 (STBR + $ZnSO_4$ foliar @ 0.025 %), T_6 (POP + $ZnSO_4$ foliar @ 0.05 %), T_3 (POP + Zn @ 2.5 kg ha^{-1}) and T_5 (POP + $ZnSO_4$ foliar @ 0.025 %). The lowest value was registered for T_7 (STBR + Zn @ 2.5 kg ha^{-1}).

The Zn content of bhusa (Table 18) was significantly influenced by different treatments. The mean values ranged from 18.08 to 70.94 mg kg^{-1}. The treatment T_6 (POP + $ZnSO_4$ foliar @ 0.05 %) recorded the highest value of 70.94 mg kg^{-1} followed by T_5 (POP + $ZnSO_4$ foliar @ 0.025 %)(59.23 mg kg^{-1}) which were significantly superior to all other treatments. The treatment T_4 (POP + Zn @ 5 kg ha^{-1}) was found to be on par with T_{10} (STBR + $ZnSO_4$ foliar @ 0.05 %) and T_8 (STBR + Zn @ 5 kg ha^{-1}). The lowest value of 18.80 mg $kg^{-1}$for bhusa Zn was reported in treatment T_1 (POP) which was found to be on par with T_2 (STBR) and T_3 (POP + Zn @ 2.5 kg ha^{-1}).

The treatments significantly influenced the Cu content of bhusa (Table 18). The mean values ranged from 2.72 to 5.58 mg kg^{-1}. Treatment T_3 (POP + Zn @ 2.5 kg ha^{-1}) recorded the highest mean value which was on par with T_4 (POP + Zn @ 5 kg ha^{-1}). Lowest value of 2.72 mg kg^{-1} was recorded by T_1 (POP) which was found to be on par with T_7 (STBR + Zn @ 2.5 kg ha^{-1}), T_9 (STBR + $ZnSO_4$ foliar @ 0.025 %), T_8 (STBR + Zn @ 5 kg ha^{-1}) and T_2 (STBR).

Table 18. Effect of treatments on Fe, Mn, Zn and Cu contents of yard long bean bhusa

Treatments	Fe	Mn	Zn	Cu
	(mg kg^{-1})			
T_1 – POP - N: P: K @ 20:30:10 kg ha^{-1}	79.12	425.73	18.80	2.72
T_2 – STBR – N: P: K @ 18.2: 7.5: 2.5 kg ha^{-1}	85.83	462.03	23.12	3.53
T_3 - POP + Zn_{soil} @ 2.5 kg ha^{-1}	90.91	359.17	27.50	5.58
T_4 - POP + Zn_{soil} @ 5 kg ha^{-1}	84.66	270.23	46.67	4.53
T_5 - POP + $ZnSO_4$ foliar @ 0.025 %	65.41	335.40	59.23	3.94
T_6 - POP + $ZnSO_4$ foliar @ 0.05 %	91.82	383.40	70.94	4.43
T_7 - STBR + Zn_{soil} @ 2.5 kg ha^{-1}	71.75	221.37	32.02	3.12
T_8 - STBR + Zn_{soil} @ 5 kg ha^{-1}	76.90	241.40	46.07	3.47
T_9 - STBR + $ZnSO_4$ foliar @ 0.025 %	61.37	405.97	33.39	3.41
T_{10} - STBR + $ZnSO_4$ foliar @ 0.05 %	114.70	406.43	46.59	3.99
CD (0.05)	19.470	129.129	11.168	1.066

4.8. SCORING FOR PEST AND DISEASES

No severe pest and disease incidence was noticed during the period of crop growth. Stem borer attack was observed during initial crop period and it was controlled by the application of Oxuron @ 5 ml L^{-1}. During the pod yielding stage, pod borer attack and incidence of aphids were observed and it was controlled by the application of Neem Kernel Suspension (NKS) @ 5 percent.

4.9. ECONOMICS OF CULTIVATION

B: C ratio was calculated by taking into consideration the cost of cultivation and returns for each treatment and the results are presented in Table 19.The mean values ranged from 1.48 to 2.02.From the analysis of data T_{10}(STBR + $ZnSO_4$ foliar @ 0.05 %) registered the highest value of 2.02 which was on par with T_9 (STBR + $ZnSO_4$ foliar @ 0.025 %), T_8(STBR + Zn @ 5 kg ha^{-1}), T_6 (POP + $ZnSO_4$ foliar @ 0.05 %), T_7 (STBR + Zn @ 2.5 kg ha^{-1}) and T_5(POP + $ZnSO_4$ foliar @ 0.025 %). Lowest B: C ratio was reported by the treatment T_1 (POP).

Table 19.Economics of cultivation

Treatments	Pod Yield (kg ha^{-1})	Gross returns (Rs. ha^{-1})	Net returns (Rs. ha^{-1})	B : C Ratio
T_1 – POP - N: P: K @ 20:30:10 kg ha^{-1}	9023	180461	59530	1.48
T_2 – STBR – N: P: K @ 18.2: 7.5: 2.5 kg ha^{-1}	11455	229102.7	86192	1.59
T_3 - POP + Zn_{soil} @ 2.5 kg ha^{-1}	11297	225940	82490	1.55
T_4 - POP + Zn_{soil} @ 5 kg ha^{-1}	11854	237093.3	93,643	1.62
T_5 - POP + $ZnSO_4$ foliar @ 0.025 %	13344	266890.7	123440	1.80
T_6 - POP + $ZnSO_4$ foliar @ 0.05 %	13852	277053.3	133603	1.86
T_7 - STBR + Zn_{soil} @ 2.5 kg ha^{-1}	13236	264717.3	121807	1.85
T_8 - STBR + Zn_{soil} @ 5 kg ha^{-1}	14104	282080	139170	1.96
T_9 - STBR + $ZnSO_4$ foliar @ 0.025 %	14272	285433.3	142523	1.99
T_{10} - STBR + $ZnSO_4$ foliar @ 0.05 %	14484	289673.3	146763	2.02
CD (0.05)	1818			0.23

Discussion

5. DISCUSSION

The investigationtitled ‘Zinc biofortification for enhancing yield and quality of yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt) in ferralitic soils’ has been carried out at the Department of Soil Science and Agricultural Chemistry, College of Agriculture, Vellayani during October, 2013 to January, 2014. The present study was envisaged to assess the effect of zinc biofortification on zinc availability, pod yield and quality of yard long bean in ferralitic soils. A brief interpretation of results pertaining to the field study conducted is presented in this chapter.

5.1. EFFECT OF ZINC BIOFORTIFICATION (AGRONOMIC) ON POD YIELD AND GROWTH CHARACTERISTICS OF YARD LONG BEAN

The pod yield and yield attributes were significantly influenced by the differenttreatments and the treatment T_{10} (STBR + $ZnSO_4$ foliar @ 0.05 %) recorded the highest values for pod yield and for most of yield attributes which were on par with all the STBR based treatments receiving Zn. The B:C ratio was also highest for the above treatment indicating the highest returns from this treatment. The total dry matter production also followed the same trend. This has indicated the need for soil test based fertilizer application. From the above results it was clear that, foliar application of Zn is the best technique to address Zn nutrition as evidenced from the data on pod yield (Fig. 3), zinc content (Fig. 4) and economical parameters (Fig. 5).In most of the studies on zinc nutrition, it has been reported that, foliar application was found to be better than soil application in terms of yield, zinc content and profit. Khan and Weaver (1989) had reported that, foliar application of Zn was more effective than soil application in providing Zn for transport to soya bean grains and thus enhancing Zn concentration in grains. According to Savithri (2001) foliar spray of zinc improved the grain yield appreciably in pulses.

Fig 3. Effect of treatments on pod yield of yard long bean

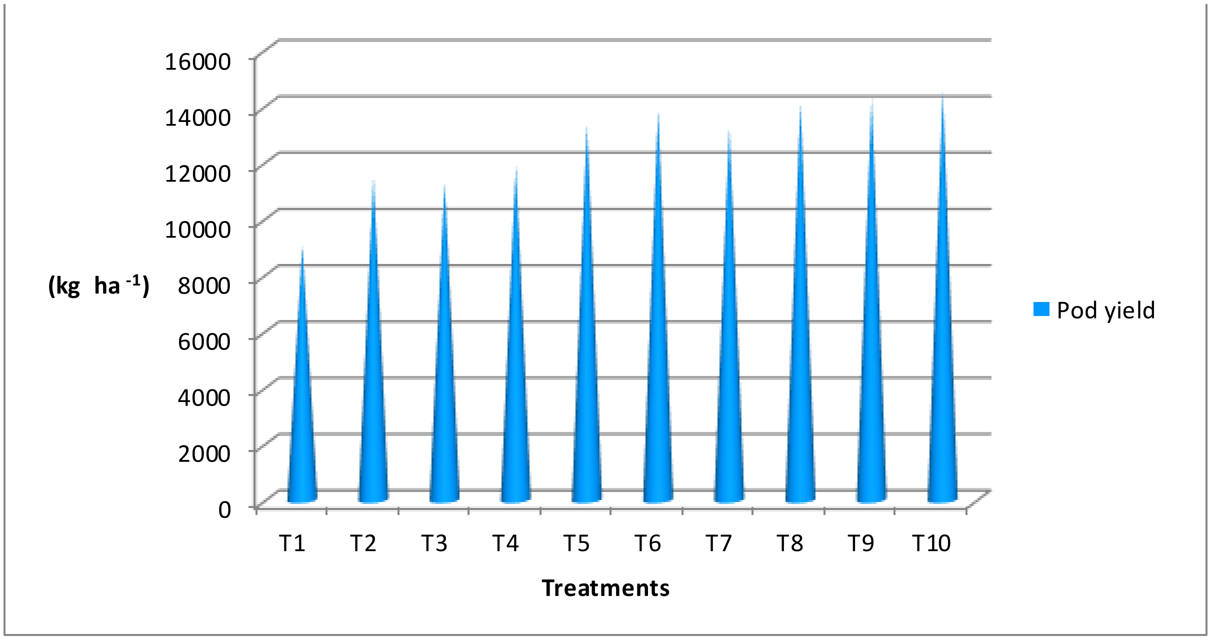
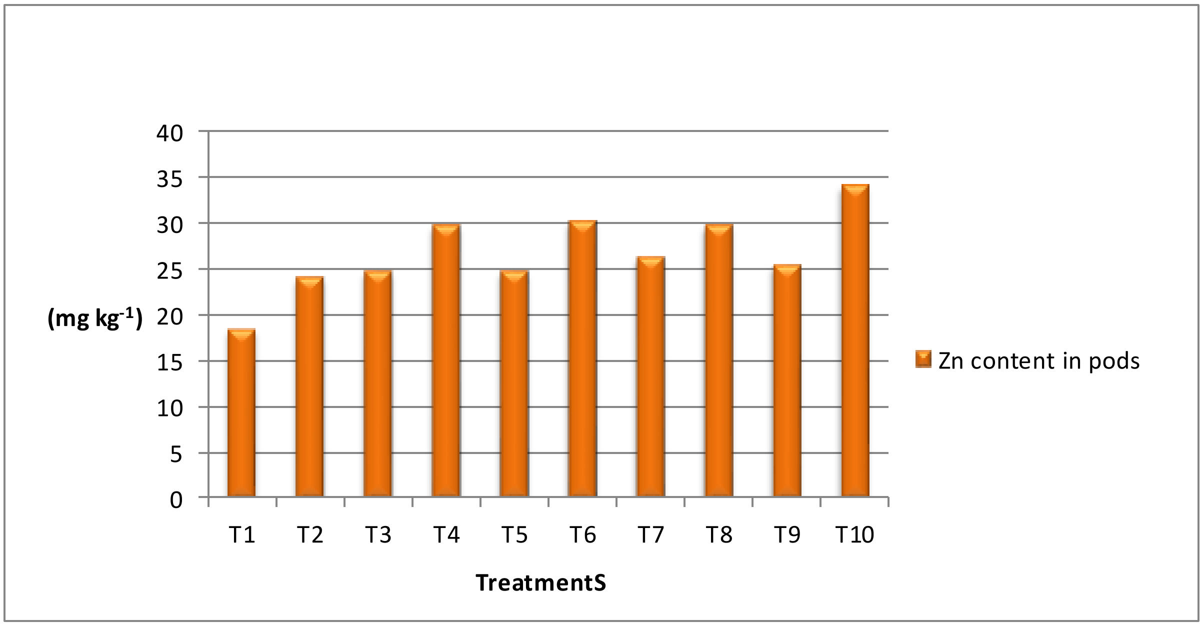


Fig 4. Effect of treatments on zinc content of pods of yard long bean



Although direct uptake of Zn by leaves is possible through foliar Zn application, the primary source of Zn for plants is through root uptake from soil. Plant root uptake of Zn is influenced by several root-related processes such as release of phytosiderophores, proton exudation, rhizosphere oxidation, mychorrizal colonization and root architecture (Graham and Rengel, 1993).Foliar Zn application has the conceptual advantage of avoiding soil chemistry problems that make Zn less available to crops and the crop performance was better at lower concentrations of Zn compared to higher concentrations when applied as foliar (Slaton*et al.*, 2005). Foliar application is the simplest way for increasing Zn density in maize (Grzebisz*et al.*, 2008) and green gram (Pathak and Pandey, 2010). It is the ideal technique for making quick correction of plant nutritional status and enhancement of Zn content in seeds for improved dietary intake by humans (Pathak *et al.*, 2012).

Among the plant characteristics, root characteristics alone were significantly influenced by agronomic biofortification of zinc. In general, all the root characteristics *viz.*, root length, volume and weight were higher for treatments receiving foliar application of Zn. Enhanced root growth has been reported under moderate Zn availability, since under such conditions, more energy is expended by the plant for root growth to facilitate nutrient extraction from more volume of soil (Chen *et al.*, 2009).Though not significant, application of Zn either to soil or foliar increased the vegetative growth and in response to that, the plants need more nutrients and hence better exhibition of root characteristics. Zinc application resulted in more vegetative growth for legumes in acid soils (Singh *et al.*, 1992;Khan *et al.*, 2000)and thereby produced more dry matter, derived mainly from increase in the number of pods (Brennen *et al.*, 2001;Valenciano*et al.*, 2007).The nodulation was also better for STBR based treatments receiving Zn either through soil or foliar, definitely due to balanced level of soil nutrients, especially that of N. Zn plays an important role in legume nodulation and nitrogen fixation (Edwards, 1971).

Fig 5. Effect of treatments on net returns of yard long bean per hectare

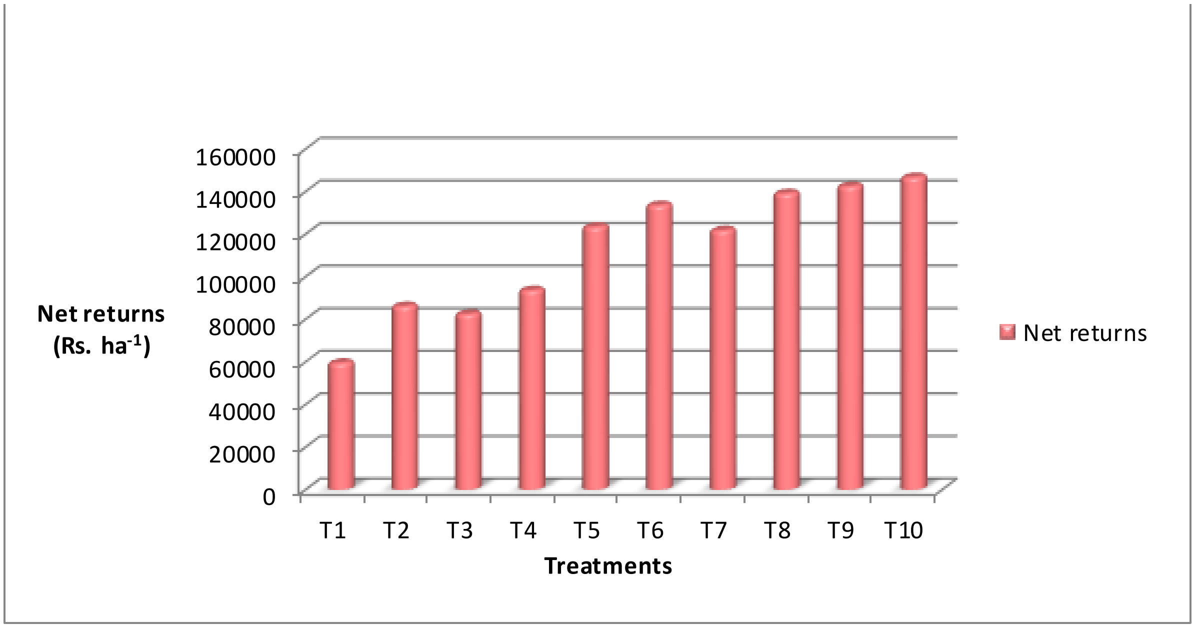
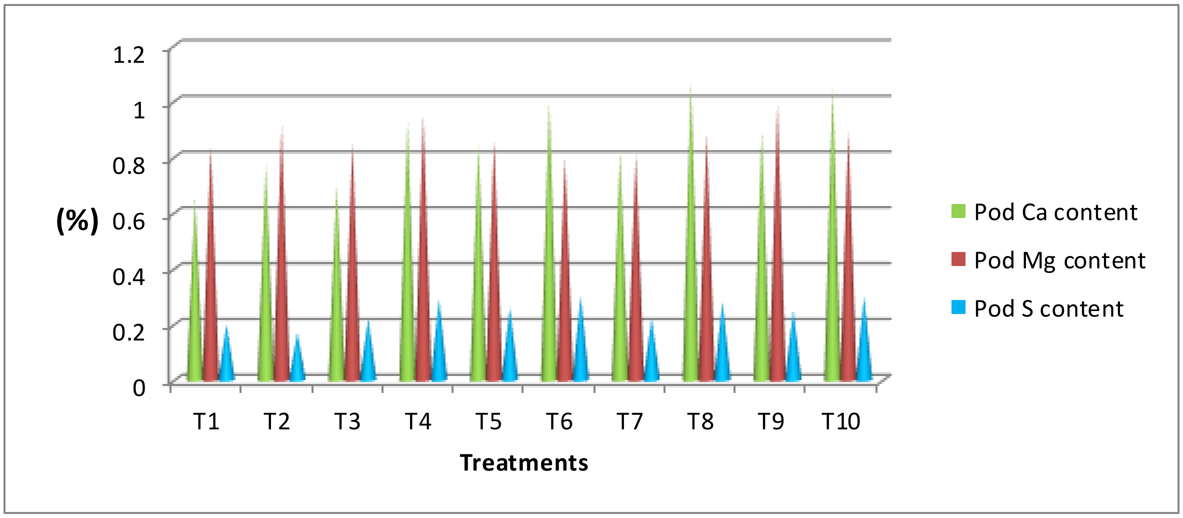


Fig 6. Effect of treatments on Ca, Mg, and S contents of yard long bean pods



5.2. EFFECT OF ZINC BIOFORTIFICATION (AGRONOMIC) ON NUTRIENT DENSITY OF YARD LONG BEAN

Among the primary nutrients, only phosphorus content was significantly influenced by agronomic biofortification of zinc. The antagonistic reaction between soil phosphorus and zinc has been reported by numerous workers and it is a well established fact.

The treatment that received Zn @ 5 kg ha^{-1} along with POP (T_4) recorded the highest P content of 0.63 per cent in the pods and was on par with treatment T_6 (POP + $ZnSO_4$ foliar @ 0.05 %). In general, the POP based treatments had more P content in pods. This was due the higher quantity of P fertilizers applied to soil which was already high in available P status. Even the highest quantity of Zn applied to soil had not adversely influenced the P content of pods (Table 12).The high content of available P in the experimental soil might have masked the antagonistic effect of Zn on P under such conditions. In soils with high available P content, soil application of Zn fertilizers had not shown any antagonistic effect on P uptake by the plants but high P reduces Zn availability due to formation of $Zn_3(PO_4)_2$(Singh *et al.*, 1986).

Among the secondary nutrients, Ca and S were significantly influenced by agronomic biofortification of zinc. Application of Zn either to soil or foliar, had enhanced the pod Ca content, exhibiting a positive relation with Zn, contrary to its negative effect as pointed out by several workers. Better soil availability of Ca due to application of CaO in soil might have helped better absorption of Ca by plant roots irrespective of the antagonistic effect with Zn. Zn shows antagonistic effect with Fe, Mn, Cu, Ca and Mg *etc.* which reduces Zn availability(Edwards, 1971).In case of S also, addition of Zn fertilizers had shown positive relation. Of course, it is proportional to the addition of $ZnSO_4$ (Fig. 6).

Among the micronutrients, Zn biofortification (agronomic) had significant effect on iron and zinc contents of pod only (Fig. 7). As regards to iron content, a

Fig 7. Effect of treatments on Fe and Zn contents of yard long bean pods

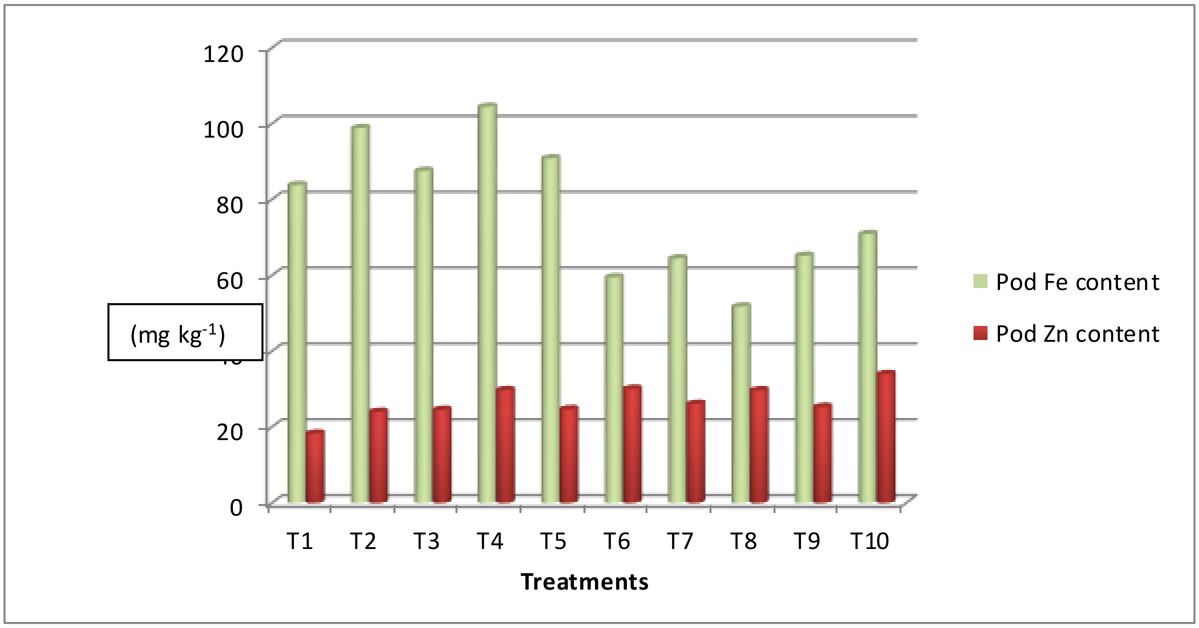
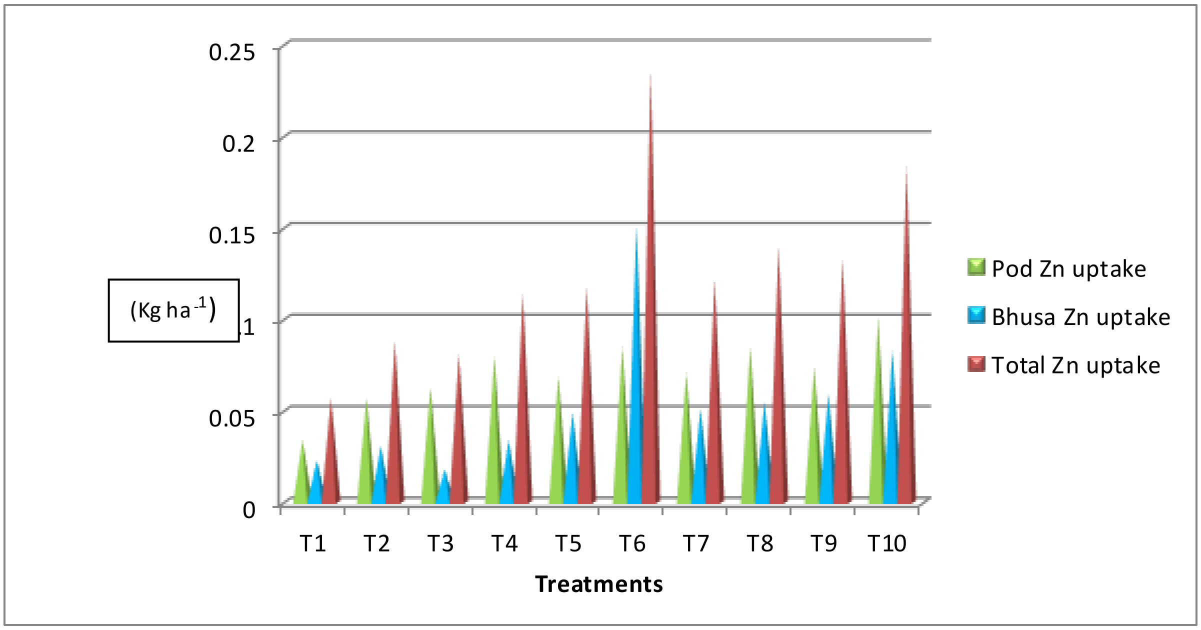


Fig 8. Effect of treatments on zinc uptake in yard long bean



static relationship with the quantity of zinc applied was not observed. In general, the high content of available iron in soil decreases the zinc extraction by the roots due to the competition for the same absorption sites. Zn application had an adverse effect on Fe concentration in plant tissue (Imtiaz *et al.*, 2003).But such a relation was not observed here, and the highest iron content was observed in the treatment receiving highest quantity of Zn along with POP. In general the soil of the experimental area is rich in available iron and this might have masked the antagonistic effect of Zn on Fe. But when the major nutrients were applied as per soil test data, the behaviour of zinc and iron showed the antagonistic relation and the treatment receiving highest quantity of Zn along with soil test based N,P and K recommendation showed the lowest iron content. The excess quantity of major nutrients especially that of phosphorus might be responsible for such a behaviour.

Zinc biofortification (agronomic) had significantly influenced the Zn content of the pod (Table14). The zinc content had increased from 18.33 mg kg^{-1} (T_1 - POP) to 34.01 mg kg^{-1}in T_{10} (STBR + $ZnSO_4$ foliar @ 0.05 %) due to zinc biofortification. An increase of 85.5 per cent of zinc in pod was observed due to foliar application of $ZnSO_4$ @ 0.05 per cent along with soil test based application of primary nutrients compared to that of POP recommendation. When Zn fertilizers are applied to foliage, it enters leaf apoplast and can be taken up by plant cells (Cakmak, 2009). When it is applied to soil, Zn uptake is limited by its phytoavailability and acquisition by roots (White and Broadley, 2009). This might be the reason for better Zn content under foliar application (Fig 8). The Zn content had increased under treatment receiving POP alone to soil test based recommendations without application of Zn fertilizers due to low phosphorous application in STBR (7.5 kg ha^{-1}) compared to POP (30 kg ha^{-1}). High P reduces Zn availability due to formation of $Zn_3(PO_4)_2$ (Singh *et al.*, 1986).However, soil or foliar application of Zn fertilizers increased Zn concentration in phloem fed tissues such as seeds and fruits (White and Broadley, 2009; White *et al.*, 2009;Bouis and Welch, 2010;Cakmak*et al.*, 2010).It was also evident from the data (Fig. 7) that, even soil test based application of primary nutrients alone could increase 31.1 per cent of pod zinc content. Better zinc removal under STBR treatment might be due the effect of balanced nutrient application which might have been able to counter act the antagonistic effect of excess fertilizers applied, especially that of phosphorus. Application of the highest doses of Zn either Zn @ 5 kg ha^{-1} or foliar spray of 0.05 per cent of $ZnSO_4$ at branching and flowering stage alone could enhance the zinc content of pods. The soil test based treatments also showed the same trend. For both recommendations, either POP or STBR, application of foliar spray of 0.05 per cent of $ZnSO_4$ at branching and flowering stages of yard long bean enhanced the zinc density of pods. It was also observed that, as the quantity of Zn applied increased it was positively reflected on the pod zinc content for both soil and foliar application. As expected, the Zn concentration was higher in the plants supplied with Zn fertilizer, than in the plants without Zn fertilizer (Singh *et al.*, 2005).

5.3. EFFECT OF ZINC BIOFORTIFICATION(AGRONOMIC) ON POD QUALITY AND ZINC BIOAVAILABILITY

Agronomic biofortification of zinc had slightly enhanced the crude protein content of pods. But the effect was not significant. The favourable role of zinc in the functioning of enzymes which are actively involved in protein synthesis might be responsible for this minor increase. Application of Zn fertilizers in combination with N fertilizers increased the Zn content of seeds and these two nutrients maintain a synergistic relationship (White and Broadley, 2011).

The antinutritional factor phytate content was significantly reduced by zinc application. The lowest value was observed for T_{10} (STBR + $ZnSO_4$ foliar @ 0.05 %). Foliar application was found to be better for reducing the phytate content compared to soil application (Table 15).Increasing seed concentration of Zn by soil and/or foliar application of zinc also brings several agronomic benefits for crop production. Applying zinc to plants grown under potentially zinc-deficient soils is effective in reducing uptake and accumulation of phosphorus (and thus phytate) in plants (Mirvat *et al.*, 2006;Cakmak, 2008).

Phytate:Zn ratio also followed the same trend since the above treatment has highest Zn content and lowest phytate content. Foliar application maintained lesser ratio compared to soil application of zinc. Hence, from the above treatment the estimated bioavailability of Zn will be more. Application of Zn reduced the accumulation of P as phytate and this agronomic side effect of zinc fertilization resulted in better bioavailability of zinc in the human digestive system(Mirvat *et al.*, 2006; Cakmak, 2008).

The inhibitory effect of phytate on the estimated bioavailability of zinc was determined by measuring their molar ratios. The high phytate content may impair the bioavailability of the minerals in the body. Phytic acid is an essential food component that has crucial negative impact on the absorption of Zn (Oberleas *et al.*, 1961). Zinc concentration in grains increased linearly with increasing Zn application rate in the soil and P concentration as phytate decreased directly with increased Zn levels (Singh *et al.*, 2005).

The Zinc bioavailability is significantly reduced due to a high intake of phytate which significantly affect the absorption of Zn in the body (Alloway, 2008). Phytate :zinc ratio of <5:1, 5-15:1and >15:1 are considered an index of bioavailability high, medium and low Zn (Graham, 1984). If phytate:zinc ratio exceeds 15:1 the absorption is low. As per the above criteria, the availability of Zn to human beings will be highest for the treatment receiving $T_{10}$with the application of $ZnSO_4$ foliar @ 0.05 per cent along with STBR.

5.4. EFFECT OF BIOFORTIFICATION (AGRONOMIC) OF ZINC ON NUTRIENT DENSITY OF YARD LONG BEAN BHUSA

The bhusa of the legume crops is valued because it can be used as a good animal feed as well as green leaf manure. Hence, the nutrient content of bhusa is discussed here. Zn application had significantly influenced the primary nutrient content of bhusa. In general, Zn application had enhanced the P and K contents compared to the non-treated ones. Application of Zn either to soil or foliar have facilitated better removal of nutrients from soil and their transportation and to plant parts and its accumulation in leaves, fruits and seeds (Cakmak, 2008;Samreen*et al.*, 2013).

Zn application had significantly influenced the secondary nutrient contents of bhusa. Ca and Mg did not show a definite pattern of variation with respect to Zn treatments. But S content of bhusa was higher for Zn applied treatments, mainly due to the contribution of sulphur from $ZnSO_4$ added to the crop.

Zn application had significantly influenced the micronutrient contents of bhusa, but did not show a definite pattern of variation in zinc content with respect to treatments due to unknown reasons. The Zn content in bhusa was highest for POP based treatment receiving Zn @2.5 kg ha^{-1} (T_3). The Zn uptake and its translocation to plant parts is a complicated phenomenon and the less translocation to pods might be responsible for higher Zn content in bhusa of the above treatment (Table 18).

5.5. EFFECT OF ZINC BIOFORTIFICATION(AGRONOMIC) ON ZINC UTILIZATION EFFICIENCY OF YARD LONG BEAN

Effect of zinc biofortification on zinc utilization efficiency in yard long bean was evaluated by computing Biofortification Recovery Efficiency (BRE_{Zn}) index(Shivay*et al.*, 2008).

Biofortification Recovery Efficiency (BRE_{Zn}) was used to compare Zn use efficiency of yard long bean under different modes and levels of Zn application. It is analogous to Recovery Efficiency of Zn, and is defined as the increase in Zn uptake in the edible part of the Zn treated plant over untreated plant part per unit quantity of applied Zn, expressed as percentage. BRE_{Zn} was highest for foliar application compared to soil application. N, P and K fertilizer application based on soil test values (STBR) were found to have better biofortification efficiency compared to POP based treatments (Fig. 9). This indicates the need for balanced

Fig 9. Effect of treatments on agronomic biofortification recovery efficiency of Zn

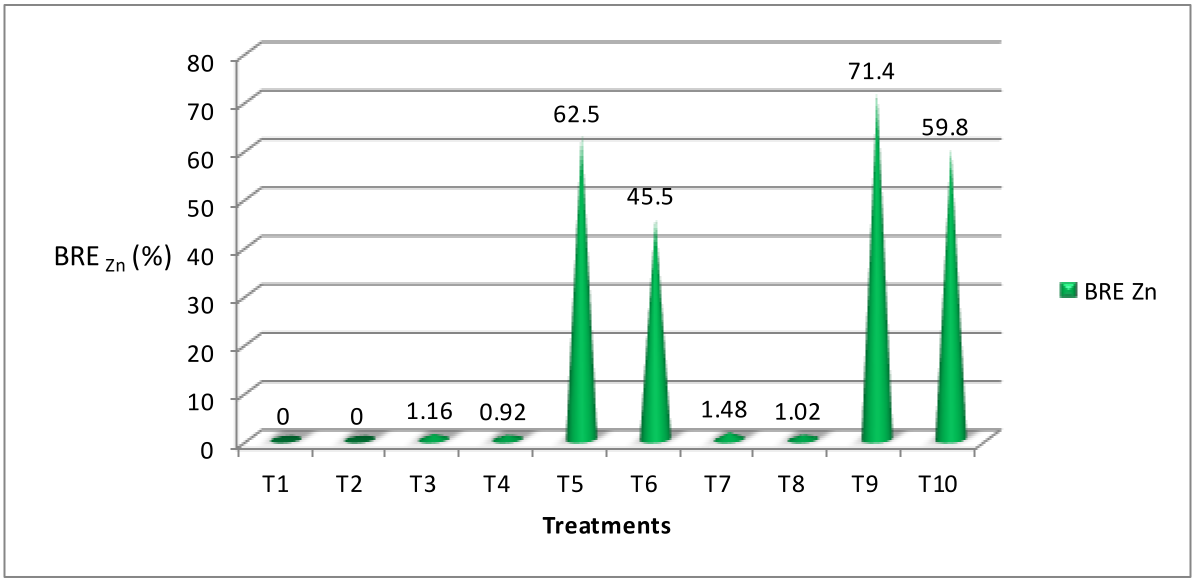
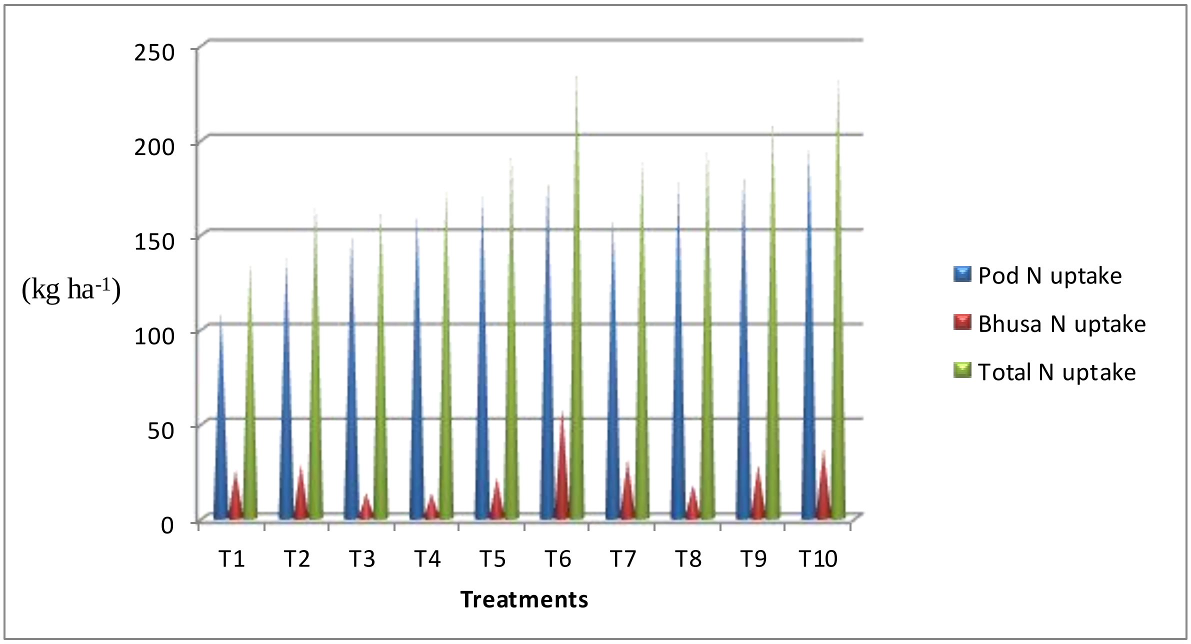


Fig 10. Effect of treatments on pod, bhusa and total uptake of N



nutrition for enhancing the Zn content of yard long bean pods. It was observed that, as the quantity of Zn applied increased either to soil or foliar, the biofortification recovery efficiency decreases. This is quite natural since better utilization efficiency was always associated with lower concentrations. Slaton *et al.* (2001) indicated that, the agronomic efficiency, recovery efficiency and partial factor productivity can usually be further improved by applying Zn at their active growing stages, when the plants can take up more Zn, rather than basally. The same fertilization techniques that improve recovery efficiency for soil-applied Zn fertilizers are expected to be more effective in biofortifying the edible parts with Zn. When the plant is able to access Zn at a time when it is able to take it up (*i.e.* later than basal), it is more likely to be taken up in higher quantities and therefore may possibly result in higher Zn concentration(White and Broadley, 2011).In case of foliar application, this might have happened (Impa *et al.*, 2012).Foliar Zn fertilization would be expected to affect grain Zn concentration only in genotypes with high remobilization capability (movement of Zn from leaves to grain)(Rozane *et al.*, 2008;Slaton *et al.*, 2001).

By supplying plants with Zn, either through soil application, foliar spray, or seed treatment, increased yield, quality and Zn use efficiency. In consideration of the important role of Zn in promoting and maintaining human health, more research is needed to determine the advantages of using the optimum level of Zn (Malakouti, 2008).

5.6. EFFECT OF ZINC BIOFORTIFICATION (AGRONOMIC) ON AVAILABILITY OF NUTRIENTS IN SOIL AND THEIR PHYTOAVAILABILITY

In general, an increase in soil pH, EC and organic carbon content of soil was found on application of Zn either to the soil or to the foliage. The above properties showed an increase towards the harvest compared to that of initial status in response to soil application of lime,$ZnSO_4$ and organic manure respectively. The root exudations and release of protons from roots and nutrient release from the decaying foliage might have favourably influenced the above soil properties. Suzuki *et al*. (2009) reported the favourable role of root exudations, release of protons and formation of fine roots on soil chemical properties. Yard long bean is a well known soil building crop and there by its cultivation will improve soil properties.

Available N, P and K content also showed an increase towards final harvest compared to initial soil status. Application of fertilizers resulted in an enhanced available nutrient status in soil at the time of final harvest so that the plant might not have suffered the dearth of nutrients. Uptake of N, P and K by yard long bean (Fig. 10, 11, 12) supported the above data and the phytoavailability is also high for Zn treated plants. Addition of FYM and the effect of decaying fallen leaves on soil organic carbon content are also responsible for the increased nutrient content in soil.

Application of lime and $ZnSO_4$ to soil increased the available Ca and S contents respectively. Soil application of $ZnSO_4$ raised the level of S in soil and foliar application also influenced the available S content. Mg content in soil at the time of final harvest in STBR treatments was low compared with POP treatments. This might be due to better Mg uptake by the STBR treatments as evidenced from the Fig. 13.

5.7. AVAILABLE MICRONUTRIENT CONTENT

The increase in available micronutrient status of soil at the time of harvest compared to initial status might be due to the influence of added organic matter and other nutrients to the crop. Organic manure is a good source of micronutrients including Zn and its constant use avoid development of Zn deficiency in soil (Biswas and Benbi, 1997).Contribution by the production of organic acids from the decomposing foliage and root secretions also might have favourably influenced their contents. In general, STBR based treatments maintained higher micronutrient contents.

Fig 11. Effect of treatments on pod, bhusa and total uptake of P

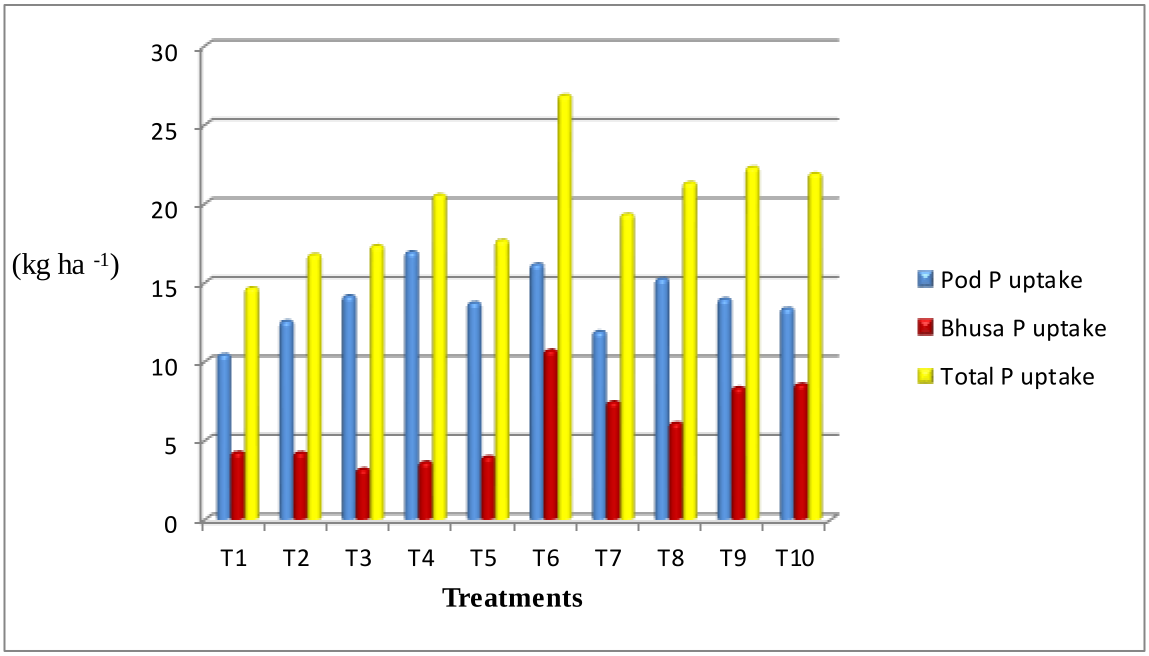


Fig 12. Effect of treatments on pod, bhusa and total uptake of K

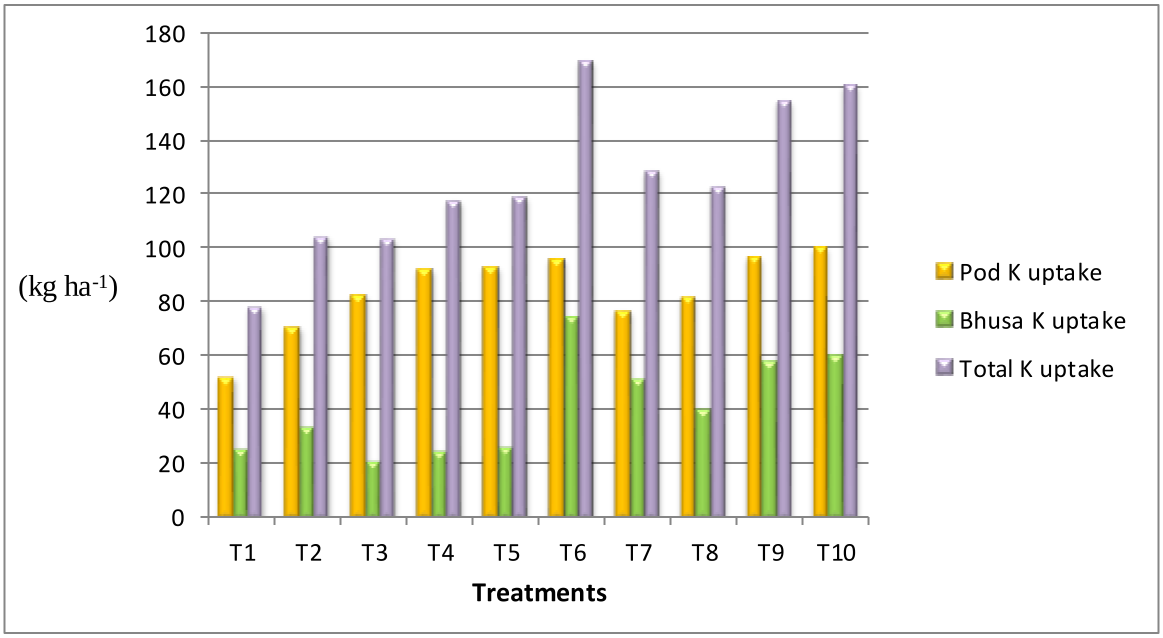
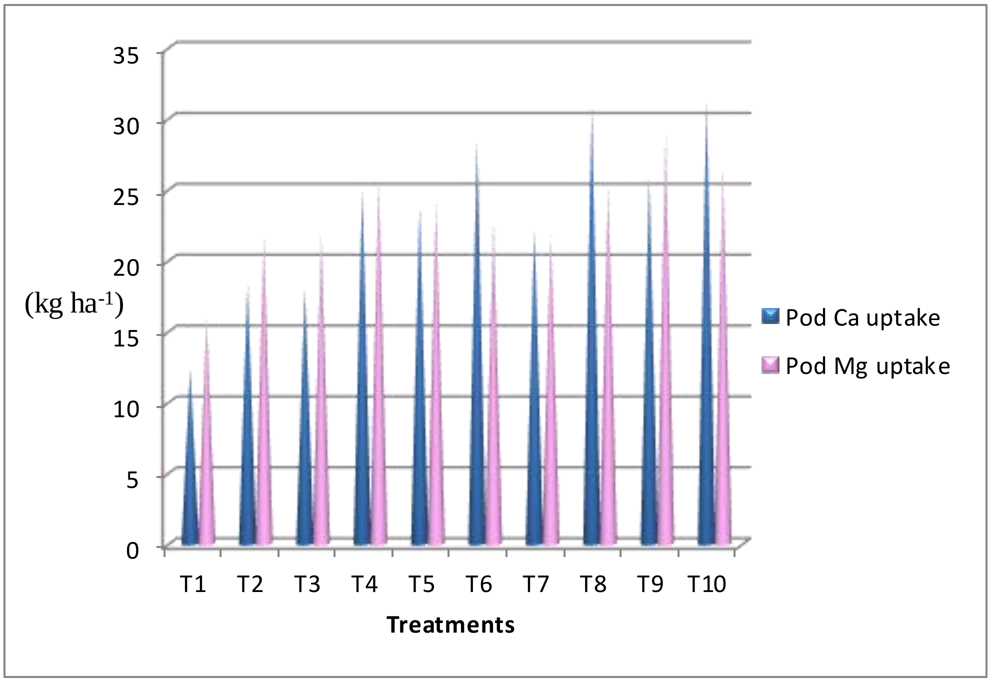


Fig 13. Effect of treatments on Ca and Mg uptake by pods



5.7.1. Available Zn

An increase in available Zn content was observed towards the end of the experiment compared to the pre sowing values especially for the treatments receiving Zn. This was in response to addition of FYM and Zn fertilizer. The treatments receiving Zn @ 5 kg ha^{-1}maintained highest quantity of available Zn in soil, evidently due to the addition of highest quantity of Zn to soil. As the quantity of Zn added decreases, phytoavailability of Zn alsodecreased (White and Broadley,2011). This clearly indicates the retention of Zn in soil for longer periods. Soil Zn occurs in three primary fractions: (i) water-soluble Zn (including Zn^{2+} and soluble organic fractions); (ii) adsorbed and exchangeable Zn in the colloidal fraction (associated with clay particles, humic compounds and Al and Fe hydroxides); and (iii) insoluble Zn complexes and minerals of which the first two fractions are easily available to the plants (Alloway, 1995; Barber, 1995).The foliar treatments also showed higher values for available Zn compared to the treatments receiving POP and STBR alone. This might due the contributions from the fallen droplets of the foliar spray and that from decaying defoliated leaves.

The treatment receiving soil test based N, P and K fertilizer recommendation along with foliar application of 0.05per cent $ZnSO_4$ at branching and flowering stages (T_{10}) was rated as the best treatment with regard to pod yield, Zn density in pods and B:C ratio for yard long bean var. Vellayani Jyothika. However, the treatment receiving POP based N, P and K recommendation with Zn as in the above case (T_6) was also found to be equally good in all the above aspects with slightly lesser values but statistically on par with the above.

The foliar application of $ZnSO_4$@ 0.05 per cent to yard long bean at branching and flowering stages was able to increase pod yield and zinc density and can reduce the phytate content of pods, both at POP and STBR based treatments of N, P and K fertilizers. Hence it can be concluded that whatever be the rate of application for N, P and K, *i.e.* either POP or Soil Test Based Recommendation, the foliar application of $ZnSO_4$@ 0.05 per cent at branching and flowering stages was found to be the best as regards to pod yield, Zn content and quality parameters compared to other methods and rate of zinc application.

Summary

6. SUMMARY

An experiment titled 'Zinc biofortification for enhancing yield and quality of yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt) in ferralitic soils' had been carried out at the Department of Soil Science and Agricultural Chemistry, College of Agriculture, Vellayani during October, 2013 to January, 2014. A field experiment was conducted in RBD to study the effect of zinc biofortification on zinc bioavailability, pod yield and quality of yard long bean in ferralitic soils. Levels of Zn @ 2.5 and 5.0 kg ha^{-1} to soil and foliar application @0.025 and 0.05 per cent $ZnSO_4$ along with POP and soil test based recommendations of N, P and K (STBR) were tested to study the effect of zinc biofortification on yard long bean. Thesalient results emerged from the study are summarized below.

- On evaluating the soil properties at the time of final harvest of yard long bean, it was observed that agronomic zinc biofortification had significantly influenced soil pH and organic carbon content. Soil pH was highest for the treatment receiving soil test based recommendation for N, P and K along with soil application of Zn @ 5 kg ha^{-1} (T_8). The highest value for soil organic carbon was recorded by T_6 (POP + $ZnSO_4$ foliar @ 0.05 %). Electrical conductivity of the soil was not significantly influenced by treatments.

- As regards to the effect of zinc biofortification (agronomic) on availability of primary nutrients, an increase was observed in their status by the time of harvest compared to initial values before sowing the crop, evidently due to the influence of added manures and fertilizers. Availability of N and K were highest for the treatment receiving foliar application of $ZnSO_4$ @ 0.05 per cent along with soil test based fertilizer recommendation (T_{10}). In the case of available P, treatment receiving POP based fertilizer recommendation along with foliar application of$ZnSO_4$ @ 0.05 per cent(T_6) recorded the highest value.

- On analyzing the effect of agronomic zinc biofortification on availability of secondary nutrients, it was observed that exchangeable Ca and available S were highest for treatment T_8 (STBR + Zn @ 5 kg ha^{-1}) and exchangeable Mg content in T_6 (POP + $ZnSO_4$ foliar @ 0.05 %).

- In the case of soil micronutrient availability, agronomic zinc biofortification had significantly influenced the Fe, Mn and Zn contents. Their availability have increased from that of initial values towards the end of the experiment, might be due to the influence of added organic manures and other nutrients to the crop. Contribution by the production of organic acids from the decomposing foliage and root secretions also might have favourably influenced their contents. In general, STBR based treatments maintained higher micronutrient contents.

- Available Fe was highest for the treatment T_{10} (STBR + $ZnSO_4$ foliar @ 0.05 %) and T_3 (POP + Zn @ 2.5 kg ha^{-1}) for Mn and T_4 (POP + Zn @ 5 kg ha^{-1}) for Zn. The treatments receiving Zn @ 5 kg ha^{-1} maintained highest quantity of available Zn in soil, evidently due to the addition of highest quantity of Zn to soil.

- The biometric characteristics of shoot *viz*., vine length and branches per plant were not significantly influenced by the treatments. Days to fifty per cent flowering was also not significantly influenced by the treatments.

- The treatments had significantly influenced the root characteristics. In general, all the root characteristics *viz*., root length, volume and weight were higher for treatments receiving foliar application of Zn. Enhanced root growth has been reported under moderate Zn availability, since under such conditions, more energy is expended by the plant for root growth to facilitate nutrient extraction from more volume of soil.

- The pod yield and yield attributes were significantly influenced by the treatments and the treatment T_{10} (STBR + $ZnSO_4$ foliar @ 0.05 %) recorded the highest values for pod yield and for most of the yield attributes which were on par with all the STBR based treatments receiving Zn. The total dry matter production also followed the same trend. This has indicated the need for soil test based fertilizer application. From the above results, it was clear that, foliar application of Zn is the best technique to address Zn nutrition.

- Among the primary nutrients, only phosphorus content in the pod was significantly influenced by Zn biofortification (agronomic). The antagonistic reaction between soil phosphorus and zinc has been reported by numerous workers and it is a well established fact. The treatment that received Zn @ 5 kg ha^{-1} along with POP (T_4) recorded the highest P content of 0.63 per cent in the pods and was on par with treatment T_6 (POP + $ZnSO_4$ foliar @ 0.05%). In general, the POP based treatments had more P content in pods. This was due the higher quantity of P fertilizers applied to soil which was already high in available P status.

- Among the secondary nutrients, Ca and S content of the pods were significantly influenced by Zn biofortification. Application of Zn either to soil or foliar, had enhanced the pod Ca content, exhibiting a positive relation with Zn, contrary to its negative effect as pointed out by several workers. The highest value for Ca and S contents were noticed with the treatment T_8 (STBR + Zn @ 5 kg ha^{-1}) and T_{10} (STBR + $ZnSO_4$ foliar @ 0.05 %) respectively.

- Among the micronutrients, Zn biofortification had significant effect on Fe and Zn contents of pod only. As regards to iron, a static relationship with the quantity of zinc applied was not observed. The highest values for Fe and Zn were noticed for the treatments T_4 (POP + Zn @ 5 kg ha^{-1}) and T_{10} (STBR + $ZnSO_4$ foliar @ 0.05 %) respectively. The zinc content had increased from 18.33 mg kg^{-1} (T_1 - POP) to 34.01 mg $kg^{-1}$$T_{10}$ (STBR + $ZnSO_4$ foliar @ 0.05 %) due to zinc biofortification. An increase of 85.5 per cent of zinc in pod was observed due to foliar application of $ZnSO_4$ @ 0.05 per cent along with soil test based application of primary nutrients compared to that of POP recommendation.

- For both recommendations *i.e.* either to the POP or STBR, application of foliar spray of 0.05 per cent of $ZnSO_4$ at branching and flowering stages of yard long bean enhanced the zinc density of pods. It was also observed that, as the quantity of Zn applied increased it was positively reflected on the pod zinc content for both soil and foliar application.

- The zinc biofortification had not significantly influenced crude protein content of yard long bean pods.

- The antinutritional factor phytate content was significantly reduced by zinc biofortification. The lowest value was observed for the treatment T_{10} (STBR + $ZnSO_4$ foliar @ 0.05 %). Foliar application was found to be better for reducing the phytate content compared to soil application.

- Phytate:Zn ratio was also lowest for the treatment T_{10} (STBR + $ZnSO_4$ foliar @ 0.05 %) since the above treatment had highest Zn content and lowest phytate content. The foliar application maintained lesser ratios compared to soil application of zinc. The bioavailability of Zn to human beings will be highest for the treatment receiving foliar application of $ZnSO_4$ @ 0.05 per cent along with STBR (T_{10}).

- N, P and K contents of bhusa of yard long bean were significantly influenced by the treatments. The highest value for N and K contents were recorded by the treatment T_6 (POP + $ZnSO_4$ foliar @ 0.05 %) and P content was recorded by the treatment T_8 (STBR + Zn @ 5 kg ha^{-1}).

- The treatment imparted significant effect on the Ca, Mg and S contents in bhusa. The highest value for bhusa Ca content was recorded for treatments $T_3$with the application of POP + Zn @ 2.5 kg $ha^{-1}$and T_5 (POP + $ZnSO_4$ foliar @ 0.025 %). Highest value for Mg and S contents in bhusa were recorded by the treatments T_6 (POP + $ZnSO_4$ foliar @ 0.05 %) and T_{10} (STBR + $ZnSO_4$ foliar @ 0.05 %) respectively. Ca and Mg did not show a definite pattern of variation with respect to Zn treatments. But S content of bhusa was higher for Zn applied treatments, mainly due to the contribution of sulphur from $ZnSO_4$ added to the crop.

- Fe, Mn, Zn and Cu contents of bhusa were significantly influenced by treatments. The highest values for Fe content in bhusa was recorded by T_{10} with the application of STBR + $ZnSO_4$ foliar @ 0.05 per cent. The Zn content was highest for POP based treatment receiving Zn @2.5 kg ha^{-1} (T_3). The Zn uptake and its translocation to plant parts is a complicated phenomenon and the less translocation to pods might be responsible for higher Zn content in bhusa of the above treatment.

- From the analysis of data on B:C ratio, T_{10}(STBR + $ZnSO_4$ foliar @ 0.05 %) registered the highest value and was on par with the treatment receiving POP based N, P and K recommendation with $ZnSO_4$ foliar @ 0.05 per cent (T_6).

CONCLUSION

The treatment receiving soil test based N, P and K fertilizer recommendation along with foliar application of 0.05per cent $ZnSO_4$ at branching and flowering stages (T_{10}) was rated as the best treatment as regards to pod yield, Zn density in pods and B:C ratio for yard long bean var. Vellayani Jyothika. However, the treatment receiving POP based N, P and K recommendation with Zn at the above rate as foliar (T_6) was also found to be equally effective in all aspects with slightly lesser values but statistically on par.

Foliar application of $ZnSO_4$@ 0.05 per cent to yard long bean at branching and flowering stages was able to increase pod yield and zinc density and can reduce the phytate content of pods, both at POP and STBR based treatments of N, P and K fertilizers. Hence it can be concluded that whatever be the rate of application for N, P and K, *i.e.* either POP or soil test based recommendation, the foliar application of $ZnSO_4$@ 0.05 per cent at branching and flowering stages was found to be the best as regards to pod yield, Zn content and quality compared to other methods and rate of zinc application.

FUTURE LINE OF WORK

Higher concentrations of zinc for foliar application have to be tested with respect to soil and plant critical levels of Zn for this crop. Simultaneously to address the micronutrient malnutrition, other micronutrients also have to be tested along with Zn to study their combined/ synergetic interaction effect on micronutrient density of edible plant parts.

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Zinc Biofortification for Enhancing Yield and Quality of Yard Long Bean (*Vigna unguiculata* subsp. *Sesquipedalis* (L.) Verdcourt) in Ferralitic Soils

by

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ABSTRACT

The study titled ‘Zinc biofortification for enhancing yield and quality of yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt) in ferralitic soils’ has been carried out at the Department of Soil Science and Agricultural Chemistry, College of Agriculture, Vellayani during 2013-14. The above investigation was under taken to study the effect of zinc biofortification through mineral fertilization on pod yield and quality of yard long bean and bioavailability of zinc.

The experiment was laid out in Instructional Farm, Vellayani in Randomized Block Design with ten treatments and three replications during October, 2013 to January, 2014. The treatments included two methods of Zn fertilizer application *viz*., soil application of Zn @ 2.5 kg ha^{-1} and 5 kg ha^{-1} and foliar application of $ZnSO_4$ @ 0.025 per cent and 0.05 per cent along with Package of Practices Recommendations of KAU (POP) and Soil Test Based Recommendations (STBR).

The treatment effect was statistically not significant for the biometric characteristics of yard long bean. Regarding the pod yield, foliar application of $ZnSO_4$ @ 0.05 per cent along with STBR (T_{10}) recorded the highest yield per plant. The same treatment showed the highest value for single pod weight plant^{-1} and number of seeds pod^{-1}as well as B:C ratio and total dry matter production. However, the harvest index was highest for the T_5 (POP + $ZnSO_4$ foliar @ 0.025 %) which on par with all other treatments except T_1 (POP).

The results of the soil analysis at the time of final harvest revealed a general increase in available nutrient content compared to that of initial status. The soil application of Zn @ 5 kg ha^{-1} along with POP (T_4) recorded the highest value for available Zn followed by the treatment with STBR + Zn @ 5 kg ha^{-1} (T_8).

The chemical analysis of pods revealed that, foliar application of $ZnSO_4$ @ 0.05 per cent along with STBR (T_{10}) recorded the highest concentration of Zn and lowest value for phytate. The increase in Zn content was 41.47 per cent over its counterpart

without Zn application. The same treatment (T_{10}) showed the lowest phytate P: Zn ratio indicating highest bioavailability of Zn. The performance of treatment T1 (POP) was comparatively poor in these aspects. The Zn content of bhusa showed a different pattern with POP based treatments receiving foliar application of Zn showed highest values.

Evaluating the performance on methods and levels of Zn application for biofortification in yard long bean var. Vellayani Jyothika, it was observed that, the treatment receiving STBR based N, P and K fertilizers along with 0.05 per cent $ZnSO_4$ foliar spray at branching and flowering stages (T_{10}) recorded the highest pod yield, B:C ratio and Zn content with lowest values for phytate and phytate: Zn ratio. Considering the lowest nutrient input addition and more benefit, the same was found to be the best treatment. However, the treatment receiving POP based N, P and K recommendation with Zn as foliar treatment at 0.05% (T_6) was also found to be equally effective in all aspects with slightly lesser values but statistically on par with the above.

The foliar application of $ZnSO_4$ @ 0.05 per cent to yard long bean at branching and flowering stages was able to increase pod yield and zinc density and can reduce the phytate content of pods, both at POP and STBR based treatments of N, P and K fertilizers. Hence it can be concluded that whatever be the rate of application for N, P and K, *i.e.* either POP or soil test based recommendation, the foliar application of $ZnSO_4$ @ 0.05 per cent at branching and flowering stages was found to be the best as regards to pod yield, Zn content and quality compared to other methods and rate of zinc application.

Appendices

APPENDIX I

Weather parameters at weekly intervals at Vellayani during October, 2013 – January, 2014

SI No	Standard Week	Dates	Temp. Max (^{0}C)	Temp. Min (^{0}C)	RF (mm)	RH (%)
	2013					
1	40	01.10.13 – 07.10.13	30.5	22.6	6.7	94.0
2	41	08.10.13 – 14.10.13	30.6	23.3	5.7	91.4
3	42	15.10.13 – 21.10.13	30.7	23.7	16.5	92.1
4	43	22.10.13 – 28.10.13	30.7	23	18.1	95.0
5	44	29.10.13 – 04.11.13	30.7	23.6	10.3	93.9
6	45	05.11.13 – 11.11.13	30.9	23.7	1.6	97.0
7	46	12.11.13 – 18.11.13	30.3	23.4	46.3	97.7
8	47	19.11.13 – 25.11.13	30.6	23.7	14.5	97.3
9	48	26.11.13 – 02.12.13	30.8	23	16.6	97.3
10	49	03.12.13 – 09.12.13	30.9	22.8	1.4	98.6
11	50	10.12.13 – 16.12.13	30.3	22.6	26.0	96.7
12	51	17.12.13 – 23.12.13	31.2	21.7	47.0	97.7
13	52	24.12.13 – 31.12.13	31	20.2	-	96.6
	2014					
14	1	01.01.14 – 07.01.14	30.9	21.5	14.0	94.9
15	2	08.01.14 – 14.01.14	29.0	22.3	-	94.4
16	3	15.01.14– 21.01.14	31.0	21.8	-	94.1
18	4	22.01.14 –28.01.14	31.3	20.7	0.5	90.4
19	5	29.01.14 – 31.01.14	-	-	-	-