

**IRON AND ZINC FORTIFICATION IN
AMARANTHUS (*Amaranthus tricolor*) THROUGH
BIOAUGMENTATION**

by

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THESIS

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DECLARATION

I hereby declare that this thesis entitled “**Iron and Zinc fortification in amaranthus (*Amaranthus tricolor*) through bioaugmentation**” is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

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LIST OF ABBREVIATIONS

%	per cent
µg	microgram
µm ²	micro square metre
B: C	Benefit: Cost
B	Boron
Ca	Calcium
CD	Critical Difference
CEC	Cation Exchange Capacity
cm	centimeter
DAS	Days After Sowing
DOI	Days of Incubation
<i>et al</i>	And others
Fe	Iron
Fig.	Figure
FYM	Farm Yard Manure
g	gram
g plant ⁻¹	gram per plant
g plot ⁻¹	gram per plot
h	hour
ha ⁻¹	Per hectare
K	Potassium
KAU	Kerala Agricultural University
kg	Kilogram
kg plant ⁻¹	kilogram per plant
kg ha ⁻¹	kilogram per hectare
Mg	Magnesium
m	metre
mg	milligram

min	minutes
ml	millilitre
MOP	Murate of potash
MSL	Mean Sea Level
mm	millimeter
N	Nitrogen
nm	nanometer
no.	number
P	Phosphorus
P ₂ O ₅	Phosphate
POP	Package of Practices
Ppm	parts per million
RP	Rock phosphate
S	Sulphur
s	seconds
SOC	Soil Organic Carbo
t	tonnes
Var.	variety
<i>viz.</i>	namely
Zn	Zinc

Introduction

1.INTRODUCTION

Producing nutritious and safe foods sufficiently and sustainably is the ultimate goal of modern agriculture. Green revolution has made self sufficiency in food grain production, but the increased production and productivity has greatly enhanced the demand on soil nutrition. The higher crop production per unit area has resulted in greater depletion of soil available nutrients. The traditional fertilization practices were designed to meet the need for only the major nutrients. Eventually micronutrient deficiency has become a limiting factor for crop production. Micronutrient deficiencies in soils not only limit crop production, but they also have negative effects on human nutrition and health. Among the micronutrients zinc and iron are the most dominant element showing human malnutrition. The WHO has estimated that over 3 billion people in the world suffer from micronutrient malnutrition and that about 2 billion of these have Fe deficiency. Hence enhancing the concentration of the prime microelements like Fe and Zn in crop produces has become an urgent task (Lombi *et al.*, 2009., Zhao and McGrath, 2009)

Mineral nutrition can be addressed through mineral supplementation, biofortification, food fortification, dietary diversification, etc. Mineral supplementation, food fortification and dietary diversification were not so successful since a major section of society could not access to it. 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 any approach which could increase root growth and result in a high transfer of minerals from soil to plants either genetically or agronomically.

All the nutrients that humans consume are derived from the soil–plant system, and a new approach to tackling the problem of micronutrient deficiencies

in the diet has consisted of increasing the density and bioavailability of micronutrients in edible parts of plants through biofortification. This approach has proved to be sustainable, can be implemented at a relatively low cost, is highly efficacious and has a large coverage, especially in the poorer regions of the world.

Micronutrient biofortification in the soil–plant system can be defined as increasing the density and bioavailability of micronutrients in the edible parts of crop plants through both plant biotechnology and nutritional management of the soil–plant system with the aim of improving human nutrition and health. Biofortification is an ecofriendly tool for enhancing the crop yield as well as the mineral concentration of edible portions. The current strategies for biofortification includes mineral fertilization, bioaugmentation, conventional breeding and transgenic approaches. Bioaugmentation and mineral fertilization are generally resorted to attain immediate benefits, while breeding and transgenic approaches are of long term interest (White and Broadley, 2009).

Bioaugmentation is a method where suitable microorganisms are introduced to a site for multiplication and growth which are capable of assisting plants for nutrient uptake. Some of the crop plants possess hyper accumulation capacity for certain metals. This property can be enhanced by bioaugmentation. *Amaranthus* sp is such a crop that posses hyperaccumulation capacity for Fe and Zn (Reeves and Baker, 2000) which is the most popular leafy vegetable in Kerala.

The plants differ in their micronutrient concentration in edible parts depending on crop species and variety. The plants of both Brassicaceae and Amaranthaceae possess hyperaccumulation capacity and are generally employed for detoxification of moderately contaminated areas (Reeves and Baker, 2000). The same property of crop plants are exploited here for enriching them with the micronutrients by biological assistance (biofortification) under graded doses of micronutrients.

Leafy vegetables are one of the major sources for the micronutrient nutrition for humans and amaranthus is the most popular leafy vegetable in Kerala. Hence, it is ideal to identify suitable methods for the enrichment of amaranthus with nutritionally important micronutrients like Fe and Zn so that it can contribute towards nutritional security of the nation. The present study attempts to identify most appropriate ecofriendly technology for biofortifying the amaranth foliage with two most deficient micronutrients viz., Fe and Zn with the following objective.

To study the effect of bioaugmentation of soil with microbial additives for nutrient enrichment in amaranthus particularly for Fe and Zn.

Review of literature

2. REVIEW OF LITERATURE

2.1 Nutritional quality and need for high nutrient density in edible crops

Producing nutritious and safe foods sufficiently and sustainably is the ultimate goal of modern agriculture. Past efforts have focused mainly on increasing crop yield but enhancing the concentration of mineral micronutrients has become an urgent task because the world population is facing the micronutrient hunger, especially for the prime microelements like Fe and Zn (Lombi *et al.*, 2009., Zhao and McGrath, 2009).

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 micro-nutrients from soil. Crop production in these soils has remained low due to inherent low soil fertility and aberrant weather conditions. 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. Micronutrient malnutrition is affecting a large segment of population mainly women, infants and children in the resource poor families of the country (Prasad, 2010).

Micronutrient deficiencies in humans mainly result from low concentrations and low availabilities of micronutrients in daily diets. Micronutrient malnutrition is most prevalent in developing countries, with deficiencies of Fe, Zn and vitamin A being among the ten leading causes of illness and disease in low-income countries. More than two billion people suffer from 'hidden hunger' a term used to describe malnutrition of micronutrients (WHO, 2002).

Past attempts to solve these dietary deficiencies have included supplementation products and the fortification of food with micronutrients. However, this approach to addressing micronutrient malnutrition has not been ideal due to its high cost and low coverage, even though such programs have been effective in treating severely deficient people. All of the nutrients that humans consume are derived from the soil–plant system, and a new approach to tackling the problem of micronutrient deficiencies in the diet has consisted of increasing the density and bioavailability of micronutrients in edible parts of plants through biofortification. This approach has proved to be sustainable, can be implemented at a relatively low cost, is highly efficacious and has a large coverage, especially in the poorer regions of the world (Prasad, 2010).

World over screening of hyper accumulators or supra cultivars having high micronutrient density in seed has been found of much help in mobilization micronutrients from seed to seed (soil to root, root to shoot, shoot to seed) by solubilizing soil native Fe, Mn, Zn and Cu to overcome malnutrition (Welch,1999). Thus, identification and cultivation of micronutrient dense crop varieties, understanding the physiological mechanisms controlling variable efficiency for mobilizing higher amounts of micronutrients and their uptake, agronomic interventions for efficient biofortification into seeds and enhancing bioavailability and reducing antinutrients are of topical research interest in minimizing Zn, Fe, Cu and Mn malnutrition in human and animals.

Zn deficiency diseases are more common in children and its deficiency is very much responsible for weak immune system and retarded mental growth (Hambridge, 2000., WHO, 2005). It affects on an average, one-third of the world's population, ranging from 4 to 73 % in different countries (Hotz and Brown, 2004). Zn deficiency expressed as retarded growth and weak immunity in children is quiet common and is next to iron anemia (Alloway, 2008). Zn is the second most micronutrient element showing hidden hunger. The Zn deficiency has assumed bigger dimension, affecting nearly half of the world population (Sharma, 2008).

The World Health Organization (WHO) has estimated that nearly 3.7 billion people are iron (Fe) deficient, with 2 billion of these so severely deficient in Fe that they can be described as being anemic. National Diet and Nutrition Survey reported that mean daily intakes of Fe from food sources were less than the lower recommended nutrient intake in 25% of adult women, and of Zn in 4% of all adults (Singh, 2009).

2.2 Mineral elements required by humans

Minerals are inorganic substances, present in body tissues and fluids and their presence is necessary for the maintenance of certain physicochemical processes which are essential to life. Although they yield no energy, they have important roles to play in many activities in the body (Malhotra, 1998).

Humans require at least 22 mineral elements for their wellbeing. These can be supplied by an appropriate diet (Welch and Graham, 2004., Graham *et al.*, 2007). Trace elements are required in small concentrations as essential components of biological enzyme systems or of structural portions of biologically active constituents (Arinola *et al.*, 2008).

It is estimated that over 60% of the world's 6 billion people are iron (Fe) deficient, over 30% are zinc (Zn) deficient, 30% are iodine (I) deficient and 15% are selenium (Se) deficient (White and Broadley, 2009)

The micronutrient malnutrition is attributed to crop production in areas with low mineral phytoavailability and/or consumption of (staple) crops with inherently low tissue mineral concentrations, compounded by a lack of fish or animal products in the diet (Welch and Graham, 2005., Grusak and Cakmak, 2005., Graham *et al.*, 2007). Currently, mineral malnutrition is considered to be among the most serious global challenges to humankind (Copenhagen Consensus, 2004).

Iron is involved in synthesis and packaging of neurotransmitters, their uptake and degradation into other iron-containing proteins which may directly or indirectly alter brain function (Beard, 2001). Fe is required for making haemoglobin and it is a pro-oxidant which is also needed by microorganisms for proliferation (Galan *et al.*, 2005).

Zn is a co factor for many enzymes and it is also involved in synthesis of some hormones and proteins. It is also required for transport of vitamin A, wound healing, sperm production and foetal development. Therefore adequate Zn uptake is essential for normal healthy growth and development (Bouis *et al.*, 2003).

2.3 Phytoavailability of iron and zinc

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 a 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 and Broadley, 2009).

Mineral elements can be present in the soil as free ions, or ions adsorbed onto 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, and activity of microbes, soil structure, organic matter and water content. Indeed, although high concentrations of Fe and Zn occur in many soils, the phytoavailability of these mineral elements is often restricted by soil properties which predetermine both genetic and agricultural strategies for their effective utilization. (Shuman, 1998., Frossard *et al.*, 2000).

Concentrations of Fe and Zn in the rhizosphere solution are determined by soil-specific precipitation, complexation and adsorption reactions, and high pH is often the major factor limiting the phytoavailability of these elements. 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 and Cu^{2+} concentrations range from 10^{-9} 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 (Abadía *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 biofortification is effective (White and Broadley, 2009).

2.4 Uptake, distribution and accumulation of iron and zinc by plants

2.4.1 Iron

The mineral concentration in a plant part depends on several factors like soil availability, uptake ability of plant roots, translocation and accumulation ability etc. To increase mineral concentrations in edible tissues, without loss of yield, there must be increased uptake by roots or leaves, effective redistribution

within the plant to the edible portion, and accumulation in edible tissues in a nontoxic form (Welch and Graham, 2005).

Roots of plants that belong to non graminaceous species, acidify the rhizosphere and release organic acids and phenolic compounds to increase Fe^{3+} concentrations in the soil solution. These compounds chelate Fe^{3+} , which is subsequently reduced to Fe^{2+} by ferric reductases in the plasma membrane of root epidermal cells, which are encoded by members of the ferric reductase oxidase (*FRO*) gene family (Wu *et al.*, 2005., Mukherjee *et al.*, 2006). Members of the zinc-regulated transporter (*ZRT*)-, iron-regulated transporter (*IRT*)-like protein (*ZIP*) family, such as *AtIRT1* in *Arabidopsis*, then mediate Fe^{2+} influx to root cells (Vert *et al.*, 2002., Ishimaru *et al.*, 2006).

Proteins that load Fe into the xylem have not been identified yet, but they are believed to transport Fe^{2+} . Within the xylem, Fe is transported as Fe^{3+} -citrate (Welch, 1995., von Wirén *et al.*, 1999., Abadía *et al.*, 2002., Mukherjee *et al.*, 2006). *FRD3*, a member of the multidrug and toxin efflux (*MATE*) family present in the root pericycle, is important for Fe transport from root to shoot and appears to be involved in loading citrate into the xylem in *Arabidopsis* (Durrett *et al.*, 2007., Haydon and Cobbett, 2007., Puig *et al.*, 2007). Presumably, members of the *ZIP* family are responsible for Fe^{2+} uptake by shoot cells. Members of the natural resistance-associated macrophage protein (*NRAMP*) family are not thought to be responsible for Fe uptake from the soil, but have been implicated in Fe homeostasis within plant cells. In particular, *NRAMP3* and *NRAMP4* are thought to facilitate Fe^{2+} release from the vacuole (Thomine *et al.*, 2003., Gross *et al.*, 2003., Hall and Williams, 2003., Lanquar *et al.*, 2005., Grotz and Guerinot, 2006., Puig *et al.*, 2007), opposing the activity of the vacuolar iron transporter 1 (*VIT1*) protein which catalyses Fe influx to the vacuole (Kim *et al.*, 2006). In leaves of plants overloaded with Fe, and in some seeds, Fe can accumulate as Fe-chelates in the vacuole (Pich *et al.*, 2001., Lanquar *et al.*, 2005., Kim *et al.*, 2006). However, under most environmental conditions, the majority of cellular Fe is

located in the plastid, where it is associated with the Fe-storage protein ferritin (Briat *et al.*, 1999., Petit *et al.*, 2001).

2.4.2 Zinc

It is commonly assumed that mainly 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 (White *et al.*, 2002., 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 (Pence *et al.*, 2000., Assunção *et al.*, 2001., López-Millán *et al.*, 2004., Colangelo and Guerinot, 2006., 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).

It is thought that 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 nicotianamine (Welch, 1995., von Wirén *et al.*, 1999., White *et al.*, 2002., 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 (Welch, 1995., Haslett *et al.*, 2001).

2.5 Variation in tissue concentrations of mineral elements among plant species

Tissue concentrations of mineral elements can differ markedly between plant species growing in the same environment. Relatively little variation in concentration of shoot Fe and Zn occurs at the ordinal level or above. Indeed, some plant species ‘hyperaccumulate’ Zn and Fe can contain leaf concentrations of these elements several orders of magnitude greater than those in closely-related

species growing on the same substrate (Reeves and Baker, 2000., Broadley *et al.*, 2001., 2007., White *et al.*, 2007). In addition to the phenomenon of hyperaccumulation, there are general differences among angiosperm orders in their shoot Zn concentrations (Broadley *et al.*, 2001, 2007; White *et al.*, 2004, 2007). Broadley *et al.* (2007) reported that the highest shoot Zn concentrations occurred in the Amaranthaceae.

In addition to the phylogenetic heritage of different plant species affecting their ability to accumulate essential mineral elements, the concentrations of mineral elements in edible tissues are also influenced by their mobility within the plant. Since Fe and Zn has little phloem mobility, phloem-fed tissues such as fruits, seeds and tubers are often poor sources of Fe and Zn, whilst leafy vegetables are rich sources of these elements (Welch, 1999., White and Broadley, 2005).

2.6 Iron and zinc status of soil

2.6.1 India

Status of total and available iron content of Indian soils is high, ranging from 4000 to 273000 mg kg⁻¹ and that of available iron 0.36 to 174 mg kg⁻¹ soil. Acid and laterite soils had still high available iron content. Even then wide spread iron deficiency anemia has been reported widely from India, indicating the influence of other factors regulating the iron absorption and its bioavailability (Singh, 2009).

The lack of micronutrients such as Fe and Zn is a widespread nutritional and health problem in developing countries. Absence of sufficient quantities of the above nutrients in soil or their unavailability due to some antagonistic factors/ transformation to less soluble forms are the major reasons for their low contents 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).

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 2960 mg kg⁻¹ and 0.1 to 24.6mg kg⁻¹, respectively with an average deficiency of 12 to 87 %. 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).

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 % found in the year 1970 to 63 % 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 buildup of zinc and decline in deficiency. It is estimated that to correct zinc and iron deficiency, India need 324 and 130 t ha⁻¹ yr⁻¹ of fertilizer zinc and iron respectively by the year 2025 (Singh, 2009).

2.6.2 Kerala

About 34 % of Kerala Soils are deficient in Zn, 31% in Cu and less than 1% in Fe and no deficiency for Mn (Singh, 2009). He also reported that Zn

deficiency is expected to increase from 49 to 63 % by 2025. Laterite soils of Kerala sufficient in available iron except 8.9% deficiency soils of Thiruvananthapuram. Deficiency of zinc ranges from 2.3 to 50 % in ten districts of Kerala (Mathew and Aparna, 2012).

2.7 Historical trends in the concentrations of iron and zinc in edible tissues

Analyses of historical data have suggested that the concentrations of Fe in the dry matter of horticultural produce has declined significantly since the mid twentieth century (White and Broadley, 2005), and that the Zn concentration in the dry matter of cereal products, vegetables and fruits have declined over the last 25 years (Ekholm *et al.*, 2007). White and Broadley (2005) suggested that this phenomenon might be a consequence of the adoption of modern varieties and/or agronomic practices.

Recent research has focused on the effects of increased yield, whether achieved by agronomic or genetic improvement, on the concentrations of mineral elements in produce. It has long been appreciated that environmental factors accelerating plant growth rates, such as higher temperatures, light intensity, CO₂ concentrations and irrigation, often result in reduced concentrations of mineral elements in plant tissues (Loladze, 2002), and a number of recent studies have shown that the concentrations of various mineral elements are lower in higher yielding genotypes. In leafy vegetables, Farnham *et al.* (2000) found a strong negative relationship between Ca and Mg concentrations and head weight among 27 broccoli (*Brassica oleracea* var. *italica*) genotypes. Graham *et al.*, (2001) reported that there are no significant relationships between seed Fe and Zn concentrations and yield in common bean (*Phaseolus vulgaris*). These observations suggest that the biofortification of crops with mineral elements can be achieved without compromising yield.

2.8 Bioavailability of iron and zinc

The research on bioavailability of iron and zinc was mainly limited to grain crops. Pulses and millets are rich source of micronutrients. The bioavailability of iron 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 bioavailability of iron. Bioavailability of zinc was only 50 % from different types food consumed. It has been reported that consumption of zinc fertilized rice grains reduced the prevalence of zinc deficiency in Thailand (Welch and Graham, 2005)

2.9 Methods for enhancing nutrient density (Fe and Zn) in edible portion

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. Biofortification or fitting plants to the soil is a good approach, rather than ameliorating soil to support normal plant growth. Zn content in grains of rice, maize and wheat was found to increase 2 to 5 times by zinc fortification (Harvestplus, 2007). Pulses and vegetable are better sources of zinc, their enrichment with iron and zinc will be more helpful in addressing iron and zinc malnutrition (Sing, 2009).

2.10 Agronomic biofortification strategies for iron and zinc

The target concentration for a specific mineral element in the edible portion of a biofortified crop will be determined by the amount of that element required in the human diet, the deficit of the mineral element in the diet of an affected population, the number of crops that will be biofortified, the bioavailability of the mineral element following processing and cooking, and the contributions of each biofortified crop to the diet of the affected population. Thus, strategies for addressing mineral malnutrition through biofortification and, therefore, target concentrations of mineral elements in edible produce will depend greatly upon local diet and culinary customs. When more than one mineral element is lacking in the diet, biofortification strategies must deliver all of them to the affected population. However, when a mineral or vitamin deficiency is induced by the lack of another mineral or vitamin, as occurs among Fe, Zn and provitamin A carotenoid deficiencies (Hess *et al.*, 2005) and between Se and I deficiencies (Lyons *et al.*, 2004), it can be corrected by the biofortification of edible crops with the appropriate mineral and/or vitamin that is lacking in the diet.

In developing countries, it has been suggested that biofortification strategies should focus on the staple foods that dominate people's diets (Bouis, 2000; Pfeiffer and McClafferty, 2007). The argument is simple: if the concentrations of mineral elements in staple foods can be increased, then the delivery of mineral elements to vulnerable populations can be increased *pro rata* to their contribution to the diet, without a change in behaviour (Bouis, 1999., Bouis *et al.*, 2000.,Graham *et al.*, 2007).

2.11 Biofortification through mineral supplementation

Mineral nutrition can be addressed through mineral supplementation, biofortification, food fortification, dietary diversification etc. Mineral supplementation, food fortification and dietary diversification were not so

successful since a major section of society could not access to it. 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 staple crops through any approach which could increase root growth and result in a high transfer of minerals from soil to plants.

Biofortification is an ecofriendly tool for enhancing the crop yield as well as the mineral concentration of edible portions. The current strategies for biofortification includes mineral fertilization, bioaugmentation, conventional breeding and transgenic approaches (White and Broadley, 2009).

The plants differ in their micronutrient concentration in edible parts depending on crop species and variety. The plants of both Brassicaceae and Amaranthaceae possess hyperaccumulation capacity and are generally employed for detoxification of moderately contaminated areas (Reeves and Baker, 2000). The same property of crop plants is exploited here for enriching them with the micronutrients by biological assistance (biofortification) under graded doses of micronutrients.

Bioaugmentation is a method where suitable microorganisms are introduced to a site for multiplication and growth which are capable of assisting plants for nutrient uptake. Some of the crop plants possess hyper accumulation capacity for certain metals. This property can be enhanced by bioaugmentation. *Amaranthus sp* is such a crop that possesses hyperaccumulation capacity for Fe and Zn (Reeves and Baker, 2000) which is the most popular leafy vegetable in Kerala.

The supply and phytoavailability of mineral elements in the rhizosphere solution ultimately limit the accumulation of elements by crop plants. This is mainly controlled by physicochemical and biological properties of soil. If the soils are deficient in mineral elements, either they have to be added or otherwise increase their phytoavailability in rhizosphere by appropriate methods to enhance their plant uptake and translocation (Mackowiak and Gross, 1999). Non

graminaceous plants generally acidify the rhizosphere by releasing organic acids and phenolic compounds which may form metal chelates and assist in nutrient translocation (Wu *et al.*, 2005). Several crop plants possess the capacity for hyper accumulation and about 400 species of plant possess the above capacity. Members of Brassicaceae, Amaranaceae and Asteraceae are capable of tolerating higher levels of metals in above ground parts (Reeves and Baker, 2000). *Amaranth crentus* was found to accumulate Zn when it was grown in Zn rich soils (Santos *et al.*, 2010)

Availability and uptake of P and other nutrients were enhanced by the use of P solubilisers along with rock phosphate in pulses and resulted a yield increase of 10 per cent (Khan *et al.*, 2010). Efficient P solubilizers have been isolated from Kerala soils by extensive isolation programmes and were found useful (Meenakumari *et al.*, 2008)

Glick (2003) reported the synergistic effect of microorganisms on metal extraction. Kuiper *et al.*, (2004) had mentioned the beneficial effect of bioaugmentation for enhancing the metal uptake by plants. Overall uptake of metals by plants can be increased by enhancement of the mobility of metals in porous medium by the action of microbes that produces bio-surfactants (Mulligan *et al.*, 2001). To date studies devoted to microorganism assisted plant for metal extraction from soil are rather scarce.

Welch and Graham (2005) viewed agriculture as an instrument for public health. The high demand of marginalized section of society and those who require more nutrients than others may not be entirely met by biofortified crops alone. Need is to aim for innovating holistic diet approaches combining high yielding nutrient dense crops, inhibiting adverse effects of anti-nutrient factors, nutrient enhancing food enrichment techniques and supplementation programmes to tackle hunger and malnutrition.

Biofortification is considered to be potentially more cost-effective than other ways to deliver the benefits of micronutrient enhancement to the rural populations in developing countries (Nestel *et al.*, 2006., Mayer *et al.*, 2008).

Agronomic strategies to increase the concentrations of mineral elements in edible tissues generally rely on the application of mineral fertilizers and/or improvement of the solubilization and mobilization of mineral elements in the soil.

When crops are grown where mineral elements become immediately unavailable in the soil, targeted application of soluble inorganic fertilizers to roots or to leaves is practiced. In situations where mineral elements are not readily translocated to edible tissues, foliar applications of soluble inorganic fertilizers are made (Graham *et al.*, 2001., 2007)

Soils often contain large amounts of Fe, but little of this is phytoavailable. The application of inorganic Fe fertilizers to such soils is usually ineffective as it rapidly becomes unavailable to plant roots through adsorption, precipitation and oxidation reactions. For this reason, Fe-chelates are often used as soil Fe fertilizers (Shuman, 1998., Rengal *et al.*, 1999., 2001).

In addition, the availability of Fe in the rhizosphere can be increased by soil acidification with elemental S (Shuman, 1998). This has the added benefit of crop S fertilization. Foliar applications of Fe fertilizers are often made to crops growing in Fe-deficient soils, but, because Fe is not readily translocated within plants, these must be repeated throughout the growing season (Cakmak, 2002). Nevertheless, by appropriate Fe fertilization, Fe concentrations in edible portions of cereals, vegetables and fruits can be increased (Shuman, 1998., Rengal *et al.*, 1999, Cakmak, 2002).

Zinc is commonly applied to crops as ZnSO₄ or as synthetic chelates (Shuman, 1998., Broadley *et al.*, 2007., Cakmak, 2008., 2009). The application of

Zn fertilizers to the soil is effective in increasing grain Zn concentrations in cereals growing on most, but not all, soils and foliar applications of either ZnSO₄ or Zn-chelates can increase grain Zn concentrations in plants with adequate Zn mobility in the phloem (Cakmak, 2002.,2008). Similarly, soil and/or foliar applications of Zn fertilizers can increase leaf, tuber and fruit Zn concentrations (Shuman, 1998., Rengal *et al.*, 1999., Broadley *et al.*, 2007). In some soils, the residual effects of a single application of Zn fertilizer can be appreciated over several years.

The application of inorganic fertilizers can undoubtedly increase the concentrations of mineral elements commonly lacking in human diets in edible produce. However, these fertilizers must be applied regularly and can be costly to manufacture, distribute and apply. Furthermore, the manufacture and use of inorganic fertilizers can incur environmental costs, such as those caused by the production of greenhouse gasses and mineral enrichment of the environment. The supply of certain mineral elements especially Zn may become limiting in the future (USDS, 2007).

2.12 Biofortification through bioaugmentation

The total mineral concentrations of Fe and Zn in most soils would be sufficient to support mineral-dense crops, if these elements were phytoavailable (Frossard *et al.*, 2000, Rengal, 2001). Hence, there is considerable interest in developing management systems that exploit soil and fertilizer sources of mineral elements more effectively (Lynch, 2007). This work aims to improve both the acquisition of mineral elements and their physiological utilization in the plant for improved yields (Lynch, 2007).

The low phytoavailability of Fe and Zn limits crop yields on many calcareous soils of the world and this can be improved by investing more biomass in the root system, by producing a greater number and more even spread of roots, by developing a more extensive root system, with longer, thinner roots with more

root hairs, and by proliferating lateral roots in mineral-rich patches (White *et al.*, 2005., Lynch, 2007., Kirkby and Johnston, 2008). In addition, the efflux of organic acids, which displace cations from their binding sites in the soil, and the secretion of enzymes capable of degrading organic compounds, such as phytate, that chelate cations can also improve the acquisition of Fe and Zn (Lynch, 2007).

Soil micro-organisms can also be exploited to increase the volume of soil explored by crop plants and the phytoavailability of mineral elements (Renga *et al.*, 1999; Lynch, 2007; Kirkby and Johnston, 2008). Many crops are associated with mycorrhizal fungi, which have the potential to increase the volume of soil exploited for the acquisition of immobile mineral elements, and release organic acids, siderophores and enzymes capable of degrading organic compounds (Rengel *et al.*, 1999., Smith and Read, 2007). Recently, He and Nara (2007) suggested that the agricultural management of mycorrhizal fungi could be used to increase mineral concentrations in edible produce, and several studies have found that mycorrhizal associations increase Se, Fe, Zn and Cu concentrations in crop plants (Rengel *et al.*, 1999., Larsen *et al.*, 2006., Cavagnaro, 2008). However, because the symbiotic relationship between plants and mycorrhizal fungi is fuelled by photosynthate from plants, such associations can reduce yields in well-fertilized soils (Lynch, 2007).

Arbuscular mycorrhizal fungi-roots can greatly enhance acquisition of mineral nutrients in host plants (Marschner, 1995). The exudates from plant roots and mycorrhizal fungi can provide carbon for other soil microbes that affect the phytoavailability of mineral elements. Hence, inoculants of growth-promoting bacteria can increase the acquisition of Fe, Zn and Cu by plant roots, tissue mineral concentrations, plant growth and yield (Rengal, 2001., Barea *et al.*, 2005).

2.13 Human bioavailability of iron and zinc

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). In addition, it was found that the consumption of Fe-biofortified rice improved the Fe status of non-anaemic Filipino women. And that replacing conventional varieties with *lpa* mutants in people's diets improved their Fe, Zn and Ca status, especially when consumption of dietary minerals was low (Mendoza *et al.*, 1998, Hambidge *et al.*, 2004., 2010)

2.14 Economics of agronomic biofortification

Biofortification of edible produce through mineral supplementation is potentially cost effective and will deliver most benefits to the 40% of the world's population who rely primarily on their own food for sustenance. Most economic analyses suggest that biofortification through mineral supplementation is 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 of wheat in Turkey suggested a cost-to-benefit quotient of greater than 20 over two decades (Bouis, 1999), and cost-to-benefit quotients of between 20 and 30 for Fe biofortification of rice in South Asia and for Fe biofortification of rice and wheat in Bangladesh and India over the same period (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 Fe-deficiency anaemia in India is 4 million lost DALYs and that Fe biofortification may reduce this burden significantly. Similarly, it is 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% (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).

2.15 Environmental impact and 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 antinutrients 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.

Materials and Methods

3. MATERIALS AND METHODS

The present study entitled “Iron and Zinc fortification in amaranthus (*Amaranthus tricolor*) through bioaugmentation” has been carried out at the College of Agriculture, Vellayani during the months May to August, 2012. A pot culture experiment was conducted to study the effect of Fe and Zn application and bioaugmentation of soil with microbial additives on yield and nutrient composition of amaranthus particularly with Fe and Zn.

3. Pot culture experiment

The pot culture experiment was done at Department of Soil Science and Agricultural Chemistry to study the effect of bioaugmentation with micro-organisms for nutrient enrichment in amaranthus particularly for Fe and Zn using laterite soil.

3.1. Soil

The soil for pot culture experiment was collected from the Instructional Farm, Vellayani. Soil belongs to the Vellayani series (Loamy Kaolinitic Isohyperthermic Rhodic Kandustult). Soils collected in bulk were air dried and sieved through 2 mm sieve. Each pot was filled with 4 kg of this soil. Important physicochemical parameters of the soil analysed as per standard procedures are presented in Table 1.

3.2. Season

The crop was grown from 14th May to 25th August of the year 2012. The weekly averages of the weather parameters *viz.* maximum and minimum temperature, relative humidity and rainfall received during the cropping period collected from the Observatory of College of Agriculture, Vellayani are given in Table 2.

Table 1. Physicochemical parameters of the soil

Sl No	Chemical properties	Content
1	pH	5.80
2	EC (dS m ⁻¹)	0.18
3	Organic Carbon (%)	0.45
4	Available N (kg ha ⁻¹)	188.16
5	Available P (kg ha ⁻¹)	48.16
6	Available K (kg ha ⁻¹)	125.89
7	Available Fe (mg kg ⁻¹)	6.12
8	Available Zn (mg kg ⁻¹)	1.11

Table 2. Weather parameters at weekly intervals Vellayani during the year 2010

Sl. No.	Standard Week	Dates	Temp. Max (°C)	Temp. Min (°C)	RF (mm)	RH (%)
1	20	14.05.12 - 20.05.12	31.5	26.1	22.0	91.4
2	21	21.05.12 - 27.05.12	31.5	25.8	0.0	91.7
3	22	28.05.12 - 03.06.12	31.5	26.1	1.0	90.0
4	23	4.06.12 - 10.06.12	31.3	24.7	3.6	91.4
5	24	11.06.12 -17.06.12	30.4	23.9	7.0	93.6
6	25	18.06.12 -24.06.12	29.4	24.3	3.5	94.4
7	26	25.06.12 -01.07.12	29.8	23.8	6.0	87.0
8	27	02.07.12 - 08.07.12	29.5	23.9	7.4	95.1
9	28	09.07.12 - 15.07.12	29.6	24.0	7.9	88.9
10	29	16.07.12 - 22.07.12	30.3	23.5	5.3	92.3
11	30	23.07.12 - 29.07.12	30.1	25.1	5.8	94.4
12	31	30.07.12 - 05.08.12	30.2	24.6	0.0	94.0
13	32	06.08.12 - 12.08.12	30.3	23.7	1.5	87.7
14	33	03.08.12 -19.08.12	29.7	23.5	17.0	91.3
15	34	20.08.12 - 26.08.12	29.8	23.9	2.0	92.6
	Mean		30.3	24.5	6.0	91.7

3.3. Variety and planting material

The variety chosen for the study was Arun, red coloured amaranthus variety. The seeds of the variety Arun were purchased from Instructional Farm, Vellayani.

3.4. Manures and fertilizers

Urea (46 % N), Factamphos (20 N: 20 P₂O₅) and MOP (56 % K₂O) were used as sources of N, P and K respectively. N content of factamphos was accounted in the calculation of N requirement. Fe and Zn were given to the crop through ferrous sulphate (FeSO₄.7H₂O) and zinc sulphate (ZnSO₄.7H₂O) respectively. Vermicompost as per Package of Practices Recommendations of Kerala Agricultural University (KAU, 2011) was applied to all treatments.

3.5. Microbial additives

AMF, P and K solubiliser purchased from the Department of Agricultural Microbiology, College of Agriculture, Vellayani were used for the experiment.

3.6. Design and layout of the experiment

Crop : Amaranth
 Variety : Arun
 Design : Factorial CRD
 Treatments : 64
 Replication : 2

Treatments

4 x 4 x 4 Factorial CRD with levels of factors as indicated below

I. Augmentation methods – 4 levels

M1 - POP + AMF

M2 - POP + P solubiliser

M3 - POP + K solubiliser

M4 - POP

II. Levels of Fe - 4 levels

F0 - Zero

F1 - 5 mg kg⁻¹ of soil

F2 - 10 mg kg⁻¹ of soil

F3 - Foliar application of 1 mg kg⁻¹ ferrous sulphate

III. Levels of Zn – 4 levels

Z0 - Zero

Z1 - 5 mg kg⁻¹ of soil

Z2 - 10 mg kg⁻¹ of soil

Z3 - Foliar application of 0.5 mg kg⁻¹ zinc sulphate

3.7. Details of cultivation**3.7.1. Sowing in the nursery**

Amaranthus seeds were sown on 14.05.2012, in separate pots containing potting mixture (soil, sand and vermicompost in 3:1:1 proportion) with the required quantity of microbial additives as per treatments. Sprinkling of water was carried out at regular intervals. Seed germination was noted on third day.

3.7.2. Transplanting to pots

On 25th day seedlings were transplanted to the pots with appropriate microbial treatments as suggested in the technical programme.



Plate 1. A general view of the experiment



Plate 2. A general view of the experiment

3.7.3. Application of treatments

Iron treatments were applied to the transplanted seedling on seventh leaf stage and one week after that zinc treatment was given. One week after taking the first harvest, the treatments were repeated.

3.7.5. After cultivation and irrigation

The crop received timely management practices as per the package of practices of KAU. Irrigation was done daily and the pots were irrigated to field capacity.

3.7.8. Plant protection

No pest and disease infestation was noticed.

3.7.9. Harvesting

First harvest was taken 45 days after transplanting and second harvest 30 days after the first one.

3.9. Chemical analysis

3.9.1. Soil Analysis

Soil samples for chemical analysis were drawn before sowing and at the time of each cutting. The samples were air dried under shade, sieved through 2 mm sieve and used for the analysis of organic carbon and available N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu using standard procedures given in Table 3.

Microbiological properties:

Rhizosphere microbial count (bacteria, fungi and actinomycetes and AMF colonization) were estimated as per the procedures mentioned in the Table 3.

3.9.2. Plant analysis

Shoot samples were taken at the time of each harvesting and root samples at the time of final harvest. The collected samples were washed, separated in to shoot and root, air dried, powdered and subjected to chemical analysis to find out their chemical composition. The methods used for each analysis are presented in Table 4

3.10.1. Chemical analysis of soil

Table 3. Standard analytical methods followed in soil analysis

Sl. No	Properties	Method	Reference
	<i>Chemical properties</i>		
1.	pH	pH meter	Jackson (1973)
2.	EC	Conductivity meter	Jackson (1973)
3.	Organic carbon	Walkley and Black rapid titration method	Walkley and Black (1934)
4.	Available nitrogen	Alkaline potassium permanganate method	Subbiah and Asija(1956)
5.	Bray No.1 extractable phosphorus	Spectrophotometry	Jackson (1973)
6.	Neutral normal NH ₄ OAC extractable potassium	Flame photometry	Jackson (1973)
7.	Neutral normal NH ₄ OAC extractable calcium and magnesium	Versanate titration method	Hesse (1971)
8.	0.5 N HCl extractable iron, manganese, zinc and copper	AAS	O'Connor (1988)
9.	0.01N Ca(PO ₄) ₂ extract able sulphur	Turbidimetry	Chesnin and Yien (1950)

<i>B. Microbiological properties</i>			
10.	Bacterial population	Serial dilution and plate count method	Waksman (1992)
11.	Fungal population	Serial dilution and plate count method	Waksman (1992)
12.	Actinomycetes population	Serial dilution and plate count method	Waksman (1992)
13.	AMF colonization	Staining method	Phillips and Hayman(1970)

3.10.2. Chemical analysis of plant samples (Shoot and root)

Table 4. Standard analytical methods followed in plant analysis

Sl. No	Properties	Method	Reference
Elemental Composition			
1	Nitrogen	Micro Kjeldahl method	Jackson (1973)
2	Phosphorus	Nitric- perchloric acid (9:4) digestion and spectrophotometry using vanadomolybdophosphoric 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 & turbidimetry	Chesnin and Yien (1950)
6	Iron, manganese, zinc and copper	Nitric- perchloric acid (9:4) digestion and AAS	Jackson (1973)
7	Crude protein	Multiplication with %N content	Simpson (1965)
8	Beta carotene	Acetone - petroleum ether	Srivastava and

	(Fresh leaf)	extraction and spectrophotometry	Kumar (1996)
9.	Vitamin C (Fresh leaf)	Titrimetric method	Sadasivam and Manickam (1996)
10.	Nitrate	Silver sulphate extraction and spectrophotometry	Middleton (1958).
11.	Oxalate content	Actetate buffer (pH 4.5) method	AOAC (1984)
12	Phenol	Folin-Ciocalteu reagent method	Sadasivam and Manickam (1996)

3.10.3. Computed indices (Chaney *et al.*, 1995)

$$\text{Bioconcentration factor (BCF)} = \frac{\text{Concentration in plant part}}{\text{Concentration in growing medium}}$$

$$\text{Translocation index} = \frac{\text{Concentration in shoot}}{\text{Concentration in root}}$$

3.11.1. Biometric observations

1. Height of plant

Height of the plant was measured from the ground level to the top most leaf bud and expressed in centimeters.

2. Number of branches

The total number of branches of each plant was counted and reported.

3. Girth of stem

The girth of main stem at the collar region was taken using a twine and expressed in centimeters.

4. Number of leaves per plant

The total number of leaves per plant, with various treatments were counted and the number of leaves per plant worked out.

5. Root length (maximum)

Plants from each pot 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 expressed in centimeters.

6. Root weight or Root biomass

The fresh weight of the washed roots were noted and expressed in g plant¹.

7. Root volume

Root volume per plant was found out by water displacement method. The roots of the sample 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 expressed in cm³.

3.11.2. Yield characteristics

1. Total Biomass yield

Leaves, stem and root of different sample plants were collected and fresh weight was recorded.

2. Total Shoot biomass

Total shoot biomass per plant was found out by adding leaf and stem biomass at each harvest was expressed in g plant¹.

3. Total root biomass

The roots of the sample plants were washed free of adhering soil with water, roots were separated, cleaned, weighed and expressed in g plant⁻¹.

4. Total leaf weight

The leaf weight per cutting was summed up and the total leaf weight was worked out.

5. Total stem weight

The stem weight per cutting was added up and the total stem weight was calculated.

6. Leaf: stem ratio

Leaf: stem ratio was obtained by dividing the weight of leaves by weight of stem. Leaf: stem ratio was worked out for each harvest.

3.12. Scoring for pest and diseases

No pest and disease incidence was noticed during the period of crop growth.

3.13. BC ratio

Benefit – cost ratio as computed using the formula.

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

3.14. Statistical Analysis

Data generated from the experiment were subjected to statistical analysis (Cochran and Cox, 1965). ANOVA was done in factorial CRD with two replications.

Results

4. RESULTS

An experiment entitled “Iron and Zinc fortification in amaranthus (*Amaranthus tricolor*) through bioaugmentation” has been carried out at the Department of Soil Science and Agricultural Chemistry, College of Agriculture, Vellayani. A pot culture experiment with 64 treatments in two replications was conducted to study the effect of Fe and Zn application and bioaugmentation of soil with microbial additives on yield and nutrient composition of amaranthus particularly with Fe and Zn. The results of the experiment are presented in this section.

4.1 Effect of treatments on growth characteristics

The data on plant height, girth of stem, no. of branches plant⁻¹ and no. of leaves plant⁻¹ are presented in Tables 5 to 8 respectively.

Statistical analysis of the data on plant height (Table 5) reveals that plant height was significantly influenced only by the individual effect of bioaugmentation treatments. The highest value was recorded by M4 (POP) which was on par with M3 (K solubilizer) and the lowest value was for M1 (AMF). Girth of the stem (Table 6) was not significantly influenced by the treatments.

No. of branches per plant (Table 7) was significantly influenced only by the individual effects of bioaugmentation, levels of iron and zinc. Among the interactions, iron x bioaugmentation (F x M) alone was significant. Evaluating the individual effect of bioaugmentation methods, the highest value was recorded by M4 (POP). Application of iron had reduced the no. of branches while zinc had increased it.

Considering the number of leaves per plant (Table 8), the treatment effect was statistically significant only for bioaugmentation methods (M) and its interaction with Fe (M x F). Evaluating the individual effect of bioaugmentation methods, M4 (POP) recorded the highest value which was significantly superior

Table 5. Effect of treatments on plant height of amaranthus (mean values in cm)

Treatments	M1	M2	M3	M4	Mean				
F0Z0	35.00	59.15	66.25	56.25	54.16				
F0Z1	51.00	53.25	60.50	59.75	56.13				
F0Z2	52.00	57.75	61.25	63.50	58.63				
F0Z3	48.00	61.75	59.00	44.00	53.19				
F1Z0	43.00	50.25	62.25	65.50	55.25				
F1Z1	52.50	54.50	47.50	65.75	55.06				
F1Z2	53.00	62.25	56.25	58.50	57.50				
F1Z3	50.75	37.50	55.75	53.75	49.44				
F2Z0	39.50	42.50	60.00	62.00	51.00				
F2Z1	33.50	58.80	54.50	61.75	52.14				
F2Z2	40.75	50.50	57.00	65.50	53.44				
F2Z3	42.25	50.00	44.25	66.75	50.81				
F3Z0	38.50	43.00	53.50	58.75	48.44				
F3Z1	39.25	49.75	55.25	59.75	51.00				
F3Z2	39.75	43.00	57.75	59.75	50.06				
F3Z3	37.00	57.50	55.75	59.50	52.44				
	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	46.50	49.81	39.00	38.63	39.00	44.06	46.38	44.50	43.48
M2	57.98	51.13	50.45	48.31	48.73	54.08	53.38	51.69	51.97
M3	61.75	55.44	53.94	55.56	60.50	54.44	58.06	53.69	56.67
M4	55.88	60.88	64.00	59.44	60.63	61.75	61.81	56.00	60.05
MEAN	55.53	54.31	51.85	50.48	52.21	53.58	54.91	51.47	
CD(0.05)M- 4.1113									

Table 6. Effect of treatments on girth of amaranthus stem (mean values in cm)

Treatments	M1	M2	M3	M4	Mean				
F0Z0	2.40	4.00	3.90	3.30	3.45				
F0Z1	3.00	3.85	4.10	3.35	3.57				
F0Z2	3.05	4.20	4.20	3.30	3.69				
F0Z3	3.25	3.50	3.90	2.05	3.18				
F1Z0	2.80	4.40	4.00	4.25	3.86				
F1Z1	3.55	4.10	4.35	4.05	4.01				
F1Z2	3.00	3.95	3.90	3.85	3.68				
F1Z3	2.75	3.20	4.45	3.70	3.52				
F2Z0	3.95	3.85	4.75	4.00	4.14				
F2Z1	2.70	5.50	4.40	3.95	4.14				
F2Z2	3.35	3.05	4.55	3.90	3.71				
F2Z3	3.65	4.10	3.55	4.10	3.85				
F3Z0	3.05	3.40	3.60	3.65	3.43				
F3Z1	3.20	4.45	3.55	3.75	3.74				
F3Z2	3.80	3.50	3.95	3.45	3.68				
F3Z3	3.10	4.10	4.25	3.45	3.73				
	F0	F1	F2	F3	Z0	Z1	Z2	Z3	MEAN
M1	2.93	3.03	3.41	3.29	3.05	3.11	3.30	3.19	3.16
M2	3.89	3.91	4.13	3.86	3.91	4.48	3.68	3.73	3.95
M3	4.03	4.18	4.31	3.84	4.06	4.10	4.15	4.04	4.09
M4	6.75	3.96	3.99	3.58	3.30	3.78	3.63	3.33	4.57
MEAN	4.40	3.77	3.96	3.64	4.64	3.87	3.69	3.57	
NS									

Table 7. Effect of treatments on no. of branches per plant (mean values)

Treatments	M1	M2	M3	M4	Mean				
F0Z0	2.50	2.75	3.50	2.75	2.88				
F0Z1	2.25	3.75	3.00	3.50	3.13				
F0Z2	3.00	3.75	3.75	3.50	3.50				
F0Z3	3.00	4.25	3.75	3.25	3.56				
F1Z0	2.50	3.50	2.25	3.75	3.00				
F1Z1	2.50	3.25	2.25	3.25	2.81				
F1Z2	2.75	2.50	2.75	3.50	2.88				
F1Z3	3.25	2.00	2.50	3.00	2.69				
F2Z0	2.00	1.75	2.50	3.75	2.50				
F2Z1	2.00	3.75	2.50	3.75	3.00				
F2Z2	2.00	3.25	3.00	3.25	2.88				
F2Z3	2.50	3.50	3.00	4.50	3.38				
F3Z0	2.00	1.25	2.50	3.00	2.19				
F3Z1	2.25	3.00	2.75	3.75	2.94				
F3Z2	2.50	2.25	3.00	4.00	2.94				
F3Z3	2.50	3.25	2.75	4.25	3.19				
	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	2.69	2.75	2.13	2.31	2.25	2.25	2.56	2.81	2.47
M2	3.63	2.81	3.06	2.44	2.31	3.44	2.94	3.25	2.98
M3	3.50	2.44	2.75	2.75	2.69	2.63	3.13	3.00	2.86
M4	3.25	3.38	3.81	3.75	3.31	3.56	3.56	3.75	3.55
Mean	3.27	2.84	2.94	2.81	2.64	2.97	3.05	3.20	
CD(0.05) F / Z- 0.2821					CD(0.05) FM- 0.5642				

Table 8. Effect of treatments on no. of leaves plant⁻¹ (mean values)

Treatments	M1	M2	M3	M4	Mean				
F0Z0	21.50	27.50	29.25	25.50	25.94				
F0Z1	26.75	23.25	24.50	28.00	25.63				
F0Z2	25.75	31.00	31.00	34.00	30.44				
F0Z3	21.50	38.00	29.50	28.00	29.25				
F1Z0	20.25	31.75	23.50	38.25	28.44				
F1Z1	26.25	25.00	23.75	34.50	27.38				
F1Z2	22.00	22.75	26.00	26.50	24.31				
F1Z3	24.75	19.00	26.00	28.50	24.56				
F2Z0	24.25	17.00	24.25	33.25	24.69				
F2Z1	20.50	27.75	24.00	29.50	25.44				
F2Z2	22.25	25.25	23.25	31.75	25.63				
F2Z3	16.00	26.25	23.00	37.00	25.56				
F3Z0	18.75	13.25	21.75	33.00	21.69				
F3Z1	21.75	23.50	24.00	35.25	26.13				
F3Z2	18.50	18.25	27.00	36.50	25.06				
F3Z3	13.75	30.25	25.75	32.25	25.50				
	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	23.88	23.31	20.75	18.19	21.19	23.81	22.13	19.00	21.53
M2	29.94	24.63	24.06	21.31	22.38	24.88	24.31	28.38	24.98
M3	28.56	24.81	23.63	24.63	24.69	24.06	26.81	26.06	25.41
M4	28.88	31.94	32.88	34.25	32.50	31.81	32.19	31.44	31.98
Mean	27.81	26.17	25.33	24.59	25.19	26.14	26.36	26.22	
CD(0.05)FM- 4.9018					CD(0.05)M- 2.4509				

to other three treatments. Considering the interaction effect of bioaugmentation with Fe, (F x M) the highest value was showed by M4F3 (POP and foliar application of iron) which was on par with several other treatments.

4.2 Root characteristics

The data on root length, root weight and root volume are presented in Tables 9 to 11 respectively.

Data on root length (Table 9) reveals that it was significantly influenced by bioaugmentation methods (M) and its interaction with Fe and Zn ie., (M x F x Z) and with Zn alone (M x Z). Root length was highest for the combination of M4F2Z2 (POP x Fe @ 10 mg kg⁻¹ x Zn @ 10 mg kg⁻¹). Evaluating the effect of bioaugmentation methods, the root length was highest for M2 (P solubilizer) which was on par with M4 (POP) and was significantly superior to M1 (AMF) and M3 (K solubilizer).

Data on root weight (root biomass) reveals (Table 10) that it was significantly influenced by bioaugmentation methods and its interaction with Fe and Zn I, (M x F x Z) and with Zn alone (M x Z). Root weight was highest for the combination of M4F0Z2 (POP x Fe @ zero x Zn @ 10 mg kg⁻¹). Evaluating the individual effect of bioaugmentation methods, the M4 (POP) showed the highest value, and was significantly superior to other three.

Individual as well as interaction effects were significant for root volume (Table 11). Treatment combination M2F2Z3 (P solubilizer x Fe @ 10 mg kg⁻¹ x Zn as foliar) recorded the highest value which was on par with M1F2Z2 (AMF x Fe @ 10 mg kg⁻¹ x Zn @ 10 mg kg⁻¹) and M4F0Z2 (POP x Fe @ zero x Zn @ 10 mg kg⁻¹). Considering the individual effects, the addition of P solubilizer (M2) had shown the highest value, which was significantly superior to other three treatments. Levels of Fe showed a negative relation whereas Zn had shown a positive relation to root volume.

Table 9. Effect of treatments on root length (cm) of amaranthus

Treatments	M1	M2	M3	M4	Mean				
F0Z0	13.75	16.50	15.50	15.00	15.19				
F0Z1	10.50	15.50	12.50	21.50	15.00				
F0Z2	16.00	19.50	13.50	17.50	16.63				
F0Z3	10.50	27.50	16.00	10.50	16.13				
F1Z0	17.75	15.00	14.00	20.50	16.81				
F1Z1	16.00	18.50	11.50	15.50	15.38				
F1Z2	13.50	19.75	14.50	12.00	14.94				
F1Z3	11.00	11.50	19.00	13.00	13.63				
F2Z0	14.00	11.00	14.50	14.50	13.50				
F2Z1	16.50	21.50	11.00	14.00	15.75				
F2Z2	17.00	15.00	17.50	23.00	18.13				
F2Z3	16.00	21.00	13.50	16.50	16.75				
F3Z0	17.50	13.00	16.00	13.00	14.88				
F3Z1	11.00	22.25	12.00	16.90	15.54				
F3Z2	10.50	13.50	13.00	15.50	13.13				
F3Z3	10.50	17.50	13.00	17.00	14.50				
	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	12.69	14.56	15.88	12.38	15.75	13.50	14.25	12.00	13.88
M2	19.75	16.19	17.13	16.56	13.88	19.44	16.94	19.38	17.41
M3	14.38	14.75	14.13	13.50	15.00	11.75	14.63	15.38	14.19
M4	16.13	15.25	17.00	15.60	15.75	16.98	17.00	14.25	15.99
Mean	15.73	15.19	16.03	14.51	15.09	15.42	15.70	15.25	
CD(0.05)M- 1.5810			CD(0.05)ZM- 3.1621			CD(0.05) FZM- 6.3242			

Table.10 Effect of treatments on root weight (root biomass) per plant (g)

TREATMENTS	M1	M2	M3	M4	Mean				
F0Z0	18.50	20.30	11.00	22.40	18.05				
F0Z1	16.35	26.10	16.55	33.60	23.15				
F0Z2	17.35	35.25	18.90	37.80	27.33				
F0Z3	20.15	14.20	29.75	27.25	22.84				
F1Z0	13.70	13.60	15.00	31.90	18.55				
F1Z1	26.30	24.90	14.55	26.75	23.13				
F1Z2	12.10	28.50	17.05	24.60	20.56				
F1Z3	15.60	13.50	22.30	17.25	17.16				
F2Z0	9.30	10.45	21.90	34.55	19.05				
F2Z1	23.55	13.15	14.70	22.20	18.40				
F2Z2	29.45	16.40	12.45	18.75	19.26				
F2Z3	10.50	32.05	10.35	28.25	20.29				
F3Z0	19.80	3.35	11.10	24.35	14.65				
F3Z1	24.35	28.30	20.15	23.70	24.13				
F3Z2	28.75	14.20	22.50	30.90	24.09				
F3Z3	9.35	18.20	19.25	32.00	19.70				
	18.44	19.53	17.34	27.27					
	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	18.09	16.92	18.20	20.56	15.33	22.64	21.91	13.90	18.44
M2	23.96	20.13	18.01	16.01	11.93	23.11	23.59	19.49	19.53
M3	19.05	17.22	14.85	18.25	14.75	16.49	17.72	20.41	17.34
M4	30.26	25.13	25.94	27.74	28.30	26.56	28.01	26.19	27.27
Mean	22.84	19.85	19.25	20.64	17.58	22.20	22.81	20.00	
CD(0.05)M/F/Z- 2.1961			CD(0.05)ZM/FZ- 4.3917			CD(0.05) FZM- 8.7835			

Table.11 Effect of treatments on root volume (ml) of amaranthus

Treatments	M1	M2	M3	M4	Mean					
F0Z0	8.95	11.80	5.65	5.00	7.85					
F0Z1	8.40	12.50	6.30	7.50	8.68					
F0Z2	8.50	14.20	7.95	15.00	11.41					
F0Z3	10.00	6.30	12.25	3.50	8.01					
F1Z0	6.35	5.25	7.15	10.00	7.19					
F1Z1	10.75	12.10	7.20	5.00	8.76					
F1Z2	6.55	14.25	8.15	7.50	9.11					
F1Z3	7.75	6.15	12.20	5.00	7.77					
F2Z0	4.50	5.60	11.65	5.00	6.69					
F2Z1	10.25	5.95	6.40	5.00	6.90					
F2Z2	16.25	8.05	6.10	5.00	8.85					
F2Z3	5.65	17.10	5.35	5.00	8.27					
F3Z0	8.90	6.10	5.55	5.00	6.39					
F3Z1	9.55	12.65	11.15	7.50	10.21					
F3Z2	10.80	5.10	11.55	7.50	8.74					
F3Z3	4.50	7.30	10.65	5.00	6.86					
	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean	
M1	8.96	7.85	9.16	8.44	7.17	9.74	10.53	6.98	8.60	
M2	11.20	9.44	9.17	7.79	7.19	10.80	10.40	9.21	9.40	
M3	8.04	8.68	7.38	9.73	7.50	7.76	8.44	10.11	8.45	
M4	7.75	6.88	5.00	6.25	6.25	6.25	8.75	4.63	6.47	
Mean	8.99	8.21	7.68	8.05	7.03	8.64	9.53	7.73		
CD(0.05)M/F/Z- 0.7103			CD(0.05)ZM/FZ/ FM -1.4206				CD(0.05) FZM- 2.8411			

4.3 Yield characteristics

The data on yield characteristics viz., leaf weight, stem weight, shoot biomass and leaf: stem ratio per plant are presented in Tables 12 to 15 respectively.

The treatments had significantly influenced the leaf weight per plant (Table 12). Individual effect of bioaugmentation methods, levels of Zn and different interactions (F x Z, M x Z, and F x Z x M) were significant. The treatment combination M4F0Z2 (POP x Fe @ zero x Zn @ 10 mg kg⁻¹) showed the highest value followed by M4F0Z0 (POP x Fe @ zero x Zn @ zero). Individual effect of iron was not significant, while the effect of Zn was significant. Leaf weight per plant showed a slight increase with levels of Zn. Z2 (Zn @ 10 mg kg⁻¹) showed the highest value and was significantly superior to Z0 (Zn @ zero).

Stem weight (Table 13) was significantly influenced by the bioaugmentation methods and levels of Zn. The interaction effects were also significant for bioaugmentation methods and levels of Fe (M x F) and with levels of Fe and Zn (M x F x Z). Stem weight was highest for the combination M2F2Z2 (P solubilizer x Fe @ 10 mg kg⁻¹ x Zn @ 10 mg kg⁻¹). Comparing the individual effect of bioaugmentation methods M4 (POP) recorded the highest value for stem weight. In the case on Zn, Zn @ 5 mg kg⁻¹ (Z1) recorded the highest value which was significantly superior only to zinc @ zero.

The treatments had significantly influenced the shoot biomass (Table 14). Both the interaction and individual effects were significant except with that of Fe and its interaction with Zn (F x Z). The combination M4F0Z2 (POP x Fe @ zero x Zn @ 10 mg kg⁻¹) recorded the highest shoot biomass. Considering the individual effects, the bioaugmentation method (POP) was significantly superior to other three treatments. Among the levels of Zn, Zn @ 5 mg kg⁻¹ (Z1) showed the highest value and was on par with all other Zn treatments except Z0 (Zn @ zero).

Table.12 Effect of treatments on leaf weight of amaranthus (g plant⁻¹)

Treatments	M1		M2		M3		M4		Mean	
F0Z0	67.60		68.40		57.40		71.50		66.23	
F0Z1	58.90		62.55		60.05		85.00		66.63	
F0Z2	57.25		83.95		74.45		109.95		81.40	
F0Z3	57.00		75.35		89.90		82.70		76.24	
F1Z0	59.65		58.80		74.55		98.50		72.88	
F1Z1	63.60		92.60		48.90		95.20		75.08	
F1Z2	71.05		66.10		66.20		90.55		73.48	
F1Z3	52.00		62.15		82.65		62.75		64.89	
F2Z0	38.60		55.80		43.10		90.45		56.99	
F2Z1	80.45		81.25		53.60		82.05		74.34	
F2Z2	65.60		80.75		56.70		68.55		67.90	
F2Z3	46.50		83.60		64.40		90.55		71.26	
F3Z0	56.60		56.35		48.60		72.20		58.44	
F3Z1	54.75		79.95		60.05		82.85		69.40	
F3Z2	53.40		61.55		62.80		86.55		66.08	
F3Z3	56.85		74.05		72.15		93.20		74.06	
	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean	
M1	60.19	61.58	57.79	55.40	55.61	64.43	61.82	53.09	58.74	
M2	72.56	69.91	75.35	67.98	59.84	79.09	73.09	73.79	71.45	
M3	70.45	68.08	54.45	60.90	55.91	55.65	65.04	77.27	63.47	
M4	87.29	86.75	82.90	83.70	83.16	86.28	88.90	82.30	85.16	
Mean	72.62	71.58	67.62	66.99	63.63	71.36	72.21	71.61		
CD(0.05)M /Z- 5.7246			CD(0.05)ZM/FZ- 11.4492				CD(0.05) FZM- 22.8985			

Table.13 Effect of treatments on stem weight of amaranthus (g plant⁻¹)

Treatments	M1	M2	M3	M4	Mean				
F0Z0	115.00	123.05	123.60	171.30	133.24				
F0Z1	121.90	145.85	135.60	185.60	147.24				
F0Z2	136.25	190.35	164.60	208.05	174.81				
F0Z3	123.50	139.50	197.50	185.80	161.58				
F1Z0	108.40	119.95	157.10	203.30	147.19				
F1Z1	144.45	209.05	141.35	189.80	171.16				
F1Z2	109.00	140.85	148.70	147.00	136.39				
F1Z3	135.85	140.95	182.85	134.80	148.61				
F2Z0	92.80	105.20	156.50	190.90	136.35				
F2Z1	117.50	182.55	122.90	162.55	146.38				
F2Z2	132.85	213.60	119.40	157.80	155.91				
F2Z3	90.45	203.65	102.95	173.65	142.67				
F3Z0	147.10	136.10	114.75	152.80	137.69				
F3Z1	153.10	197.55	141.40	155.40	161.86				
F3Z2	107.55	116.15	151.65	193.30	142.16				
F3Z3	96.65	147.90	151.25	202.50	149.58				
	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	124.16	124.43	108.40	126.10	115.83	134.24	121.41	111.61	120.77
M2	149.69	152.70	176.25	149.43	121.08	183.75	165.24	158.00	157.02
M3	155.33	157.50	125.44	139.76	137.99	135.31	146.09	158.64	144.51
M4	187.69	168.72	171.23	176.00	179.58	173.34	176.54	174.19	175.91
Mean	154.22	150.84	145.33	147.82	138.62	156.66	152.32	150.61	
CD(0.05)M /Z- 9.0687			CD(0.05)FM- 18.1375			CD(0.05) FZM-36.2750			

Table.14 Effect of treatments on shoot biomass of amaranthus (g plant⁻¹)

Treatments	M1	M2	M3	M4	Mean				
F0Z0	182.60	191.44	180.95	242.81	199.45				
F0Z1	180.80	208.41	195.66	270.59	213.86				
F0Z2	193.50	274.29	239.06	318.03	256.22				
F0Z3	180.49	214.84	287.42	268.47	237.80				
F1Z0	168.05	178.73	231.63	301.77	220.05				
F1Z1	208.03	301.66	190.27	285.00	246.24				
F1Z2	180.05	206.95	214.92	237.57	209.87				
F1Z3	187.85	203.09	265.48	197.54	213.49				
F2Z0	131.36	161.00	199.65	281.37	193.35				
F2Z1	197.92	263.82	176.52	244.62	220.72				
F2Z2	198.46	294.34	176.11	226.35	223.81				
F2Z3	136.94	287.23	167.33	264.19	213.92				
F3Z0	203.69	192.41	163.33	224.97	196.10				
F3Z1	207.86	277.45	201.44	238.20	231.24				
F3Z2	160.89	177.68	214.44	279.87	208.22				
F3Z3	153.53	221.98	223.38	295.68	223.64				
	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	184.35	186.00	166.17	181.49	171.43	198.65	183.22	164.70	179.50
M2	222.25	222.61	251.60	217.38	180.89	262.84	238.32	231.78	228.46
M3	225.77	225.57	179.90	200.65	193.89	190.97	211.13	235.90	207.97
M4	274.97	255.47	254.13	259.68	262.73	259.60	265.45	256.47	261.06
Mean	226.83	222.41	212.95	214.80	202.23	228.02	224.53	222.21	
CD(0.05)M/F -13.6891			CD(0.05)ZM/FM27.3783			CD(0.05) FZM- 54.7566			

Table.15 Effect of treatments on leaf: stem ratio in amaranthus

Treatments	M1	M2	M3	M4	Mean				
F0Z0	0.59	0.56	0.46	0.42	0.51				
F0Z1	0.48	0.43	0.44	0.45	0.45				
F0Z2	0.42	0.44	0.45	0.53	0.46				
F0Z3	0.47	0.54	0.45	0.45	0.48				
F1Z0	0.55	0.49	0.47	0.48	0.50				
F1Z1	0.44	0.45	0.34	0.50	0.43				
F1Z2	0.65	0.47	0.45	0.62	0.55				
F1Z3	0.38	0.44	0.45	0.47	0.44				
F2Z0	0.41	0.53	0.28	0.48	0.43				
F2Z1	0.68	0.44	0.44	0.50	0.52				
F2Z2	0.50	0.38	0.48	0.43	0.45				
F2Z3	0.51	0.41	0.62	0.52	0.52				
F3Z0	0.38	0.41	0.42	0.47	0.42				
F3Z1	0.36	0.40	0.43	0.53	0.43				
F3Z2	0.50	0.53	0.42	0.45	0.47				
F3Z3	0.59	0.50	0.4	0.46	0.51				
	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	0.49	0.51	0.53	0.46	0.48	0.49	0.52	0.49	0.50
M2	0.49	0.46	0.44	0.46	0.50	0.43	0.45	0.47	0.46
M3	0.45	0.43	0.46	0.43	0.41	0.41	0.45	0.50	0.44
M4	0.46	0.52	0.48	0.48	0.46	0.50	0.51	0.47	0.49
MEAN	0.47	0.48	0.48	0.46	0.46	0.46	0.48	0.48	
CD(0.05)M-0.0539			CD(0.05)ZM/FZ-)- 0.0269			CD(0.05) FZM- 0.1077			

Leaf: stem ratio (Table 15) was significantly influenced by the treatments. Lowest ratio was noted for the combination M3F2Z0 (K solubilizer x Fe @10mg kg⁻¹ x Zn @ zero) and the highest ratio for M1F2Z1 (AMF x Fe @ 10 mg kg⁻¹ x Zn @ 5 mg kg⁻¹). Individual effect of bioaugmentation was also significant and the lowest ratio was obtained for M1 (AMF) which was significantly superior to others.

4.4 Total Biomass Yield

The data on total biomass yield per plant is presented in Tables 16. Total biomass yield was significantly affected by the treatments and their interactions. It followed almost same trend as that of shoot biomass. The treatment combination M4F0Z2 (POP x Fe @ zero x Zn @ 10 mg kg⁻¹) recorded the highest biomass yield. Evaluating the individual effect of bioaugmentation methods, it was observed that M4 (POP) was significantly superior to other three microbial treatments. Individual levels of Zn had shown an increase in total biomass yield only up to 5 mg kg⁻¹ while levels of Fe had shown a negative trend.

4.5 Effect of Treatment on nutrient composition of amaranthus shoot

4.5.1 Effect on shoot nitrogen, phosphorus and potassium contents

Shoot Nitrogen content was significantly influenced by the treatments and their interactions (Table 17). Nitrogen content was highest for the combination M2F0Z1 (K solubilizer x Fe @ zero x Zn @ 5 mg kg⁻¹). Considering the individual effect of bioaugmentation methods, it was observed that the treatments receiving microbial treatments (M1, M2, and M3) were significantly superior to M4 (POP). In the case of individual effects of Fe and Zn, both up to @ 5 mg kg⁻¹ increased the shoot N content while the higher levels decreased the N content. The treatment effect was almost similar to that of first harvest with slightly higher values for nitrogen during second harvest.

At both the harvests, shoot phosphorous content was significantly influenced by the treatments and their interactions (Table 18).

Table.16 Effect of treatments on total biomass of amaranthus (g plant⁻¹)

Treatments	M1	M2	M3	M4	mean				
F0Z0	201.10	211.75	191.95	265.22	217.50				
F0Z1	197.16	234.51	212.19	304.17	237.01				
F0Z2	210.87	309.53	257.98	355.83	283.55				
F0Z3	200.65	229.03	317.17	295.71	260.64				
F1Z0	181.77	192.35	246.63	333.67	238.60				
F1Z1	234.34	326.57	204.82	311.73	269.36				
F1Z2	192.16	235.44	231.95	262.17	230.43				
F1Z3	203.44	216.61	287.78	214.80	230.65				
F2Z0	140.67	171.46	221.53	315.93	212.39				
F2Z1	221.50	276.97	191.23	266.80	239.12				
F2Z2	227.90	310.72	188.54	245.09	243.06				
F2Z3	147.44	319.27	177.68	292.43	234.20				
F3Z0	223.51	205.74	174.42	249.34	210.75				
F3Z1	232.21	305.76	221.62	261.88	255.37				
F3Z2	189.66	191.88	236.94	310.79	232.31				
F3Z3	162.88	240.17	242.63	327.68	243.34				
	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	202.44	202.93	184.38	202.06	186.76	221.30	205.14	178.60	197.95
M2	246.20	242.74	269.60	233.39	192.82	285.95	261.89	251.27	247.98
M3	244.82	242.79	194.74	218.90	208.63	207.46	228.85	256.31	225.31
M4	305.23	280.59	280.06	287.42	291.04	286.15	293.47	282.65	288.33
Mean	249.67	242.26	232.20	235.44	219.81	250.21	247.34	242.21	
CD(0.05)M/ Z-14.1987			CD(0.05)ZM/FZ- 28.3974			CD(0.05) FZM- 56.7949			

Table 17. Effect of treatments on shoot nitrogen content (%) of amaranthus

Treatments	I cut					II cut				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
F0Z0	2.55	2.33	2.55	2.44	2.47	3.25	3.03	3.25	3.14	3.17
F0Z1	2.55	3.81	2.52	2.64	2.88	3.25	4.51	3.23	3.34	3.58
F0Z2	2.72	3.42	2.72	2.66	2.88	3.42	4.12	3.43	3.37	3.58
F0Z3	2.66	3.48	2.72	2.33	2.79	3.37	4.18	3.43	3.03	3.50
F1Z0	2.61	3.34	2.78	2.47	2.80	3.31	4.03	3.48	3.17	3.50
F1Z1	2.52	2.94	3.22	2.49	2.79	3.23	3.65	3.93	3.20	3.50
F1Z2	2.75	2.69	3.20	2.33	2.74	3.45	3.40	3.90	3.03	3.44
F1Z3	2.75	2.66	3.22	2.24	2.72	3.45	3.37	3.93	2.95	3.42
F2Z0	2.72	2.32	2.58	2.05	2.41	3.43	3.03	3.28	2.75	3.12
F2Z1	2.69	2.38	2.78	2.80	2.66	3.40	3.09	3.48	3.51	3.37
F2Z2	2.80	2.52	2.66	1.99	2.49	3.51	3.23	3.37	2.69	3.20
F2Z3	3.00	2.68	2.80	2.08	2.64	3.70	1.89	3.51	2.78	2.97
F3Z0	2.89	2.10	2.47	2.38	2.46	3.59	2.81	3.17	3.09	3.16
F3Z1	3.05	2.52	2.75	2.72	2.76	3.76	3.23	3.45	3.42	3.46
F3Z2	3.45	2.55	2.55	2.50	2.76	4.15	3.25	3.25	3.20	3.46
F3Z3	3.17	2.30	2.86	2.44	2.69	3.87	3.00	3.56	3.15	3.39
CD (0.05) FZM - 0.4458						CD (0.05) FZM - 0.4432				

Two way Table

I cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	2.62	2.65	2.80	3.14	2.69	2.70	2.93	2.89	2.80
M2	3.26	2.91	2.47	2.37	2.52	2.91	2.79	2.78	2.75
M3	2.63	3.10	2.70	2.65	2.59	2.81	2.78	2.90	2.77
M4	2.51	2.38	2.23	2.51	2.33	2.66	2.37	2.27	2.41
Mean	2.75	2.76	2.55	2.67	2.53	2.77	2.72	2.71	
CD (0.05) M / Z / F -0.1115					CD (0.05) MZ / FZ / MF - 0.2229				
II cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	3.32	3.36	3.51	3.84	3.39	3.41	3.63	3.60	3.51
M2	3.96	3.61	2.81	3.07	3.23	3.62	3.50	3.11	3.36
M3	3.33	3.81	3.41	3.36	3.29	3.52	3.48	3.60	3.48
M4	3.22	3.09	2.93	3.21	3.04	3.37	3.07	2.97	3.11
Mean	3.46	3.47	3.16	3.37	3.24	3.48	3.42	3.32	
CD (0.05) M / Z/F -0.1108					CD (0.05) MZ / FZ / MF -0.2216				

Table 18. Effect of treatments on shoot phosphorous content (%) of amaranthus

Treatments	I cut					II cut				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
F0Z0	0.04	0.06	0.05	0.04	0.05	0.07	0.06	0.05	0.04	0.06
F0Z1	0.03	0.05	0.05	0.03	0.04	0.03	0.05	0.06	0.04	0.04
F0Z2	0.04	0.06	0.04	0.04	0.04	0.04	0.06	0.05	0.04	0.04
F0Z3	0.04	0.05	0.07	0.05	0.05	0.04	0.05	0.07	0.05	0.05
F1Z0	0.05	0.05	0.04	0.04	0.04	0.05	0.06	0.04	0.05	0.05
F1Z1	0.05	0.05	0.04	0.03	0.04	0.05	0.05	0.04	0.04	0.04
F1Z2	0.05	0.04	0.04	0.03	0.04	0.05	0.05	0.04	0.03	0.04
F1Z3	0.06	0.08	0.04	0.03	0.05	0.06	0.08	0.04	0.04	0.05
F2Z0	0.02	0.05	0.03	0.03	0.03	0.02	0.06	0.03	0.04	0.04
F2Z1	0.02	0.06	0.04	0.03	0.04	0.02	0.06	0.04	0.03	0.04
F2Z2	0.01	0.05	0.04	0.05	0.04	0.01	0.06	0.04	0.05	0.04
F2Z3	0.01	0.04	0.04	0.04	0.03	0.01	0.04	0.04	0.04	0.03
F3Z0	0.02	0.07	0.04	0.04	0.04	0.03	0.08	0.04	0.04	0.05
F3Z1	0.06	0.06	0.04	0.04	0.05	0.06	0.06	0.05	0.04	0.05
F3Z2	0.04	0.06	0.04	0.04	0.04	0.04	0.06	0.05	0.04	0.05
F3Z3	0.08	0.08	0.04	0.03	0.06	0.08	0.08	0.04	0.04	0.06
CD(0.05) FZM - 0.0129						CD(0.05) FZM - 0.0149				

Two way Table

I cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	0.04	0.05	0.01	0.05	0.03	0.04	0.03	0.04	0.04
M2	0.05	0.05	0.05	0.07	0.06	0.05	0.05	0.06	0.06
M3	0.05	0.04	0.04	0.04	0.04	0.04	0.04	0.05	0.04
M4	0.04	0.03	0.04	0.04	0.04	0.03	0.04	0.04	0.04
Mean	0.04	0.04	0.03	0.05	0.04	0.04	0.04	0.05	
CD (0.05) M/ Z/ F-0.0032					CD (0.05) MZ / FZ / MF -0.0032				
II cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	0.05	0.05	0.01	0.05	0.04	0.04	0.04	0.05	0.04
M2	0.06	0.06	0.05	0.07	0.06	0.06	0.05	0.06	0.06
M3	0.05	0.04	0.04	0.04	0.04	0.05	0.04	0.05	0.05
M4	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Mean	0.05	0.05	0.04	0.05	0.05	0.04	0.04	0.05	
CD (0.05) M / Z / F-0.0037					CD (0.05) MZ / FZ / MF--0.0074				

The highest P content at the first harvest was recorded by treatment combinations viz. M1F3Z3, (AMF with Fe and Zn as foliar) M2F1Z3 (P solubilizer x Fe @ 5 mg kg⁻¹ x Zn as foliar) and M2F3Z3 (P solubilizer with Fe and Zn as foliar). At the second harvest, along with the above treatments, M2F3Z0 (P solubilizer with Fe as foliar and Zn @ zero) has also recorded the same highest value. Considering the individual effects of bioaugmentation, P solubilizer had significantly increased the shoot P content at both the harvests. In the case of Fe and Zn, foliar application of both significantly influenced the P content at first harvest. At the second harvest the levels of Fe showed a positive trend for shoot P only up to Fe @ 5 mg kg⁻¹. And for the levels of Zn, soil application showed a negative relation with P content of shoot.

At both the harvests, treatments and their interactions had significantly influenced the shoot K content (Table 19). The highest value was recorded by M3F0Z3 (K solubilizer with Fe @ zero and Zn as foliar) at both the harvests. Considering the individual effect of bioaugmentation methods, addition of K solubilizer had significantly increased the K content of shoot. Evaluating the individual effect of Fe and Zn, a definite pattern of relationship with shoot K content were not observed. Same trend was observed for second harvest also.

4.5.2 Effect of treatments on calcium, magnesium and sulphur contents

The data on calcium, magnesium and sulphur contents of shoot are presented in Tables 20 to 22 respectively.

The treatments and their interactions were significant for shoot calcium content at both the harvests (Table 20). The highest values was recorded by M3F0Z1 (K solubilizer x Fe @ control x Zn @ 5 mg kg⁻¹). Considering the individual effect of bioaugmentation, K solubilizer recorded the highest value and was also on par with treatments receiving AMF or P solubilizer. A definite relation with levels of Fe and Zn were not noticed. At second harvest also, the same trend was observed.

Table 19. Effect of treatments on shoot potassium content (%) of amaranthus

Treat-ments	I cut					II cut				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
F0Z0	5.28	6.80	5.30	4.60	5.50	5.80	7.32	5.82	5.12	6.02
F0Z1	4.68	4.01	6.84	4.06	4.90	5.20	4.53	7.36	4.58	5.42
F0Z2	3.58	4.24	5.61	3.58	4.25	4.10	3.76	6.13	4.11	4.52
F0Z3	5.60	4.74	7.04	5.60	5.75	6.12	5.27	7.56	6.12	6.27
F1Z0	4.62	4.13	6.67	4.84	5.07	5.14	4.65	7.19	5.36	5.59
F1Z1	5.24	3.49	6.04	3.73	4.63	5.76	3.51	6.56	4.26	5.02
F1Z2	5.32	5.40	6.12	4.40	5.31	5.84	5.92	6.64	4.92	5.83
F1Z3	4.18	5.57	5.38	3.79	4.73	4.70	6.09	5.90	4.31	5.25
F2Z0	6.02	5.90	5.48	5.98	5.84	6.54	6.42	6.00	6.50	6.36
F2Z1	5.72	5.64	4.38	3.82	4.89	6.24	6.16	4.90	4.34	5.41
F2Z2	6.70	3.59	4.18	5.22	4.92	7.22	4.11	4.70	5.74	5.44
F2Z3	4.20	4.56	5.09	4.69	4.64	4.72	5.08	5.61	4.22	4.91
F3Z0	6.00	3.52	5.45	6.12	5.27	6.52	4.03	5.97	6.64	5.79
F3Z1	5.00	5.98	3.74	3.97	4.67	5.52	6.50	4.26	4.49	5.19
F3Z2	5.46	4.32	5.48	4.10	4.84	5.98	4.84	6.00	4.62	5.36
F3Z3	5.06	4.48	3.90	4.09	4.38	5.58	5.00	4.42	4.61	4.90
CD (0.05) FZM - 1.1096						CD (0.05) FZM - 1.2907				

Two way Table

I cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	4.79	4.84	5.66	5.38	5.48	5.16	5.27	4.76	5.17
M2	4.95	4.65	4.92	4.57	5.09	4.78	4.39	4.84	4.77
M3	6.20	6.05	4.78	4.64	5.72	5.25	5.35	5.35	5.42
M4	4.46	4.19	4.93	4.57	5.39	3.89	4.33	4.55	4.54
Mean	5.10	4.93	5.07	4.79	5.42	4.77	4.83	4.87	
CD (0.05) M / F- 0.2774					CD (0.05) MZ / FZ / MF- 0.5548				
II cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	5.31	5.36	6.18	5.90	6.00	5.68	5.79	5.28	5.69
M2	5.22	5.04	5.44	5.09	5.61	5.18	4.66	5.36	5.20
M3	6.72	6.57	5.30	5.16	6.25	5.77	5.87	5.87	5.94
M4	4.98	4.71	5.20	5.09	5.90	4.42	4.85	4.81	4.99
Mean	5.56	5.42	5.53	5.31	5.94	5.26	5.29	5.33	
CD (0.05) M / Z -0.3227					CD (0.05) MZ / FZ / MF--0.6453				

Table 20. Effect of treatments on shoot calcium content (%) of amaranthus

Treat-ments	I cut					II cut				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
F0Z0	3.80	3.880	4.520	4.520	4.050	4.24	5.44	6.72	6.72	5.78
F0Z1	4.120	4.000	5.760	5.400	4.820	5.92	5.68	9.20	8.48	7.32
F0Z2	3.760	4.320	3.280	4.400	3.940	5.20	6.32	4.24	6.48	5.56
F0Z3	4.00	3.240	4.160	3.520	3.730	5.68	4.16	6.00	4.72	5.14
F1Z0	3.960	3.720	5.000	3.080	3.940	5.60	5.12	7.68	3.84	5.56
F1Z1	4.200	4.000	3.960	3.640	3.950	6.08	5.68	5.60	4.96	5.58
F1Z2	4.080	3.720	3.240	3.720	3.690	5.84	5.12	4.16	5.12	5.06
F1Z3	5.520	4.040	4.800	3.680	4.510	8.72	5.76	7.28	5.04	6.70
F2Z0	4.920	4.680	4.280	3.520	4.350	7.52	7.04	6.24	4.72	6.38
F2Z1	4.640	3.920	3.280	3.00	3.710	6.96	5.52	4.24	3.68	5.10
F2Z2	5.080	4.040	5.560	4.000	4.670	7.84	5.76	8.80	5.68	7.02
F2Z3	4.760	4.280	3.120	3.200	3.840	7.20	6.24	3.92	4.08	5.36
F3Z0	4.320	4.320	5.280	3.880	4.450	6.32	6.32	8.24	5.44	6.58
F3Z1	4.120	4.960	4.960	4.200	4.560	5.92	7.60	7.60	6.08	6.80
F3Z2	3.360	4.520	4.680	4.040	4.150	4.40	6.72	7.04	5.76	5.98
F3Z3	3.440	4.680	4.240	4.320	4.170	4.56	7.04	6.16	6.32	6.02
CD (0.05) FZM - 0.95258						CD (0.05) FZM - 1.9051				

Two way Table

I cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	3.790	4.440	4.850	3.810	4.120	4.270	4.070	4.430	4.223
M2	3.860	3.870	4.230	4.620	4.150	4.220	4.150	4.060	4.145
M3	4.430	4.250	4.060	4.790	4.770	4.490	4.190	4.080	4.383
M4	4.460	3.530	3.430	4.110	3.750	4.060	4.040	3.680	3.883
Mean	4.135	4.023	4.143	4.333	4.198	4.260	4.113	4.063	
CD (0.05) M / Z / F-0.23814					CD (0.05) MZ / FZ / MF--0.47629				
II cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	5.26	6.56	7.38	5.30	5.92	6.22	5.82	6.54	6.13
M2	5.40	5.42	6.14	6.92	5.98	6.12	5.98	5.80	5.97
M3	6.54	6.18	5.80	7.26	7.22	6.66	6.06	5.84	6.45
M4	6.60	4.74	4.54	5.90	5.18	5.80	5.76	5.04	5.45
Mean	5.95	5.72	5.97	6.35	6.08	6.20	5.90	5.81	
CD (0.05) M -0.4763					CD (0.05) MZ / FZ / MF--0.9526				

Table 21. Effect of treatments on shoot magnesium content (%) of amaranthus

Treatments	I cut					II cut				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
F0Z0	5.76	3.41	2.11	0.53	2.95	6.34	3.99	2.69	1.11	3.53
F0Z1	3.65	4.08	1.15	0.82	2.42	4.23	4.65	1.73	1.39	3.00
F0Z2	4.56	3.32	5.48	0.91	3.57	5.14	3.88	6.05	1.49	4.14
F0Z3	4.56	5.33	3.79	1.25	3.73	5.14	5.91	4.37	0.86	4.07
F1Z0	5.33	5.52	3.31	2.26	4.10	5.90	6.10	3.89	1.54	4.36
F1Z1	5.28	4.80	4.08	0.96	3.78	5.86	5.38	4.65	0.96	4.21
F1Z2	3.08	5.81	5.56	1.49	3.99	3.65	6.38	6.15	0.82	4.25
F1Z3	4.27	5.62	3.84	1.58	3.83	4.85	6.19	4.42	1.20	4.17
F2Z0	4.18	2.78	4.56	1.96	3.37	4.08	3.36	5.14	1.11	3.42
F2Z1	3.75	5.28	3.84	2.74	3.90	4.32	5.86	4.42	1.40	4.00
F2Z2	2.69	5.28	1.88	1.88	2.93	3.26	5.86	2.45	1.39	3.24
F2Z3	3.32	4.90	4.18	1.59	3.49	3.88	5.48	4.76	2.16	4.07
F3Z0	3.46	4.13	2.30	1.58	2.87	4.03	4.71	2.88	1.44	3.26
F3Z1	4.51	4.18	3.51	1.78	3.49	5.09	4.76	4.08	1.82	3.94
F3Z2	4.99	3.41	5.14	1.63	3.79	5.57	3.99	5.72	1.35	4.15
F3Z3	5.28	5.04	7.20	1.54	4.76	5.86	5.62	7.77	1.15	5.10
CD(0.05) FZM - 1.8982						CD (0.05) FZM - 1.8903				

Two way Table

I cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	4.63	4.49	3.48	4.56	4.68	4.30	3.83	4.36	4.29
M2	4.03	5.44	4.56	4.19	3.96	4.58	4.45	5.22	4.55
M3	3.13	4.20	3.61	4.54	3.07	3.14	4.51	4.75	3.87
M4	0.88	1.57	2.04	1.63	1.58	1.57	1.48	1.49	1.53
Mean	3.17	3.92	3.42	3.73	3.32	3.40	3.57	3.95	
CD (0.05) M / Z / F - 0.4746					CD (0.05) MZ / FZ / MF-- 0.9491				
II cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	5.21	5.07	3.89	5.14	5.09	4.87	4.40	4.93	4.82
M2	4.61	6.01	5.14	4.77	4.54	5.16	5.03	5.80	5.13
M3	3.71	4.78	4.19	5.11	3.65	3.72	5.09	5.33	4.45
M4	1.21	1.13	1.51	1.44	1.30	1.39	1.26	1.34	1.32
Mean	3.68	4.25	3.68	4.11	3.64	3.79	3.94	4.35	
CD (0.05) M / Z / F - 0.4726					CD (0.05) MZ / FZ / MF-- 0.9452				

Magnesium content was significantly influenced by the treatments and their interactions (Table 21). M3F3Z3 (K solubilizer with Fe and Zn as foliar application) has recorded the highest value. Considering the individual effect, P solubilizer (M2) was significantly superior to POP (M4), which recorded the lowest value. Application of Fe @ 5 mg kg⁻¹ enhanced the Mg content. Though the Mg content increased with levels of Zn, the foliar application (Z3) showed the highest value compared to soil application. At second harvest also the same trend was followed.

Shoot sulphur content was significantly influenced by the treatments and their interactions (Table 22). The highest value was recorded by M3F1Z0 (K solubilizer x Fe @ 5 mg kg⁻¹ x Zn @ zero). Considering the individual effects, P solubilizer (M2) had increased the S content and was significantly superior to AMF (M1). A definite pattern was not observed for the levels of Fe and Zn for shoot S content. At second harvest also the same trend was repeated.

4. 5.3 Effect of treatment on shoot iron, manganese, zinc and copper contents

The data on iron, manganese, zinc and copper contents of shoot are presented in Tables 23 to 26 respectively.

Iron content in the shoot (Table 23) was significantly influenced by the bioaugmentation methods and its interaction with Fe alone (M x F) and with Fe and Zn (M x F x Z). Fe content was highest for M1F3Z0 (AMF x Fe as foliar x Zn @ zero). Among the bioaugmentation methods, AMF was significantly superior to K solubilizer (M3) and POP (M4). Soil application of Fe @ 5 mg kg⁻¹ enhanced the Fe content but not significant for first harvest. At second harvest also the behaviour of shoot Fe followed the same trend except that the individual effect of Fe became significant.

Individual as well as interactional effect of microbial treatments, were significant for shoot manganese content (Table 24). The highest value was observed for M1F3Z1 (AMF x Fe @ foliar x Zn @ 5 mg kg⁻¹). Considering the individual effect of bioaugmentation, P solubilizer (M2) and POP (M4) were on

Table 22. Effect of treatments on shoot sulphur content (mg kg⁻¹) of amaranthus

Treatments	I cut					II cut				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
F0Z0	167.3	132.7	182.3	202.9	171.3	177.7	143.2	192.8	213.4	181.7
F0Z1	192.9	182.9	200.6	170.8	186.7	203.3	192.8	211.1	181.3	197.1
F0Z2	225.4	216.0	218.8	192.9	213.3	235.9	226.5	229.2	203.4	223.7
F0Z3	177.5	264.6	127.5	173.8	185.8	188.0	275.0	138.0	184.2	196.3
F1Z0	215.0	180.0	495.2	166.9	264.3	225.5	190.5	505.7	177.3	274.7
F1Z1	220.0	237.3	71.5	183.3	178.0	230.5	247.7	81.9	193.8	188.5
F1Z2	238.3	248.5	117.5	175.0	194.8	248.8	259.0	127.9	185.5	205.3
F1Z3	175.4	180.0	58.6	172.3	146.6	185.9	190.5	69.0	182.7	157.0
F2Z0	111.3	202.3	136.0	181.3	157.7	121.7	212.8	146.5	191.7	168.1
F2Z1	160.2	226.7	181.9	191.7	190.1	170.6	237.1	192.3	202.1	200.5
F2Z2	185.8	247.1	218.1	217.3	217.1	196.3	257.5	228.6	227.7	227.5
F2Z3	149.8	188.5	192.5	178.3	177.3	160.2	199.0	203.0	188.8	187.7
F3Z0	131.3	131.7	178.1	189.8	157.7	141.7	142.1	188.6	200.2	168.1
F3Z1	133.3	174.0	191.5	229.2	182.0	143.8	184.4	201.9	239.6	192.4
F3Z2	143.6	217.7	219.8	229.0	202.5	154.0	228.2	230.2	239.4	212.9
F3Z3	135.0	208.1	215.0	208.8	191.7	145.4	218.6	225.4	219.2	202.1
CD (0.05) FZM - 82.8740						CD (0.05) FZM - 82.8723				
Two way Table										
I cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean	
M1	190.7	212.1	151.7	135.7	156.2	176.6	198.2	159.4	172.6	
M2	198.9	211.4	216.1	182.8	161.6	205.0	232.3	210.3	202.3	
M3	182.2	185.6	182.1	201.0	247.9	161.3	193.5	148.3	187.8	
M4	185.1	174.3	192.1	214.1	185.2	193.7	203.5	183.2	191.4	
Mean	189.2	195.9	185.5	183.4	187.7	184.1	206.9	175.3		
CD (0.05) M / Z - 20.7185					CD (0.05) MZ / FZ / MF-- 41.4370					
II cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean	
M1	201.2	222.6	162.1	146.1	166.6	187.0	208.7	169.8	183.0	
M2	209.3	221.8	226.5	193.3	172.1	215.4	242.7	220.7	212.7	
M3	192.7	196.1	192.5	211.5	258.3	171.7	203.9	158.8	198.2	
M4	195.5	184.8	202.5	224.5	195.6	204.1	213.9	193.7	201.8	
Mean	199.7	206.3	195.9	193.9	198.1	194.6	217.3	185.7		
CD (0.05) M / Z - 20.718					CD (0.05) MZ / FZ / MF - 41.436					

Table 23. Effect of treatments on shoot iron content (mg kg⁻¹) of amaranthus

Treat-ments	I cut					II cut				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
F0Z0	854.5	659.0	1268.0	460.5	1560.5	880.4	685.0	1294.0	486.6	836.5
F0Z1	852.5	729.0	1286.5	579.0	861.8	878.40	754.8	1312.6	554.6	875.1
F0Z2	851.0	906.0	1275.0	566.0	899.5	876.6	932.0	1300.8	567.0	919.1
F0Z3	857.0	1026.0	1275.0	581.5	934.9	1160.8	1051.8	1301.0	607.4	1030.2
F1Z0	1448.5	1010.0	880.0	908.5	1061.8	1474.2	1036.0	906.0	934.4	1087.6
F1Z1	1226.0	877.0	834.0	890.5	956.9	1251.8	903.0	893.6	916.8	991.3
F1Z2	1302.0	853.5	890.0	915.5	990.3	1328.0	879.40	916.0	941.4	1016.2
F1Z3	1492.5	1264.5	752.0	876.0	1096.3	1018.4	1290.6	777.6	901.8	997.1
F2Z0	1279.5	883.5	485.0	884.5	883.1	1305.6	909.2	510.6	910.8	909.0
F2Z1	1265.0	908.0	467.0	855.5	873.8	1290.8	934.2	493.0	881.4	899.8
F2Z2	1261.5	921.0	462.0	844.5	872.3	1312.6	947.4	488.2	870.6	904.7
F2Z3	1286.5	959.0	657.5	869.5	943.1	1312.6	985.4	683.2	895.4	969.1
F3Z0	1619.0	684.5	461.5	852.5	904.4	1644.8	710.4	487.2	878.8	930.3
F3Z1	1451.5	857.5	867.5	893.5	1017.5	1477.4	833.2	893.6	919.2	1030.8
F3Z2	1299.5	877.0	854.5	880.5	977.9	1325.2	903.0	880.4	906.2	1003.7
F3Z3	1269.0	921.0	452.5	851.0	873.4	1294.8	947.0	478.4	876.8	899.2
CD (0.05) FZM - NS						CD (0.05) FZM - 191.0144				

Two way Table

I cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	853.8	1367.3	1273.1	1409.8	1300.4	1198.8	1178.5	1226.3	1226.0
M2	1080.0	1001.3	917.9	835.0	1559.3	842.9	889.4	1042.6	953.5
M3	1276.1	839.0	517.9	659.0	773.6	863.8	870.4	784.3	823.0
M4	546.8	897.6	863.5	869.4	776.5	804.6	801.6	794.5	794.3
Mean	939.2	1026.3	893.1	943.3	1102.4	927.5	935.0	961.9	
CD (0.05) M - 65.5609					CD (0.05) MF- 531.1217				
II cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	949.1	1268.1	1305.4	1435.6	1326.3	1224.6	1210.6	1196.7	1239.5
M2	855.9	1027.3	944.1	848.4	835.2	856.3	915.5	1068.7	918.9
M3	1302	873.3	543.8	684.9	799.5	898.2	896.4	810.1	851.0
M4	553.9	923.6	889.6	895.3	802.7	818.0	821.3	820.4	815.6
Mean	915.2	1023.1	920.7	966.0	940.9	949.3	960.9	973.9	
CD (0.05) M / F- 47.7536					CD (0.05) MZ / FZ / MF -47.7536				

Table 24. Effect of treatments on shoot manganese content (mg kg⁻¹) of amaranthus

Treat-ments	I cut					II cut				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
F0Z0	86.65	92.05	136.25	144.00	114.74	87.76	93.14	137.34	145.10	115.84
F0Z1	157.80	228.75	132.10	125.95	161.15	158.90	229.80	133.18	127.02	162.23
F0Z2	150.30	227.20	164.50	124.90	166.73	151.36	228.29	165.58	125.98	167.80
F0Z3	129.10	192.30	140.40	97.55	139.84	130.20	193.36	141.48	98.60	140.91
F1Z0	87.70	183.65	83.00	164.50	129.71	88.82	184.72	84.09	165.56	130.80
F1Z1	154.75	102.05	118.55	194.40	142.44	155.82	153.10	119.62	195.52	156.02
F1Z2	149.05	127.30	101.85	213.15	147.84	150.14	128.38	102.92	214.26	148.93
F1Z3	60.00	163.15	130.35	99.00	113.13	111.04	164.26	81.44	100.06	114.20
F2Z0	91.05	87.70	112.90	191.00	120.86	92.14	88.76	113.94	692.88	246.93
F2Z1	87.20	115.65	139.05	124.30	116.55	88.28	116.72	140.12	125.40	117.63
F2Z2	67.50	123.30	153.80	157.85	125.61	68.56	124.38	154.88	158.94	126.69
F2Z3	65.15	159.40	165.40	107.70	124.41	66.22	160.50	166.48	108.78	125.50
F3Z0	161.75	201.05	174.05	187.75	181.15	162.86	202.14	175.10	188.82	182.23
F3Z1	247.35	211.50	150.95	179.05	197.21	248.40	212.62	152.02	180.14	198.30
F3Z2	202.90	84.10	155.90	128.00	142.73	203.98	185.16	156.96	129.08	168.80
F3Z3	228.10	133.30	108.40	166.95	159.19	229.16	134.42	109.48	168.00	160.27
CD (0.05) FZM - 74.9599						CD (0.05) FZM - 75.0161				

Two way Table

I cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	130.96	112.88	77.73	210.03	106.79	161.78	142.44	120.59	132.90
M2	185.08	144.04	121.51	157.49	141.11	164.49	140.48	162.04	152.03
M3	143.31	108.44	142.79	147.33	126.55	135.16	144.01	136.14	135.47
M4	123.10	167.76	270.41	165.44	129.78	155.93	155.98	117.80	150.43
Mean	145.61	133.28	153.11	170.07	126.05	154.34	145.73	134.14	
CD (0.05) M / Z / F -18.7400					CD (0.05) MZ / F / MF - 37.4799				
II cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	132.06	126.46	78.80	211.10	107.90	162.85	143.51	134.16	137.10
M2	186.15	157.62	122.59	183.59	142.19	178.06	166.55	163.14	162.48
M3	144.39	97.02	143.86	148.39	127.62	136.23	145.08	124.72	133.41
M4	124.18	168.85	271.50	166.51	298.09	157.02	157.07	118.86	182.76
Mean	146.69	137.48	154.19	177.40	168.95	158.54	153.05	135.22	
CD (0.05) M / Z / F - 18.7540					CD (0.05) MZ / FZ / MF - 37.5081				

par with each other and were significantly superior to other treatments. The relationship between Fe and Zn had not shown any definite pattern. For both the harvests, the results followed the same trend.

Individual as well as interaction effects were significant for zinc content for both the harvests (Table 25). M2F0Z3 (P solubilizer x Fe @ control x Zn @ foliar) recorded the highest Zn content. Considering the individual effects of microbial treatments, P solubilizer (M2) significantly increased the Zn content compared to other treatments. With increasing levels of Fe, the shoot Zn content decreased.

Though the Zn content increases with the levels of Zn, the highest value was observed for foliar application of Zn. The same trend was observed for the second harvest also.

Treatment effect for individual factors and their interactions were significant for shoot copper content at both the harvests (Table 26). M2F3Z3 (K solubilizer with Fe and Zn given as foliar application) recorded the highest value. Considering the individual microbial effect, AMF (M1) and P solubilizer (M2) were significantly superior to others. For the second harvest, treatment combination M2F0Z1 (P solubilizer x Fe @ control x Zn @ 5mg kg⁻¹) showed the highest value. The individual effect of levels of Fe and Zn had not shown any definite pattern on Cu content of shoot for both the harvests.

4.6 Quality aspects

4.6.1 Nutritional factors : β – carotene, vitamin C and crude protein

The data on β – carotene, vitamin-C and crude protein of shoot are presented in Tables 27 to 29 respectively. The β carotene in the shoot was significantly influenced by the treatments and their interactions for the first harvest only (Table 27). M3F2Z3 (K solubilizer x Fe @ 10 mg kg⁻¹ Zn @ foliar) recorded the highest value. Considering the individual effect of bioaugmentation methods, K solubilizer (M3) significantly increased the β carotene content. In the

Table 25. Effect of treatments on shoot zinc content (mg kg⁻¹) of amaranthus

Treat-ments	I cut					II cut				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
F0Z0	102.65	76.70	56.15	42.70	69.55	155.82	115.72	68.38	57.10	99.26
F0Z1	107.20	111.35	64.00	86.90	92.36	107.04	194.38	113.72	99.70	128.71
F0Z2	138.65	189.95	68.40	91.90	122.23	143.06	196.37	69.62	96.62	126.42
F0Z3	151.45	192.00	107.05	95.65	136.54	111.58	81.08	111.42	91.32	98.85
F1Z0	87.05	74.95	56.90	58.95	69.46	106.28	107.82	119.70	92.20	106.50
F1Z1	101.70	97.20	65.30	87.80	88.00	91.41	53.52	69.70	178.14	98.19
F1Z2	101.85	112.85	115.30	158.75	122.19	106.14	117.64	61.30	163.14	112.06
F1Z3	107.05	125.55	127.25	173.70	133.39	111.44	99.18	131.62	63.32	101.39
F2Z0	63.20	67.85	49.75	53.00	58.45	75.88	92.24	54.14	116.88	84.79
F2Z1	63.80	86.60	83.15	63.00	74.14	72.52	150.20	100.32	67.36	97.60
F2Z2	66.65	101.35	95.95	96.10	90.01	68.20	105.72	137.56	100.54	103.01
F2Z3	73.00	145.80	118.15	112.50	112.36	67.63	91.00	122.58	57.41	84.66
F3Z0	45.90	67.65	95.65	50.65	64.96	99.86	132.22	166.44	75.30	118.46
F3Z1	51.40	71.70	106.95	53.35	70.85	127.28	76.10	112.24	55.04	92.67
F3Z2	95.50	111.30	107.90	63.35	94.51	155.80	147.94	111.36	57.74	118.21
F3Z3	122.85	160.10	162.05	70.90	128.98	50.26	72.04	100.04	67.74	72.52
CD (0.05) FZM - 28.3915						CD (0.05) FZM - 36.5171				

Two way Table

I cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	124.99	99.41	66.66	78.91	74.70	81.02	100.66	113.59	92.49
M2	142.50	102.64	100.40	102.69	71.79	91.71	128.86	155.86	112.06
M3	73.90	91.19	86.75	118.14	64.61	79.85	96.89	128.63	92.49
M4	79.29	119.80	81.15	59.56	51.32	72.76	102.53	113.19	84.95
Mean	105.17	103.26	83.74	89.83	65.61	81.34	107.23	127.82	
CD (0.05) M / Z / F - 7.0979					CD (0.05) MZ / MF - 14.1958				
II cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	129.37	103.82	71.06	108.30	109.46	99.56	118.30	85.23	103.14
M2	146.89	94.54	109.79	107.08	112.00	118.55	141.92	85.83	114.57
M3	90.79	95.58	103.65	122.52	102.17	98.99	94.96	116.42	103.13
M4	86.19	124.20	85.55	63.96	85.37	100.06	104.51	69.95	89.97
Mean	113.31	104.53	92.51	100.46	102.25	104.29	114.92	89.35	
CD (0.05) M / Z / F - 9.1293					CD (0.05) MZ / FZ / MF 18.2586-				

Table 26. Effect of treatments on shoot copper content (mg kg⁻¹) of amaranthus

Treat-ments	I cut					II cut				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
F0Z0	73.75	38.90	17.10	67.25	49.25	77.24	35.04	90.75	31.66	58.67
F0Z1	63.35	37.50	20.50	32.75	38.53	77.79	122.95	21.15	36.31	64.55
F0Z2	24.10	33.50	10.55	36.70	26.21	87.40	121.57	38.80	36.81	71.14
F0Z3	59.45	29.90	29.00	35.25	38.40	28.14	47.52	14.60	17.28	26.89
F1Z0	56.10	28.45	32.40	32.80	37.44	63.48	33.94	33.00	39.29	42.43
F1Z1	65.35	46.45	56.15	21.45	47.35	60.14	32.49	36.45	31.88	40.24
F1Z2	24.50	72.05	76.05	33.90	51.63	64.38	50.46	60.20	33.82	52.21
F1Z3	27.95	25.85	31.80	23.85	27.36	28.54	76.06	38.10	30.40	43.28
F2Z0	30.90	29.05	28.95	27.85	29.19	32.02	29.90	35.85	27.90	31.42
F2Z1	17.45	33.30	21.95	55.50	32.05	34.96	33.10	33.00	31.88	33.23
F2Z2	17.70	23.25	66.10	48.20	38.81	21.50	47.28	25.95	36.65	32.85
F2Z3	26.10	34.25	27.00	41.70	32.26	21.76	27.28	37.65	22.20	27.22
F3Z0	17.05	28.80	33.25	49.25	32.09	30.14	38.26	31.05	25.75	31.30
F3Z1	77.10	46.70	30.25	25.50	44.89	21.12	32.84	37.30	33.25	31.13
F3Z2	28.20	50.70	16.25	42.90	34.51	81.12	50.78	34.25	25.85	48.00
F3Z3	31.00	86.70	37.65	42.90	49.56	32.24	52.42	15.25	29.55	32.36
CD (0.05) FZM - 16.3430						CD (0.05) FZM - 4.9077				

Two way Table

I cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	55.16	53.48	23.04	38.34	44.45	55.81	23.63	36.13	40.00
M2	34.95	43.20	29.96	53.23	31.30	40.99	44.88	44.18	40.33
M3	19.29	49.10	36.00	29.35	27.93	32.21	42.24	31.36	33.43
M4	42.99	28.00	43.31	40.14	44.29	33.80	40.43	35.93	38.61
Mean	38.10	43.44	33.08	40.26	36.99	40.70	37.79	36.90	
CD (0.05) M / Z / F - 4.0857					CD (0.05) MZ / FZ / MF - 8.1715				
II cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	67.64	54.14	27.56	41.16	50.72	48.50	63.60	27.67	47.62
M2	81.77	48.24	34.39	43.57	34.29	55.35	67.52	50.82	51.99
M3	41.33	41.94	33.11	29.46	47.66	31.97	39.80	26.40	36.46
M4	30.51	33.85	29.66	28.60	31.15	33.33	33.28	24.86	30.65
Mean	55.31	44.54	31.18	35.70	40.95	42.29	51.05	32.44	
CD (0.05) M / Z / F - 1.2269					CD (0.05) MZ / FZ / MF - 2.4539				

Table 27. Effect of treatments on shoot beta carotene content ($\mu\text{g}/100\text{g}$) of amaranthus

Treatments	I cut					II cut				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
F0Z0	2793.7	2850.8	2874.4	2602.5	2780.3	2845.8	2902.9	2926.5	2654.7	2832.4
F0Z1	2771.3	2842.1	2902.9	2580.2	2774.1	2823.5	2894.2	2955.0	2632.3	2826.2
F0Z2	2807.3	2878.1	2856.9	2560.3	2775.7	2859.4	2930.2	2909.1	2612.5	2827.8
F0Z3	2746.5	2830.9	2838.4	2593.9	2752.4	1520.3	2883.0	2890.5	2645.9	5907.2
F1Z0	2767.6	2905.4	2993.5	2551.9	2804.6	2819.7	2957.5	3045.6	2604.0	2856.7
F1Z1	2775.0	2901.6	3033.2	2481.2	2797.7	2827.2	2953.8	3085.3	2533.3	2849.9
F1Z2	2830.9	2214.1	2756.4	2501.3	2575.7	2883.0	2266.2	2808.6	2553.4	2627.8
F1Z3	2837.1	2809.8	2921.5	2977.4	2886.4	2889.2	2861.9	2973.6	3029.5	2938.5
F2Z0	2812.3	2812.	2768.9	2941.4	2833.8	2864.4	2865.1	2820.9	2993.5	2885.9
F2Z1	2827.2	2784.9	2799.9	3193.3	2901.3	2879.3	2837.1	2852.0	3245.4	2953.5
F2Z2	2818.2	2813.5	3467.6	2880.8	2995	2870.3	2865.7	3519.7	2932.9	3047.1
F2Z3	2817.3	2778.8	3612.8	2835.9	3011.2	2869.4	2830.9	3664.9	2887.9	3063.3
F3Z0	2791.2	2829.7	2822.3	2742.8	2796.4	2843.3	2881.8	2874.4	2794.9	2848.6
F3Z1	2789.9	2829.7	2854.5	2936.4	2852.6	2842.1	2881.8	2906.6	2988.5	2904.8
F3Z2	2808.6	2858.2	2972.4	2875.6	2878.7	2860.7	2910.3	3024.5	2927.7	2930.8
F3Z3	2833.4	2868.1	2917.8	2827.2	2861.6	2885.5	2920.3	2969.9	2879.3	2913.7
CD (0.05) FZM - 212.3959						CD (0.05) FZM - NS				

Two way Table

I cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	2779.7	2802.6	2818.7	2805.7	2791.2	2790.8	2816.2	2808.6	2801.7
M2	2850.4	2708.7	2797.5	2846.4	2849.7	2839.6	2691.0	2821.9	2800.5
M3	2868.1	2926.2	3162.3	2891.7	2864.7	2897.6	3013.3	3072.6	2962.1
M4	2584.2	2627.9	2962.8	2845.5	2709.6	2797.8	2704.5	2808.6	2755.1
Mean	2770.6	2766.1	2935.3	2847.3	2803.8	2831.4	2806.2	2877.9	
CD (0.05) M / Z / F - 53.0990					CD (0.05) MZ / FZ / MF - 106.1979				
II cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	5934.5	2854.8	2870.8	287.9	2843.3	2843	2868.3	5963.3	3629.5
M2	2902.6	2759.9	2849.7	2898.5	2901.8	2891.7	2743.1	2874.0	2852.7
M3	2920.2	2978.3	3214.4	2943.8	2916.8	2949.7	3065.4	3124.7	3014.2
M4	2636.4	2680.0	3014.9	2897.6	2761.8	2849.9	2756.6	2860.7	2807.2
Mean	3598.4	2818.2	2987.5	2899.5	2855.9	2883.6	2858.4	3705.7	
CD (0.05) M / Z / F-NS					CD (0.05) MZ / FZ / MF-NS				

case of Fe, highest value was observed for Fe @ 10 mg kg⁻¹ (F2) and for Zn, Z1 (Zn @ 5 mg kg⁻¹ soil application) recorded the highest value. Compared to zero application, foliar application of Fe and Zn has enhanced the β carotene content. For the second harvest β carotene content was not significantly influenced by the treatments.

Vitamin C content in the shoot (Table 28) was significantly influenced only by the individual factors at the first harvest. Among the bioaugmentation methods, K solubilizer (M3) recorded the highest value which was significantly superior to other three treatments. Levels of Fe showed a positive relation with vitamin C content. A definite pattern of variation in vitamin C content was not observed for the levels of Zn. Vitamin C content at second harvest was significantly influenced by the individual as well as by the two way interactions viz., M x F, M x Z, and F x Z.

Crude protein content of the shoot was significant at both harvests (Table 29). Crude protein content was highest for M2F0Z1 (P solubilizer x Fe @ zero x Zn @ 5 mg kg⁻¹). Considering the individual effect of bioaugmentation methods, the highest value was observed for M1 (AMF), which was also on par with M3 (K solubilizer). In the case of individual effects of Fe and Zn, both up to @ 5 mg kg⁻¹ increased the crude protein content while the higher levels had decreased it. At second harvest also crude protein followed the same trend.

Table 28. Effect of treatments on vitamin C content (mg /100g) of amaranthus

Treat-ments	I cut					II cut				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
F0Z0	132.50	150.00	167.50	135.00	146.25	135.75	153.25	170.75	138.25	149.50
F0Z1	130.00	167.50	165.00	152.50	153.75	133.25	170.75	168.25	155.75	157.00
F0Z2	120.00	155.00	155.00	152.50	145.63	123.25	158.25	158.25	155.75	148.88
F0Z3	120.00	150.00	155.00	127.50	138.13	123.25	153.25	158.25	130.75	141.38
F1Z0	137.50	172.50	177.50	145.00	158.13	140.75	175.75	180.75	148.25	161.38
F1Z1	137.50	160.00	142.50	137.50	144.38	140.75	163.25	145.75	140.75	147.63
F1Z2	127.50	147.50	135.00	147.50	139.38	130.75	150.75	138.25	150.75	142.63
F1Z3	142.50	120.00	160.00	165.00	146.88	145.75	123.25	163.25	168.25	150.13
F2Z0	140.00	155.00	182.50	120.00	149.38	143.25	158.25	185.75	123.25	152.63
F2Z1	130.00	167.50	187.50	137.50	155.63	133.25	170.75	190.75	140.75	158.88
F2Z2	147.50	180.00	175.00	145.00	161.88	150.75	183.25	178.25	148.25	165.13
F2Z3	157.50	167.50	177.50	142.50	161.25	160.75	170.75	180.75	145.75	164.50
F3Z0	147.50	167.50	182.50	120.00	154.38	150.75	170.75	185.75	123.25	157.63
F3Z1	140.00	152.50	167.50	130.00	147.50	143.25	155.75	170.75	133.25	150.75
F3Z2	147.50	185.00	202.50	150.00	171.25	150.75	188.25	205.75	153.25	174.50
F3Z3	167.50	147.50	180.00	140.00	158.75	170.75	150.75	183.25	143.25	162.00
CD(0.05) FZM - NS					CD(0.05) FZM - NS					

Two way Table

I cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	125.6	136.3	143.8	150.6	139.4	134.4	135.6	146.9	139.1
M2	155.6	150.0	167.5	163.1	161.3	161.9	166.9	146.3	159.1
M3	160.6	153.8	180.6	183.1	177.5	165.6	166.9	168.1	169.5
M4	141.9	148.8	136.3	135.0	130.0	139.4	148.8	143.8	140.5
Mean	145.9	147.2	157.0	158.0	152.0	150.3	154.5	151.3	
CD (0.05) M / Z / F-6.5027					CD (0.05) MZ / FZ / MF- NS				
II cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	128.9	139.5	147.0	153.9	142.6	137.6	138.9	150.1	142.3
M2	158.9	153.3	170.8	166.4	164.5	165.1	170.1	149.5	162.3
M3	163.9	157.0	183.9	186.4	180.8	168.9	170.1	171.4	172.8
M4	145.1	152.0	139.5	138.3	133.3	142.6	152.0	147.0	143.7
Mean	149.2	150.4	160.3	161.2	155.3	153.6	157.8	154.5	
CD (0.05) M / F- 6.3660					CD (0.05) MZ / FZ / MF- 2.7319				

Table 29. Effect of treatments on crude protein content (%) of amaranthus

Treatments	I cut					II cut				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
F0Z0	15.92	14.53	15.92	15.23	15.40	20.28	18.91	20.28	19.59	19.77
F0Z1	15.92	23.80	15.75	16.45	17.98	20.28	28.14	20.16	20.84	22.35
F0Z2	16.98	21.35	16.98	16.63	17.98	21.34	25.71	21.40	21.03	22.37
F0Z3	16.63	21.70	16.98	14.53	17.46	21.03	26.08	21.40	18.91	21.86
F1Z0	16.28	20.83	17.33	15.40	17.46	20.65	25.15	21.72	19.78	21.82
F1Z1	15.75	18.38	20.13	15.58	17.46	20.16	22.78	24.52	19.97	21.86
F1Z2	17.15	16.80	19.95	14.53	17.11	21.53	21.22	24.34	18.91	21.50
F1Z3	17.15	16.63	20.13	14.00	16.98	21.53	21.03	24.52	18.41	21.37
F2Z0	16.98	14.53	16.10	12.78	15.09	21.40	18.91	20.47	17.16	19.48
F2Z1	16.80	14.88	17.33	17.50	16.63	21.22	19.28	21.72	21.90	21.03
F2Z2	17.50	15.75	16.63	12.43	15.58	21.90	20.16	21.03	16.79	19.97
F2Z3	18.72	7.35	17.50	12.95	14.13	23.09	11.79	21.90	17.35	18.53
F3Z0	18.03	13.13	15.40	14.88	15.36	22.40	17.53	19.78	19.28	19.75
F3Z1	19.08	15.75	17.15	16.97	17.24	23.46	20.16	21.53	21.34	21.62
F3Z2	21.53	15.92	15.92	15.58	17.24	25.90	20.28	20.28	19.97	21.61
F3Z3	19.78	14.35	17.85	15.23	16.80	24.15	18.72	22.21	19.66	21.18
CD (0.05) FZM - 2.7664						CD (0.05) FZM - 2.6672				

Two way Table

I cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	16.36	16.58	17.50	19.60	16.80	16.89	18.29	18.07	17.51
M2	20.34	18.16	13.13	14.79	15.75	18.20	17.46	15.01	16.60
M3	16.41	19.38	16.89	16.58	16.19	17.59	17.37	18.11	17.31
M4	15.71	14.88	13.91	15.66	14.57	16.63	14.79	14.17	15.04
Mean	17.20	17.25	15.36	16.66	15.83	17.33	16.97	16.34	
CD (0.05) M / Z / F-0.6916					CD (0.05) MZ / FZ / MF-1.3832				
II cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	20.75	21	21.94	24	21.19	21.31	22.69	22.50	21.89
M2	24.75	22.56	17.56	19.19	20.19	22.63	21.88	19.44	20.99
M3	20.81	23.81	21.31	21.00	20.56	22.00	21.75	22.50	347.26
M4	20.13	19.31	18.31	20.06	19.00	21.06	19.19	18.56	19.43
Mean	21.61	21.67	19.78	21.06	20.23	21.75	21.38	20.75	
CD (0.05) M / F-0.6893					CD (0.05) MZ / FZ / MF - 1.3786				

4.6.2 Anti-nutritional factors: Nitrate, phenol and oxalate

The data on nitrate, phenol and oxalate of shoot are presented in Tables 30 to 32 respectively. Individual effects of bioaugmentation methods (M), levels of Fe (F) and their interactions viz., (M x F) and (M x F x Z) showed significant influence on nitrate content of shoot (Table 30). Nitrate content was lowest for M2F0Z1 (P solubilizer x Fe @ foliar x Zn @ 5 mg kg⁻¹). Application of AMF (M1) had significantly increased the nitrate content and the same trend was observed for the second harvest also.

Phenol content (Table 31) was significantly influenced by the bioaugmentation methods (M) and with the levels of Fe (F). Lowest phenol content was recorded by M2 (P solubilizer) and the highest by M4 (POP). Phenol content increased with the levels of Fe for soil application while the foliar application decreased its content. Zn also behaved in the same manner.

Individual effect of bioaugmentation methods and levels of Fe alone had significant influence on oxalate content (Table 32) for the first harvest. Among the bioaugmentation methods, application of AMF (M1) showed the lowest value. Application of Fe @ 10 mg kg⁻¹ has enhanced the oxalate content. At second harvest also all the treatment effect was similar to the first. Interactional effects of bioaugmentation with levels of Fe and Zn were also significant.

Table 30. Effect of treatments on shoot nitrate content (%) of amaranthus

Treat-ments	I cut					II cut				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
F0Z0	0.34	0.36	0.33	0.34	0.34	0.34	0.37	0.33	0.34	0.34
F0Z1	0.36	0.29	0.39	0.36	0.35	0.36	0.29	0.39	0.36	0.35
F0Z2	0.33	0.31	0.38	0.36	0.34	0.33	0.31	0.38	0.36	0.34
F0Z3	0.34	0.37	0.33	0.33	0.34	0.35	0.38	0.34	0.33	0.35
F1Z0	0.30	0.32	0.33	0.34	0.32	0.30	0.32	0.34	0.34	0.33
F1Z1	0.31	0.33	0.37	0.35	0.34	0.32	0.34	0.38	0.35	0.35
F1Z2	0.32	0.31	0.33	0.35	0.33	0.33	0.31	0.33	0.36	0.33
F1Z3	0.33	0.32	0.35	0.34	0.33	0.33	0.32	0.35	0.35	0.34
F2Z0	0.43	0.33	0.37	0.35	0.37	0.43	0.33	0.38	0.35	0.37
F2Z1	0.42	0.33	0.34	0.33	0.35	0.42	0.33	0.35	0.33	0.36
F2Z2	0.42	0.33	0.30	0.33	0.34	0.42	0.34	0.30	0.34	0.35
F2Z3	0.43	0.33	0.33	0.35	0.36	0.43	0.33	0.34	0.35	0.36
F3Z0	0.43	0.41	0.31	0.34	0.37	0.43	0.41	0.31	0.34	0.37
F3Z1	0.33	0.40	0.35	0.31	0.35	0.34	0.41	0.36	0.31	0.35
F3Z2	0.43	0.43	0.32	0.30	0.37	0.43	0.43	0.32	0.31	0.37
F3Z3	0.37	0.38	0.34	0.35	0.36	0.37	0.38	0.34	0.35	0.36
CD(0.05) FZM - 0.0480						CD(0.05) FZM - 0.0489				

Two way Table

I cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	0.34	0.31	0.42	0.39	0.37	0.35	0.37	0.37	0.37
M2	0.33	0.32	0.33	0.40	0.35	0.34	0.34	0.35	0.34
M3	0.36	0.35	0.33	0.33	0.33	0.36	0.33	0.34	0.34
M4	0.35	0.34	0.34	0.32	0.34	0.34	0.33	0.34	0.34
Mean	0.34	0.33	0.36	0.36	0.35	0.35	0.34	0.35	
CD (0.05) M/F - 0.0120					CD (0.05) MF- 0.0240				
II cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	0.34	0.32	0.43	0.39	0.37	0.36	0.38	0.37	0.37
M2	0.34	0.32	0.33	0.41	0.36	0.34	0.35	0.35	0.35
M3	0.36	0.35	0.34	0.33	0.34	0.37	0.33	0.34	0.34
M4	0.35	0.35	0.34	0.32	0.34	0.34	0.34	0.34	0.34
Mean	0.35	0.34	0.36	0.36	0.35	0.35	0.35		
CD (0.05) M /F- 0.0122					CD (0.05) MF -0.0245				

Table 31. Effect of treatments on shoot phenol content (mg kg⁻¹) of amaranthus

Treat-ments	I cut					II cut				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
F0Z0	31.81	40.61	31.81	39.25	35.87	35.87	44.67	35.87	43.31	39.93
F0Z1	32.48	36.54	39.25	35.87	36.04	36.54	40.61	43.32	39.93	40.10
F0Z2	34.51	35.19	39.25	37.22	36.54	38.58	39.26	43.32	41.28	40.61
F0Z3	35.87	40.61	40.61	35.87	38.24	39.93	44.67	44.67	39.93	42.30
F1Z0	39.93	36.54	35.19	40.61	38.07	43.99	40.61	39.26	44.67	42.13
F1Z1	37.22	41.28	40.61	38.57	39.42	41.29	45.35	44.67	42.64	43.48
F1Z2	33.84	37.90	34.51	41.96	37.05	37.90	41.96	38.58	46.02	41.11
F1Z3	33.84	35.19	33.84	38.58	35.36	37.90	39.26	37.90	42.64	39.42
F2Z0	39.25	37.22	35.19	43.99	38.91	43.32	41.28	39.26	48.05	42.98
F2Z1	35.19	40.61	33.16	42.64	37.90	39.26	44.67	37.22	46.70	41.96
F2Z2	38.57	38.58	35.87	37.22	37.56	42.64	42.64	39.93	41.28	41.62
F2Z3	31.81	41.96	36.54	41.28	37.90	35.87	46.02	40.61	45.34	41.96
F3Z0	35.87	37.22	37.90	37.90	37.22	39.93	41.28	41.96	41.96	41.28
F3Z1	33.16	34.51	37.90	37.22	35.70	37.22	38.58	41.96	41.28	39.76
F3Z2	34.51	39.93	33.16	41.28	37.22	38.58	43.99	37.22	45.34	41.28
F3Z3	33.16	31.13	31.81	33.16	32.31	37.22	35.19	35.87	37.22	36.38
CD(0.05) FZM - 5.9915						CD(0.05) FZM - 5.9917				

Two way Table

I cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	33.67	36.21	36.21	34.18	36.71	34.51	35.36	33.67	35.06
M2	38.24	37.73	39.59	35.70	37.90	38.24	37.90	37.22	37.81
M3	37.73	36.04	35.19	35.19	35.02	37.73	35.70	35.70	36.04
M4	37.05	39.93	41.28	37.39	40.44	38.58	39.42	37.22	38.91
Mean	36.67	37.48	38.07	35.61	37.52	37.26	37.09	35.95	
CD (0.05) M / F-1.4979					CD (0.05) FZ -2.9958				
II cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	37.73	40.27	40.27	38.24	40.78	38.58	39.42	37.73	39.13
M2	42.30	41.79	43.65	39.76	41.96	42.30	41.96	41.28	41.87
M3	41.79	40.10	39.25	39.25	39.09	41.79	39.76	39.76	40.10
M4	41.11	43.99	45.34	41.45	44.50	42.63	43.48	41.28	42.97
Mean	40.73	41.54	42.13	39.67	41.58	41.32	41.16	40.01	
CD (0.05) M / F- 1.4979					CD (0.05) FZ - 2.9958				

Table 32. Effect of treatments on shoot oxalate content (%) of amaranthus

Treat-ments	I cut					II cut				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
F0Z0	0.15	0.19	0.15	0.18	0.17	0.17	0.21	0.17	0.20	0.19
F0Z1	0.15	0.17	0.18	0.17	0.17	0.17	0.19	0.20	0.19	0.19
F0Z2	0.16	0.16	0.18	0.17	0.17	0.18	0.18	0.20	0.19	0.19
F0Z3	0.17	0.19	0.19	0.17	0.18	0.19	0.21	0.21	0.19	0.20
F1Z0	0.19	0.17	0.16	0.19	0.18	0.21	0.19	0.18	0.21	0.20
F1Z1	0.17	0.19	0.19	0.18	0.18	0.19	0.21	0.21	0.20	0.20
F1Z2	0.16	0.18	0.16	0.20	0.17	0.18	0.20	0.18	0.21	0.19
F1Z3	0.16	0.16	0.16	0.18	0.17	0.18	0.18	0.18	0.20	0.18
F2Z0	0.18	0.17	0.16	0.21	0.18	0.20	0.19	0.18	0.22	0.20
F2Z1	0.16	0.19	0.15	0.20	0.18	0.18	0.21	0.17	0.22	0.20
F2Z2	0.18	0.18	0.17	0.17	0.17	0.20	0.20	0.19	0.19	0.19
F2Z3	0.15	0.20	0.17	0.19	0.18	0.17	0.21	0.19	0.21	0.20
F3Z0	0.17	0.17	0.18	0.18	0.17	0.19	0.19	0.19	0.19	0.19
F3Z1	0.15	0.16	0.18	0.17	0.17	0.17	0.18	0.20	0.19	0.19
F3Z2	0.16	0.19	0.15	0.19	0.17	0.18	0.21	0.17	0.21	0.19
F3Z3	0.15	0.15	0.15	0.15	0.15	0.17	0.16	0.17	0.17	0.17
CD (0.05) FZM - 0.0277						CD (0.05) FZM - 0.0279				

Two way Table

I cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	0.16	0.17	0.17	0.16	0.17	0.16	0.16	0.16	0.16
M2	0.18	0.18	0.18	0.17	0.18	0.18	0.18	0.17	0.18
M3	0.18	0.17	0.16	0.16	0.16	0.18	0.17	0.17	0.17
M4	0.17	0.19	0.19	0.17	0.19	0.18	0.18	0.17	0.18
Mean	0.17	0.17	0.18	0.17	0.17	0.17	0.17	0.17	
CD (0.05) M / F - 0.0069					CD (0.05) FZ - 0.0139				
II cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	0.18	0.19	0.19	0.18	0.19	0.18	0.18	0.18	0.18
M2	0.20	0.19	0.20	0.19	0.20	0.20	0.20	0.19	0.19
M3	0.19	0.19	0.18	0.18	0.18	0.19	0.19	0.19	0.19
M4	0.19	0.20	0.21	0.19	0.21	0.20	0.20	0.19	0.20
Mean	0.19	0.19	0.20	0.18	0.19	0.19	0.19	0.19	
CD (0.05) M / F - 0.0070					CD (0.05) FZ - 0.0140				

4.7 Effect of Treatment on nutrient composition of amaranthus root

4.7.1 Effect of treatment on root nitrogen, phosphorous and potassium contents

The data on nitrogen, phosphorous and potassium contents of root are presented in Tables 33 to 35 respectively. Individual effect of the treatment factors and their interactions were significant for root nitrogen content (Table 33). Highest content of N in root was observed for M1F2Z3 (AMF x Fe @ 10 mg kg⁻¹ x Zn @ foliar) followed by M1F3Z0 (AMF x Fe @ foliar x Zn @ zero). Evaluating the effect of bioaugmentation methods, AMF treatment (M1) was significantly superior to others. Levels of Fe showed an increase in nitrogen content up to Fe @ 5 mg kg⁻¹ only, while for the levels of Zn an increase in root N was observed only for Z2 (Zn @ 10 mg kg⁻¹). Foliar application of both Fe and Zn enhanced N content of root than that of control.

P content in the root was significantly influenced by the treatments as well as by their interactions (Table 34). Root P content was highest for the treatment combination *viz.* M1F1Z0 (AMF x Fe @ 5 mg kg⁻¹ x Zn @ zero), M1F0Z3 (AMF x Fe @ zero x Zn @ foliar) and M1F1Z2 (AMF x Fe @ 5 mg kg⁻¹ x Zn @ 10 mg kg⁻¹). Considering the individual effects, AMF (M1) and Fe @ 5 mg kg⁻¹ (F1) maintained the highest values. Zn application did not show any significant influence on root P.

Individual as well as interaction effect were significant for root potassium content (Table 35) and the highest value was for M1F2Z0 (AMF x Fe @ 10 mg kg⁻¹ x Zn @ zero). Considering the individual effects, AMF and Fe @ 5 mg kg⁻¹ recorded significantly highest values for root K. Zn showed a negative relation with root K content.

Table 33. Effect of treatments on root-nitrogen content (%) of amaranthus

Treatments	M1	M2	M3	M4	Mean				
F0Z0	1.29	0.99	1.13	1.32	1.18				
F0Z1	1.52	0.76	1.29	1.41	1.24				
F0Z2	1.83	1.43	1.10	1.27	1.41				
F0Z3	1.41	1.21	1.07	1.60	1.32				
F1Z0	1.74	1.43	1.32	1.29	1.45				
F1Z1	1.38	1.35	1.63	1.85	1.55				
F1Z2	1.80	1.21	1.66	1.43	1.52				
F1Z3	1.41	1.27	1.66	1.74	1.52				
F2Z0	1.29	1.15	1.24	1.46	1.29				
F2Z1	1.38	0.76	1.29	1.24	1.17				
F2Z2	1.74	0.87	1.35	1.27	1.31				
F2Z3	1.99	0.91	1.55	1.46	1.48				
F3Z0	1.88	0.71	1.66	1.66	1.48				
F3Z1	1.83	0.76	1.29	1.74	1.41				
F3Z2	1.80	0.79	1.10	1.55	1.31				
F3Z3	1.77	1.04	1.21	1.46	1.37				
	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	1.51	1.58	1.60	1.82	1.55	1.52	1.79	1.64	1.63
M2	1.10	1.31	0.92	0.82	1.07	0.91	1.08	1.11	1.04
M3	1.15	1.57	1.36	1.31	1.34	1.38	1.30	1.37	1.35
M4	1.40	1.58	1.36	1.60	1.43	1.56	1.38	1.57	1.48
Mean	1.29	1.51	1.31	1.39	1.35	1.34	1.39	1.42	
CD(0.05) M/F/Z- 0.0549			CD(0.05)FM/ZM/FZ			CD(0.05)FZM- 0.2196			

Table 34 Effect of treatments on root phosphorous content (%) of amaranthus

Treatments	M1	M2	M3	M4	Mean				
F0Z0	0.03	0.02	0.01	0.01	0.02				
F0Z1	0.04	0.03	0.01	0.01	0.02				
F0Z2	0.04	0.01	0.01	0.03	0.03				
F0Z3	0.05	0.01	0.01	0.01	0.02				
F1Z0	0.05	0.01	0.01	0.01	0.02				
F1Z1	0.04	0.02	0.01	0.01	0.02				
F1Z2	0.05	0.01	0.02	0.01	0.02				
F1Z3	0.04	0.02	0.01	0.01	0.02				
F2Z0	0.01	0.01	0.02	0.01	0.01				
F2Z1	0.01	0.01	0.01	0.01	0.01				
F2Z2	0.01	0.01	0.01	0.01	0.01				
F2Z3	0.01	0.02	0.01	0.01	0.01				
F3Z0	0.01	0.01	0.01	0.01	0.01				
F3Z1	0.01	0.02	0.01	0.01	0.01				
F3Z2	0.01	0.01	0.02	0.01	0.01				
F3Z3	0.02	0.01	0.02	0.01	0.01				
	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	0.04	0.05	0.01	0.01	0.03	0.03	0.03	0.03	0.03
M2	0.02	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01
M3	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
M4	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01
Mean	0.02	0.02	0.01	0.01	0.02	0.02	0.02	0.02	
CD(0.05) M/F -0.0015			CD(0.05) FZ/FM/ZM - 0.0029				CD(0.05)FZM- 0.0059		

Table 35. Effect of treatments on root potassium content (%) of amaranthus

Treatments	M1	M2	M3	M4	Mean				
F0Z0	5.16	4.72	3.92	5.16	4.74				
F0Z1	3.96	5.08	4.98	4.00	4.51				
F0Z2	3.66	3.28	4.54	2.44	3.48				
F0Z3	5.42	3.42	5.34	4.72	4.73				
F1Z0	4.62	5.30	4.30	3.60	4.46				
F1Z1	5.24	3.34	4.44	4.20	4.31				
F1Z2	5.32	5.22	5.10	3.38	4.76				
F1Z3	4.18	5.24	4.44	5.02	4.72				
F2Z0	6.52	4.90	3.92	3.02	4.59				
F2Z1	5.02	4.96	3.82	3.24	4.26				
F2Z2	4.90	5.02	4.46	4.04	4.61				
F2Z3	3.86	3.84	4.32	4.32	4.09				
F3Z0	5.38	4.52	3.38	3.56	4.21				
F3Z1	4.54	3.88	5.02	3.64	4.27				
F3Z2	5.18	3.98	4.30	3.18	4.16				
F3Z3	3.98	4.60	4.60	2.76	3.99				
	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	4.55	4.84	5.08	4.77	5.42	4.69	4.77	4.36	4.81
M2	4.13	4.78	4.68	4.25	4.86	4.32	4.38	4.28	4.46
M3	4.69	4.57	4.13	4.33	3.88	4.57	4.60	4.68	4.43
M4	4.08	4.05	3.66	3.28	3.84	3.77	3.26	4.21	3.77
Mean	4.36	4.56	4.39	4.16	4.50	4.34	4.25	4.38	
CD (0.05) M / F - 0.2278			CD (0.05) FZ - 0.4556			CD (0.05) FZM- 0.9113			

4.7.2 Effect of treatments on root calcium, magnesium and sulphur content

The data on calcium, magnesium and sulphur contents of root are presented in Tables 36 to 38 respectively. Individual as well as interaction effect of treatment factors were significant for root calcium content (Table 36). Highest Ca content was observed by M1F1Z0 (AMF x Fe @ 5 mg kg⁻¹ x Zn @ zero) and M2F0Z2 (P solubilizer x Fe @ zero x Zn @ 10 mg kg⁻¹). Considering the individual microbial effect, application of K solubilizer (M3) significantly decreased the root Ca content. Soil application of Fe decreased the root Ca content while Zn had enhanced it.

Individual as well as interaction effect of treatment factors were significant for root magnesium content (Table 37). The highest value was recorded by M2F0Z2 (P solubilizer x Fe @ zero x Zn @ 10 mg kg⁻¹). Considering the individual effect of bioaugmentation methods, M4 (POP) recorded the highest value. Zn application had enhanced the Mg content significantly, while Fe decreased it.

Sulphur content in the root was significantly influenced by individual factors as well as by their interaction effects (Table 38). Highest value was recorded by the treatment combination M3F0Z2 (K solubilizer x Fe @ zero x Zn @ 10 mg kg⁻¹). Considering the individual microbial effect, K solubilizer (M3) was significantly superior to others. Levels of Fe increased root S content up to 5 mg Fe kg⁻¹, which was on par with Fe @ zero mg kg⁻¹. Zn had showed significant and positive relation with root S content.

4.7.3 Effect of treatment on root iron, manganese, zinc and copper

The data on iron, manganese, zinc and copper contents are presented in Tables 39 to 42 respectively. Iron content of root was significantly influenced by the treatments (Table 39). The treatment combination M4F1Z0 (AMF x Fe @ 5 mg kg⁻¹ x Zn @ zero) recorded the highest value which was significantly superior to all others. Considering the individual effect of bioaugmentation methods, highest value was recorded by P solubilizer (M2) which was on par with K

Table 36. Effect of treatments on root calcium content (%) of amaranthus

Treatments	M1		M2		M3		M4		Mean
F0Z0	3.400		2.400		2.280		2.880		2.740
F0Z1	3.280		3.400		2.400		3.240		3.080
F0Z2	3.440		3.680		2.360		3.120		3.150
F0Z3	3.160		2.600		2.760		3.320		2.960
F1Z0	3.680		2.600		2.440		3.320		3.010
F1Z1	2.640		2.080		2.720		3.040		2.620
F1Z2	2.880		2.600		3.280		2.360		2.780
F1Z3	3.320		2.360		2.880		2.720		2.820
F2Z0	2.440		2.080		1.920		2.920		2.340
F2Z1	2.520		2.600		2.440		2.840		2.600
F2Z2	3.480		2.840		2.760		2.960		3.010
F2Z3	2.400		3.600		2.720		2.720		2.860
F3Z0	2.160		3.320		2.560		2.640		2.670
F3Z1	2.680		3.080		2.760		2.640		2.790
F3Z2	2.720		3.120		3.00		2.720		2.890
F3Z3	3.040		3.120		2.560		3.160		2.970
	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	3.320	3.130	2.710	2.650	2.920	2.780	3.130	2.980	2.953
M2	3.020	2.410	2.780	3.160	2.600	2.790	3.060	2.920	2.43
M3	2.450	2.830	2.460	2.720	2.300	2.580	2.850	2.730	2.615
M4	3.140	2.860	2.860	2.790	2.940	2.940	2.790	2.980	2.913
Mean	2.983	2.808	2.703	2.830	2.690	2.773	2.958	2.902	
CD(0.05) M/F/Z - 0.09453			CD(0.05) FZ/ZM/ FM- 0.18907				CD(0.05)FZM)- 0.37814		

Table 37. Effect of treatments on root magnesium content (%) of amaranthus

Treatments	M1	M2	M3	M4	Mean				
F0Z0	1.416	1.176	1.560	1.752	1.476				
F0Z1	1.776	2.136	1.464	2.424	1.950				
F0Z2	1.584	2.664	1.488	1.392	1.782				
F0Z3	1.656	1.536	2.544	1.704	1.860				
F1Z0	2.06	1.464	1.128	1.704	1.578				
F1Z1	1.320	1.320	1.272	1.680	1.398				
F1Z2	1.512	1.368	1.152	1.920	1.488				
F1Z3	1.536	1.368	2.040	2.496	1.860				
F2Z0	1.512	1.032	1.440	2.424	1.602				
F2Z1	1.080	1.056	1.560	2.088	1.446				
F2Z2	2.400	1.200	2.232	2.424	2.064				
F2Z3	1.128	1.344	1.776	1.704	1.488				
F3Z0	1.320	1.344	1.200	2.328	1.548				
F3Z1	1.320	1.416	1.224	2.472	1.608				
F3Z2	1.584	1.464	1.824	1.728	1.650				
F3Z3	1.824	1.416	1.824	1.968	1.758				
	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	1608	1596	1530	1512	1566	1374	1770	1536	1562
M2	1878	1380	1158	1410	1254	1482	1674	1416	1457
M3	1764	1398	1752	1518	1332	1380	1674	2046	1608
M4	1818	1950	2160	2124	2052	2166	1866	1968	2013
Mean	1767	1581	1650	1641	1551	1600	1746	1741	
CD (0.05) M / F / Z - 0.08522			CD(0.05) FZ/MZ/FM - 0.17045				CD(0.05) FZM -0.34089		

Table 38. Effect of treatments on root sulphur content (mg kg⁻¹) of amaranthus

Treatments	M1	M2	M3	M4	Mean				
F0Z0	128.55	71.25	210.21	120.63	132.66				
F0Z1	175.21	127.09	246.67	170.63	179.90				
F0Z2	318.13	262.29	372.50	195.63	287.14				
F0Z3	170.63	103.13	236.04	140.21	162.50				
F1Z0	91.04	89.17	238.54	154.38	143.28				
F1Z1	134.58	205.63	276.88	238.33	213.85				
F1Z2	217.08	350.42	206.46	255.83	257.45				
F1Z3	131.46	187.08	131.67	216.46	166.67				
F2Z0	112.29	83.75	171.46	176.04	135.89				
F2Z1	93.34	108.13	182.50	191.05	143.75				
F2Z2	108.96	122.71	390.41	257.08	219.79				
F2Z3	111.25	102.50	159.38	176.04	137.29				
F3Z0	103.54	158.13	289.59	185.63	184.22				
F3Z1	117.50	161.67	358.95	166.46	201.15				
F3Z2	140.21	190.42	256.88	240.21	206.93				
F3Z3	107.09	159.38	277.71	113.75	164.48				
	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	198.13	143.54	106.46	117.08	108.85	130.16	196.09	130.11	141.30
M2	140.94	208.07	104.27	167.40	100.57	150.63	231.46	138.02	155.17
M3	266.35	213.39	225.94	295.78	227.45	266.25	306.56	201.20	250.36
M4	156.77	216.25	200.05	176.51	159.17	191.61	237.19	161.61	187.40
Mean	190.55	195.31	159.18	189.19	149.01	184.66	242.83	157.73	
CD(0.05) / F/Z - 11.8529			CD (0.05) FZ /FM/MZ- 23.7059			CD (0.05) FZM- 47.4118			

Table 39. Effect of treatments on root iron content (mg kg⁻¹) of amaranthus

Treatments	M1	M2	M3	M4						Mean
F0Z0	1647.1	1284.4	845.2	1120.4						1224.3
F0Z1	1248.3	1264.7	2057.2	1150.4						1430.1
F0Z2	1247.1	1647.2	2098.0	1483.2						1618.9
F0Z3	2066.3	1253.6	2444.8	1089.6						1713.6
F1Z0	839.1	3316.8	1368.4	3953.2						2369.4
F1Z1	852.3	2061.2	1296.4	1897.6						1526.9
F1Z2	890.3	3252.0	1327.6	3088.4						2139.6
F1Z3	923.5	2092.8	2222.0	1929.2						1791.9
F2Z0	1407.1	1644.8	1645.6	1481.2						1544.7
F2Z1	1683.5	2468.8	3336.4	2305.2						2448.5
F2Z2	1811.5	1293.2	2424.0	1129.6						1664.6
F2Z3	1255.1	1662.8	2081.6	1499.2						1624.7
F3Z0	1294.0	1321.6	1662.0	1158.0						1358.9
F3Z1	1269.0	1271.2	1604.0	1107.6						1312.9
F3Z2	1273.6	1456.0	1289.2	1292.4						1327.8
F3Z3	1246.0	1748.8	1287.9	1585.2						1467.0
	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean	
M1	1552.2	876.3	1539.3	1270.6	1296.8	1263.2	1305.6	1372.7	1309.6	
M2	1362.4	2680.7	1767.4	1449.4	1891.9	1766.4	1912.1	1689.5	1815.0	
M3	1861.3	1553.6	2371.9	1460.8	1380.3	2073.5	1784.7	2009.1	1811.9	
M4	1210.9	2717.1	1603.8	1285.8	1928.2	1615.2	1748.4	1525.8	1704.4	
Mean	1496.7	1956.9	1820.6	1366.6	1624.3	1679.6	1687.7	1649.3		
CD(0.05) M / F / Z - 0.2278			CD (0.05) FZ / FM / MZ - 8.9443				CD (0.05) FZM- 17.8885			

solubilizer (M3) and lowest value was recorded by M1 (AMF). Soil application of Fe enhanced root Fe content compared to control (F0), but the highest level of Fe decreased it and the lowest value was recorded by foliar application (F3). Zn maintained a significant and positive relation with Fe content in the root.

Root manganese content was significantly influenced by the treatments (Table 40). M3F0Z3 (P solubilizer x Fe @ zero x Zn @ foliar) recorded the highest value, which was on par with M2F1Z3 (P solubilizer x Fe @ 5 mg kg⁻¹ x Zn @ foliar) M2F0Z1 (P solubilizer x Fe @ zero x Zn @ 5 mg kg⁻¹) and M3F2Z3 (K solubilizer x Fe @ 10 mg kg⁻¹ x Zn @ foliar). Evaluating the individual effect, P solubilizer (M2) was significantly superior to others. Levels of Fe maintained a positive relationship only with soil application of Fe @ 5 mg kg⁻¹ and the concentration decreased with increasing levels. The foliar application (F3) recorded the lowest value for root Mn. Zn levels maintained a positive relation with root Mn.

Individual as well as interaction effects were significant for root Zn content (Table 41). M3F1Z1 (K solubilizer x Fe @ 5 mg kg⁻¹ x Zn @ 5 mg kg⁻¹) recorded the highest value and was on par with M3F1Z0 (K solubilizer x Fe @ 5 mg kg⁻¹ x Zn @ zero) and M3F1Z2 (K solubilizer x Fe @ 5 mg kg⁻¹ x Zn @10 mg kg⁻¹). The lowest value was showed by M2F0Z0 (P solubilizer x Fe @ zero x Zn @ zero). With regard to the individual effect, significantly highest value was observed for K solubilizer (M3) and significantly lowest value for P solubilizer (M2). Fe levels showed a negative relationship and for levels of Zn, no consistent pattern was observed.

Only the interaction effect among the variables was significant for root copper content (Table 42). The treatment combination M4F0Z2 (POP x Fe@ zero x Zn @ 10 mg kg⁻¹) recorded the highest value for root Cu content whereas the lowest value was recorded by M4F0Z0 (POP x Fe @ zero x Zn @ zero).

Table 40. Effect of treatments on root manganese content (mg kg⁻¹) of amaranth

Treatments	M1	M2	M3	M4	Mean				
F0Z0	61.86	127.22	309.54	102.42	150.26				
F0Z1	166.38	262.34	151.62	237.54	204.47				
F0Z2	167.18	242.38	139.06	217.58	191.55				
F0Z3	239.18	165.54	278.74	140.74	206.05				
F1Z0	243.26	199.18	187.42	174.38	201.06				
F1Z1	243.58	163.22	177.22	138.42	180.61				
F1Z2	163.54	247.62	181.66	222.82	203.91				
F1Z3	167.30	271.74	122.82	246.94	202.20				
F2Z0	167.30	114.34	219.50	89.54	147.67				
F2Z1	199.22	159.22	247.94	134.42	185.20				
F2Z2	203.26	207.26	205.86	182.46	199.71				
F2Z3	199.26	239.54	270.78	214.74	231.08				
F3Z0	207.26	199.54	159.50	174.74	185.26				
F3Z1	127.38	262.14	108.65	237.34	183.87				
F3Z2	123.26	194.30	175.18	169.50	165.56				
F3Z3	127.50	223.82	207.74	199.02	189.52				
	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M0	158.65	204.42	192.26	146.35	169.92	184.14	164.31	183.31	175.42
M1	199.37	220.44	180.09	219.95	160.07	211.73	222.89	225.16	204.96
M2	219.74	167.28	236.02	162.76	218.99	171.35	175.44	220.02	196.45
M3	174.57	195.64	155.29	195.15	135.27	186.93	198.09	200.36	180.16
Mean	188.08	196.94	190.91	181.05	171.06	188.54	190.18	207.21	
CD (0.05) M / F / Z- 4.7255			CD(0.05) FZ / ZM / FM - 9.4509				CD (0.05) FZM- 18.9019		

Table 41. Effect of treatments on root zinc content (mg kg⁻¹) of amaranthus

Treatments	M1	M2	M3	M4	Mean				
F0Z0	230.98	89.02	206.34	220.54	186.72				
F0Z1	185.02	104.94	156.94	174.58	155.37				
F0Z2	190.22	126.30	166.54	179.78	165.71				
F0Z3	148.86	162.14	249.82	138.42	174.81				
F1Z0	141.90	145.50	262.06	131.46	170.23				
F1Z1	149.86	166.58	274.18	139.42	182.51				
F1Z2	161.10	98.14	273.82	150.66	170.93				
F1Z3	142.70	168.46	166.22	132.26	152.41				
F2Z0	142.70	104.06	139.18	132.26	129.55				
F2Z1	143.10	100.98	234.46	132.66	152.80				
F2Z2	118.26	98.38	97.46	137.82	112.98				
F2Z3	167.54	105.86	237.22	157.10	166.93				
F3Z0	168.26	122.06	166.54	157.82	153.67				
F3Z1	152.14	113.02	156.78	141.70	140.91				
F3Z2	152.02	151.66	162.14	141.58	151.85				
F3Z3	151.26	98.46	205.90	140.82	149.11				
	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M0	188.77	148.89	142.90	155.92	170.96	157.53	155.40	152.59	159.12
M1	120.60	144.67	102.32	121.30	115.16	121.38	118.62	133.73	122.22
M2	194.91	244.07	177.08	172.84	193.53	205.59	174.99	214.79	197.23
M3	178.33	138.45	139.96	145.48	160.52	147.09	152.46	142.15	150.55
Mean	170.65	169.02	140.56	148.89	160.04	157.90	150.37	160.81	
CD (0.05) M / F 4.2220			CD (0.05) FZ / FM / MZ- 8.4440				CD (0.05) FZM- 16.8880		

Table 42. Effect of treatments on root copper content (mg kg^{-1}) of amaranthus

Treatments	M1	M2	M3	M4	Mean				
F0Z0	16.02	24.05	8.01	6.29	13.60				
F0Z1	23.58	27.82	15.04	13.32	19.94				
F0Z2	15.66	11.65	16.56	89.61	33.37				
F0Z3	11.88	23.93	19.69	17.97	18.37				
F1Z0	12.04	19.65	19.69	17.97	17.34				
F1Z1	12.41	29.72	15.61	13.89	17.91				
F1Z2	11.65	27.79	16.01	14.29	17.44				
F1Z3	12.08	24.01	23.89	22.17	20.54				
F2Z0	12.08	8.01	20.45	18.73	14.82				
F2Z1	16.15	8.75	8.05	16.33	12.32				
F2Z2	15.94	10.83	12.85	11.13	12.69				
F2Z3	15.93	8.98	24.13	22.41	17.86				
F3Z0	16.02	24.86	26.87	22.15	22.47				
F3Z1	31.66	15.81	19.77	18.05	21.32				
F3Z2	23.66	17.16	15.78	14.06	17.66				
F3Z3	23.70	32.03	11.58	9.86	19.29				
	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M0	16.79	12.05	15.02	23.76	14.04	20.95	16.73	15.90	16.90
M1	21.87	25.29	9.14	22.47	19.15	20.53	16.86	22.24	19.69
M2	14.82	18.80	16.37	18.50	18.75	14.62	15.30	19.82	17.12
M3	31.80	17.08	17.15	16.03	16.28	15.40	32.27	18.10	20.51
Mean	21.32	18.31	14.42	20.19	17.06	17.87	20.29	19.02	
CD (0.05) M / F / Z - NS			CD (0.05) FZ / FM / MZ- NS				CD (0.05) FZM-26.0748		

4.8 Soil Analysis

4.8.1 Effect of treatments on pH, EC and organic carbon

The data on soil pH, EC and organic carbon are presented in Tables 43 to 45 respectively. The treatments had significantly influenced the soil pH at both the harvests (Table 43). Considering the individual effect, microbial treatments (M1, M2 and M3) had maintained the soil pH above 7.0 and were significantly superior to M4 (POP). In general the soil pH was maintained around neutrality at the time of first harvest. The highest pH was recorded by M1F1Z2 (AMF x Fe @ 5 mg kg⁻¹ x Zn @ 10 mg kg⁻¹) at both the harvests. At second harvest the mean values of pH for bioaugmentation treatments showed a slight decrease in general except P solubilizer compared to first harvest. Z0 (Zn @ zero) showed lowest pH in the case Zn levels.

EC of the samples were significantly influenced by the treatments at the first harvest (Table 44). Both the individual and interaction effects were significant. The highest value of EC was recorded by M3F3Z2 (K solubilizer x Fe @ foliar x Zn @ 10 mg kg⁻¹). The addition of P solubilizer had showed a lower EC value compared to other values. Comparing the individual effect of Fe, Fe @ zero showed the highest EC value. At second harvest, the treatments effects were not significant.

Soil organic carbon content (Table 45) was significantly influenced by the individual factors as well as their interaction effects at both the harvests. The highest soil organic carbon was recorded by M1F2Z0 (AMF x Fe @ 10 mg kg⁻¹ x Zn @ zero) for both the harvests. Evaluating the individual effects of bioaugmentation methods on SOC, the highest value was observed for AMF (M1) which was significantly superior to other three. At the second harvest also, it followed the same trend with regard to the individual effect of bioaugmentation, but the highest value was on par with M2 (P solubilizer).

Table 43 Effect of treatments on soil pH

Treatments	I cut					II cut				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
F0Z0	6.62	6.67	7.11	6.71	6.78	6.73	6.78	7.22	6.61	6.84
F0Z1	7.06	7.07	7.08	6.67	6.97	7.18	7.18	7.19	6.57	7.03
F0Z2	7.12	6.95	7.09	6.86	7.00	7.23	7.06	7.19	6.76	7.06
F0Z3	7.15	7.03	6.97	6.95	7.02	7.27	7.14	7.08	6.85	7.09
F1Z0	6.96	7.06	6.95	7.01	6.99	6.85	7.06	6.84	6.91	6.91
F1Z1	7.20	7.14	7.13	6.86	7.08	7.09	7.14	7.02	6.76	7.00
F1Z2	7.29	7.09	7.12	6.90	7.10	7.18	7.09	7.01	6.80	7.02
F1Z3	7.06	7.16	7.02	6.91	7.04	6.95	7.16	6.91	6.81	6.96
F2Z0	6.85	7.10	7.07	6.77	6.94	6.73	7.08	6.95	6.67	6.86
F2Z1	6.76	7.17	7.15	6.56	6.91	6.64	7.15	7.03	6.46	6.82
F2Z2	6.98	7.11	7.14	7.28	7.13	6.87	7.10	7.02	7.18	7.04
F2Z3	7.06	7.00	7.24	7.21	7.12	6.94	6.98	7.12	7.11	7.03
F3Z0	6.96	7.09	7.11	7.03	7.05	6.84	7.07	6.99	6.93	6.96
F3Z1	7.12	7.17	7.21	7.06	7.14	7.05	7.15	7.00	6.96	7.04
F3Z2	7.11	7.15	7.26	7.07	7.14	6.95	7.14	7.15	6.97	7.05
F3Z3	6.95	7.14	7.15	6.94	7.05	7.15	7.12	7.05	6.85	7.04
CD(0.05) FZM - 0.2512						CD(0.05) FZM - 0.2519				

Two way Table

I cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	MEAN
M1	6.98	7.13	6.91	7.03	6.85	7.03	7.12	7.05	7.01
M2	6.93	7.11	7.09	7.13	6.98	7.13	7.08	7.08	7.07
M3	7.06	7.05	7.15	7.18	7.06	7.14	7.15	7.09	7.11
M4	6.80	6.92	6.95	7.03	6.88	6.79	7.03	7.00	6.92
Mean	6.94	7.05	7.03	7.09	6.94	7.02	7.09	7.06	
CD (0.05) M/Z/F-0.0628					CD (0.05) MZ/FZ/FM-0.1256				
II cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	MEAN
M1	7.11	7.02	6.79	7.00	6.79	6.99	7.06	7.08	6.98
M2	7.04	7.11	7.08	7.12	7.00	7.16	7.10	7.10	7.09
M3	7.17	6.94	7.03	7.05	7.00	7.06	7.09	7.04	7.05
M4	6.70	6.82	6.85	6.93	6.78	6.68	6.93	6.90	6.82
Mean	7.00	6.97	6.94	7.02	6.89	6.97	7.04	7.03	
CD (0.05) M/Z/F-0.0630					CD (0.05) MZ/FM- 0.0630				

Table 44. Effect of treatments on soil EC ($\mu\text{S cm}^{-1}$)

Treat-ments	I cut					II cut				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
F0Z0	178.50	125.20	158.70	143.45	151.46	177.50	130.85	164.70	137.75	52.70
F0Z1	189.95	129.90	169.60	146.80	159.06	183.45	133.25	173.10	148.15	159.49
F0Z2	181.80	138.40	184.05	159.05	165.83	175.00	135.50	179.05	158.20	161.94
F0Z3	183.05	135.75	170.30	170.10	164.80	184.35	138.70	176.90	164.00	165.99
F1Z0	147.20	130.35	158.75	166.25	150.64	152.20	131.10	165.45	161.30	152.51
F1Z1	168.60	124.90	168.70	170.35	158.14	159.25	131.15	173.00	164.60	157.00
F1Z2	160.70	129.00	171.95	172.40	158.51	163.70	127.35	169.25	168.75	157.26
F1Z3	159.30	123.90	161.80	173.90	154.73	163.30	130.40	162.80	169.65	156.54
F2Z0	179.50	125.20	149.20	128.65	145.64	176.00	131.70	837.20	123.65	317.14
F2Z1	189.50	129.55	154.00	130.10	150.79	184.50	131.25	150.45	129.60	148.95
F2Z2	179.50	132.45	165.00	179.95	164.23	177.30	132.45	161.40	178.55	162.42
F2Z3	173.20	131.20	170.35	131.15	151.48	93.95	134.20	170.35	124.15	130.66
F3Z0	118.75	159.20	184.30	149.75	153.00	128.15	157.70	180.20	142.75	152.20
F3Z1	152.65	153.60	183.70	153.95	160.98	157.60	148.60	181.20	148.45	158.96
F3Z2	150.00	138.85	191.10	167.60	161.89	151.10	146.35	187.40	161.45	161.58
F3Z3	143.35	143.55	168.80	167.45	155.79	149.65	146.05	169.75	159.80	156.31
CD(0.05) FZM - 6.7291						CD(0.05) FZM - NS				

Two way Table

I cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	MEAN
M1	183.3	159.0	180.4	141.2	156.0	175.2	168.0	164.7	166.0
M2	132.3	127.0	129.6	148.8	135.0	134.5	134.7	133.6	134.4
M3	170.7	165.3	159.6	182.0	162.7	169.0	178.0	167.8	169.4
M4	154.9	170.7	142.5	159.7	147.0	150.3	169.8	160.7	156.9
Mean	160.3	155.5	153.0	157.9	150.2	157.2	162.6	156.7	
CD (0.05) M/Z/F-1.6823					CD (0.05) MZ/FZ/FM- 3.3646				
II cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	MEAN
M1	180.1	159.6	157.9	146.6	158.5	171.2	166.8	147.8	161.1
M2	134.6	130.0	132.4	149.7	137.8	136.1	135.4	137.3	136.7
M3	173.4	167.6	329.9	179.6	336.9	169.4	174.3	170.0	212.6
M4	152.0	166.1	139.0	153.1	141.4	147.7	166.7	154.4	152.6
Mean	160.0	155.8	189.8	157.3	193.6	156.1	160.8	152.4	
CD (0.05) M/Z/F- NS					CD (0.05) MZ/FZ/FM- NS				

Table 45. Effect of treatments on soil organic carbon (%)

Treatments	I cut					II cut				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
F0Z0	1.42	1.44	1.11	1.14	1.28	1.71	1.35	1.28	1.31	1.41
F0Z1	1.33	1.24	1.16	1.17	1.22	1.68	1.30	1.33	1.34	1.41
F0Z2	1.31	1.35	1.04	1.24	1.23	1.32	1.52	1.21	1.41	1.36
F0Z3	1.37	1.11	1.02	1.19	1.17	1.50	1.28	1.19	1.36	1.33
F1Z0	1.36	1.29	1.30	1.28	1.31	1.53	1.46	1.47	1.45	1.48
F1Z1	1.42	1.08	1.38	1.04	1.23	1.51	1.25	1.55	1.22	1.38
F1Z2	1.55	1.15	1.21	1.30	1.30	1.66	1.32	1.38	1.47	1.46
F1Z3	1.89	1.08	1.15	2.00	1.53	1.62	1.39	1.32	1.47	1.45
F2Z0	2.15	1.20	1.02	1.26	1.41	1.90	1.37	1.19	1.43	1.47
F2Z1	1.45	1.46	1.11	1.11	1.28	1.53	1.64	1.28	1.28	1.43
F2Z2	1.48	1.51	1.07	1.34	1.35	1.54	1.68	1.23	1.51	1.49
F2Z3	1.46	1.41	1.13	1.12	1.28	1.47	1.58	1.30	1.29	1.41
F3Z0	1.35	1.56	1.26	1.29	1.36	1.56	1.73	1.43	1.46	1.54
F3Z1	1.24	1.53	1.37	1.17	1.32	1.38	1.69	1.54	1.34	1.49
F3Z2	1.32	1.43	1.43	0.61	1.20	1.51	1.60	1.60	0.78	1.37
F3Z3	1.34	1.61	1.46	1.01	1.35	1.43	1.78	1.63	1.18	1.50
CD(0.05) FZM - 0.3704						CD(0.05) FZM - NS				

Two way Table

I cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	MEAN
M1	1.36	1.55	1.64	1.31	1.57	1.36	1.41	1.51	1.46
M2	1.28	1.15	1.40	1.53	1.37	1.33	1.36	1.30	1.34
M3	1.08	1.26	1.08	1.38	1.17	1.25	1.19	1.19	1.20
M4	1.18	1.41	1.21	1.02	1.24	1.12	1.12	1.33	1.20
Mean	1.22	1.34	1.33	1.31	1.34	1.27	1.27	1.33	
CD (0.05) M - 0.0926					CD (0.05) FM - 0.1852				
II cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	MEAN
M1	1.55	1.58	1.61	1.47	1.67	1.52	1.51	1.50	1.55
M2	1.36	1.35	1.57	1.70	1.48	1.47	1.53	1.50	1.49
M3	1.25	1.43	1.25	1.55	1.34	1.42	1.36	1.36	1.37
M4	1.35	1.40	1.38	1.19	1.41	1.29	1.29	1.32	1.33
Mean	1.38	1.44	1.45	1.4	1.48	1.43	1.42	1.42	
CD (0.05) M - 0.0847					CD (0.05) FM - 0.1695				

4.8.2 Effect of treatments on soil available nitrogen, phosphorous and potassium

The data on soil available nitrogen, phosphorous and potassium contents are presented in Tables 46 to 48 respectively. Soil available nitrogen content (Table 46) was significantly influenced by all the treatment factors and their interactions. The available N content was highest for M1F1Z0 (AMF x Fe @ 5 mg kg⁻¹ x Zn @ zero) for both the harvests. Considering the individual effect of application. At second harvest the available N content was significantly influenced by the interactions as well as by individual factors except for the levels of Zn. The available N content decreased considerably during this stage but it showed the same pattern of first harvest.

Interactions as well as individual effects were significant for available P content in the soil (Table 47). The highest available P content as recorded by M2F1Z1 (P solubilizer x Fe @ 5 mg kg⁻¹ x Zn @ 5 mg kg⁻¹) for both the harvests. Considering the individual microbial effect application of P solubilizer (M2) had showed a tremendous increase in available P content compared to other treatments. The same pattern was observed for the second harvest also.

Available potassium content in the soil (Table 48) was also significantly influenced by the treatments. The highest value was recorded by for M₃F₃Z₀ (K solubilizer x Fe @ foliar x Zn @zero) for both the harvests. Comparing the individual effect of microbial additives, application of K solubilizer (M3) has enhanced the available K content in the soil. At the second harvest the available K content was not significant but the application of K solubilizer has increased the available K content in the soil.

Table 46. Effect of treatments on available nitrogen in soil (kg ha⁻¹)

Treat-ments	I cut					II cut				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
F0Z0	309.40	256.25	253.13	206.28	256.26	65.65	212.55	209.40	162.50	212.53
F0Z1	262.50	225.00	225.00	187.50	225.00	218.80	181.30	181.30	143.80	181.30
F0Z2	275.03	206.28	243.78	175.00	225.02	231.25	162.50	200.00	131.30	181.26
F0Z3	225.00	256.25	268.78	171.90	230.48	181.30	212.55	225.00	128.15	186.75
F1Z0	312.50	253.15	212.50	200.03	244.54	268.80	209.40	168.80	156.25	200.81
F1Z1	331.28	265.65	215.65	218.75	257.83	287.50	221.90	171.90	175.05	214.09
F1Z2	356.28	243.75	250.00	253.13	275.79	312.50	200.05	206.30	209.40	232.06
F1Z3	253.15	200.00	259.38	278.15	247.67	209.40	156.30	215.65	234.40	203.94
F2Z0	262.50	278.15	203.15	281.25	256.26	218.80	234.40	159.40	237.55	212.54
F2Z1	221.88	259.40	212.53	265.63	239.86	178.15	215.65	168.75	221.90	196.11
F2Z2	259.38	246.90	275.00	250.00	257.82	215.65	203.15	231.30	206.30	214.10
F2Z3	240.63	203.13	318.75	250.00	253.13	196.90	159.40	275.05	206.30	209.41
F3Z0	259.38	250.00	296.90	190.65	249.23	215.65	206.30	253.15	146.90	205.50
F3Z1	287.50	278.13	206.25	221.90	248.44	243.80	234.40	162.55	178.15	204.73
F3Z2	275.00	300.00	193.78	231.25	250.01	231.30	256.30	150.00	187.55	206.29
F3Z3	259.40	309.38	184.40	250.00	250.79	215.65	265.65	140.65	206.30	207.06
CD(0.05) FZM - 33.0563						CD(0.05) FZM - 33.0440				

Two way Table

I cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	MEAN
M1	267.98	313.30	246.09	270.32	285.94	275.79	291.42	244.54	274.42
M2	235.94	240.64	246.89	284.38	259.39	257.04	249.23	242.19	251.96
M3	247.67	234.38	252.36	220.33	241.42	214.86	240.64	257.83	238.68
M4	185.17	237.51	261.72	223.45	219.55	223.44	227.34	237.51	226.96
Mean	234.19	256.46	251.77	249.62	251.58	242.78	252.16	245.52	
CD (0.05) M/F - 8.2641					CD (0.05) MZ/FZ/FM - 16.5281				
II cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	MEAN
M1	224.25	269.55	202.38	226.60	242.22	232.06	247.68	200.81	230.69
M2	192.22	196.91	203.15	240.66	215.66	213.31	205.50	198.48	208.24
M3	203.93	190.66	208.63	176.59	197.69	171.13	196.90	214.09	194.95
M4	141.44	193.78	218.01	79.72	175.80	179.73	183.64	93.79	183.24
Mean	190.46	212.73	208.04	205.89	207.84	199.06	208.43	201.79	
CD (0.05) M /F - 8.2610					CD (0.05) MZ/FZ/FM - 16.5220				

Table 47. Effect of treatments on available phosphorus in soil (kg ha⁻¹)

Treatments	I cut					II cut				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
F0Z0	117.50	297.50	110.50	214.50	185.00	115.50	254.50	109.85	219.00	174.71
F0Z1	127.50	319.00	118.00	223.00	196.88	126.00	310.45	116.80	220.50	193.44
F0Z2	130.00	220.50	104.00	265.00	179.88	129.50	218.95	103.30	286.45	184.55
F0Z3	98.00	208.00	134.00	203.00	160.75	95.50	207.65	130.55	207.35	160.26
F1Z0	108.00	310.00	120.00	190.50	182.13	114.50	303.70	119.95	191.20	182.34
F1Z1	120.50	328.00	132.00	153.00	183.38	104.50	326.80	131.60	150.55	178.36
F1Z2	191.50	322.00	164.50	98.00	194.00	184.00	318.60	163.20	96.80	190.65
F1Z3	185.50	303.00	205.00	83.50	194.25	184.50	297.00	203.35	81.90	191.69
F2Z0	163.50	309.00	199.00	151.50	205.75	178.00	308.50	199.10	147.00	208.15
F2Z1	196.00	320.00	187.50	145.00	212.13	104.00	314.70	187.15	144.35	187.55
F2Z2	199.50	300.50	175.50	101.50	194.25	118.50	295.90	174.35	101.35	172.53
F2Z3	196.00	300.50	160.50	120.50	194.38	117.50	293.30	159.40	119.85	172.51
F3Z0	127.50	300.00	82.50	86.50	149.13	121.00	300.40	91.45	86.15	149.75
F3Z1	202.50	295.50	81.00	101.00	170.00	201.50	293.90	81.85	100.25	169.38
F3Z2	203.50	288.00	200.50	116.50	202.13	202.50	287.50	199.20	115.75	201.24
F3Z3	200.00	303.50	202.00	136.50	210.50	199.00	303.75	199.40	136.10	209.56
CD(0.05) FZM - 26.1223						CD(0.05) FZM - 39.9664				

Two way Table

I cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	MEAN
M1	118.3	151.4	188.8	183.4	129.1	161.6	181.1	169.9	160.4
M2	261.3	315.8	307.5	296.8	304.1	315.6	282.8	278.8	295.3
M3	116.6	155.4	180.6	141.5	128.0	129.6	161.1	175.4	148.5
M4	226.4	131.3	129.6	110.1	160.8	155.5	145.3	135.9	149.3
Mean	180.6	188.4	201.6	182.9	180.5	190.6	192.6	190.0	
CD (0.05) M/Z/F - 6.5306					CD (0.05) MZ/FZ/FM - 13.0612				
II cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	MEAN
M1	116.63	146.88	129.50	181.00	132.25	134.00	158.63	149.13	143.50
M2	247.89	311.53	303.10	296.39	291.78	311.46	280.24	275.43	289.72
M3	115.13	154.53	180.00	142.98	130.09	129.35	160.01	173.18	148.16
M4	233.33	130.11	128.14	109.56	160.84	153.91	150.09	136.30	150.28
Mean	178.24	185.76	185.18	182.48	178.74	182.18	187.24	183.51	
CD (0.05) M - 9.9916					CD (0.05) MZ/FZ/FM - 19.9832				

Table 48. Effect of treatments on available potassium in soil (kg ha⁻¹)

Treat-ments	I cut					II cut				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
F0Z0	175.50	156.90	287.60	139.30	189.83	172.50	153.60	285.65	136.50	187.06
F0Z1	137.00	182.50	299.05	170.10	197.16	133.50	177.45	295.05	166.85	193.21
F0Z2	196.00	145.05	335.80	151.15	207.00	189.50	145.15	332.20	145.80	203.16
F0Z3	185.50	181.05	345.35	166.90	219.70	180.00	178.35	344.30	163.10	216.44
F1Z0	330.00	205.20	332.75	105.25	243.30	314.00	204.35	330.70	102.65	237.93
F1Z1	306.00	200.55	353.10	104.75	241.10	278.50	200.35	351.05	100.05	232.49
F1Z2	141.00	131.70	377.05	157.70	201.86	137.50	129.60	371.80	905.35	386.06
F1Z3	121.50	170.10	232.90	171.65	174.04	129.00	166.80	230.75	167.70	173.56
F2Z0	145.50	188.25	250.10	137.85	180.42	99.50	184.60	250.25	134.25	167.15
F2Z1	193.50	224.90	262.90	103.55	196.21	190.50	219.15	258.70	101.70	192.51
F2Z2	175.50	205.50	283.95	123.10	197.01	170.00	203.35	278.35	118.90	192.65
F2Z3	152.50	256.05	347.10	115.20	217.71	150.00	251.75	344.60	111.85	214.55
F3Z0	157.50	284.45	392.75	207.10	260.45	107.50	282.50	389.65	204.00	245.91
F3Z1	353.50	295.55	244.35	185.15	269.64	351.50	291.75	240.00	180.85	266.03
F3Z2	355.00	229.70	235.30	110.85	232.71	352.00	155.85	231.15	107.75	479.19
F3Z3	369.50	198.70	220.65	105.20	223.51	365.00	196.20	216.85	103.55	220.40
CD(0.05) FZM - 31.8934						CD(0.05) FZM - NS				

Two way Table

I cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	MEAN
M1	173.5	224.6	166.8	308.9	202.1	247.5	216.9	207.3	18.4
M2	166.4	176.9	218.7	252.1	208.7	225.9	178.0	201.5	203.5
M3	317.0	324.0	286.0	273.3	315.8	289.9	308.0	286.5	300.0
M4	156.9	134.8	119.9	152.1	147.4	140.9	135.7	139.7	140.9
Mean	203.4	215.1	197.8	246.6	218.5	226.0	209.7	208.7	
CD (0.05) M/Z/F - 7.9733					CD (0.05) MZ/FZ/FM - 15.9467				
II cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	MEAN
M1	168.9	214.8	152.5	294.0	173.4	238.5	212.3	206.0	207.5
M2	163.6	175.3	214.7	499.1	206.3	222.2	426.0	198.3	263.2
M3	314.3	321.1	283.0	269.4	314.1	286.2	303.4	284.1	296.9
M4	153.1	318.9	116.7	149.0	144.4	137.4	319.5	136.6	184.4
Mean	200.0	257.5	191.7	302.9	209.5	221.1	315.3	206.2	
CD (0.05) M/Z/F- NS					CD (0.05) MZ/FZ/FM- NS				

4.8.3 Effect of treatments on soil exchangeable calcium, magnesium and available sulphur

The data on soil exchangeable calcium, magnesium and available sulphur contents are presented in Tables 49 to 51 respectively.

Exchangeable calcium content in the soil (Table 49) was significantly influenced by the treatments and their interactions. The highest value for exchangeable calcium content was observed for M3F3Z1 (K solubilizer x Fe @ foliar x Zn @ 5 mg kg⁻¹) for both the harvests. Considering the individual effect of microbial treatments, application of K solubilizer (M3) maintained highest exchangeable calcium content in the soil which was significantly superior to the other treatments and the same trend was observed for the second harvest also. Levels of Fe and Zn had not shown a definite pattern for both the harvests.

Exchangeable magnesium content in the soil (Table 50) was also significantly influenced by the treatments and their interactions except for the individual effect of Fe. The highest value was observed for M1F2Z1 (AMF x Fe @ 10 mg kg⁻¹ x Zn @ 5 mg kg⁻¹) at both the harvests. Addition of AMF (M1) maintained significantly higher exchangeable magnesium content compared to other treatments. At second harvest the treatment effects were significant only for bioaugmentation treatments and its interaction with Fe.

The individual as well as interactional effects were significant for available sulphur content in the soil (Table 51). The highest value was recorded by the treatment combination M1F2Z2 (AMF x Fe @ 10 mg kg⁻¹ x Zn @ 10 mg kg⁻¹) for both the harvests. Considering the individual effect of microbial treatments, AMF application (M1) was significantly superior to others and the same trend was observed for the second harvest also. An increase in available sulphur content was observed with increasing levels of Fe and Zn in the first harvest but the same trend was not observed for the second harvest.

Table 49 Effect of treatments on exchangeable calcium (cmol kg⁻¹) in soil

Treat-ments	I cut					II cut				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
F0Z0	5.15	4.40	5.70	5.10	5.09	5.00	3.95	5.70	5.00	4.91
F0Z1	5.00	4.25	6.80	5.15	5.30	4.85	4.00	6.20	5.25	5.08
F0Z2	5.45	4.75	4.35	4.95	4.88	5.10	4.55	4.10	5.00	4.69
F0Z3	4.50	4.00	4.40	4.45	4.34	4.00	4.05	4.00	4.30	4.09
F1Z0	4.40	5.00	4.45	4.85	4.67	4.25	5.10	4.10	4.65	4.53
F1Z1	5.10	5.25	5.00	5.10	5.11	5.10	5.25	4.95	4.75	5.01
F1Z2	5.55	5.00	5.00	5.20	5.19	5.40	5.15	4.80	5.10	5.11
F1Z3	5.60	4.30	5.00	5.00	4.98	5.40	4.35	4.80	5.00	4.89
F2Z0	4.25	4.40	4.75	5.20	4.65	4.30	4.50	4.60	4.85	4.56
F2Z1	4.30	4.95	5.75	5.25	5.06	4.25	4.55	5.30	5.15	4.81
F2Z2	4.25	5.00	5.50	5.15	4.98	4.15	4.80	5.10	5.00	4.76
F2Z3	5.00	4.90	5.50	4.35	4.94	4.60	5.15	5.25	4.35	4.84
F3Z0	5.00	5.25	4.15	4.30	4.68	5.05	5.00	4.50	4.40	4.74
F3Z1	5.00	5.25	6.85	5.25	5.59	4.95	5.00	6.50	4.65	5.28
F3Z2	5.25	5.00	4.80	5.15	5.05	5.00	5.15	4.70	5.15	5.00
F3Z3	5.25	5.35	4.85	5.00	5.11	5.10	5.15	4.85	5.00	5.03
CD(0.05) FZM - 0.4236						CD(0.05) FZM - 0.6091				

Two way Table

I cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	MEAN
M1	5.02	5.16	4.45	5.13	4.70	4.85	5.13	5.09	4.94
M2	4.35	4.89	4.81	5.21	4.76	4.93	4.94	4.64	4.82
M3	5.31	4.86	5.38	5.16	4.76	6.10	4.91	4.94	5.18
M4	4.91	5.04	4.99	4.93	4.86	5.19	5.11	4.70	4.97
Mean	4.90	4.99	4.91	5.11	4.77	5.27	5.02	4.84	
CD (0.05) M/Z/F - 0.1059					CD (0.05) MZ/FZ/FM - 0.2118				
II cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	MEAN
M1	4.74	5.04	4.33	5.03	4.65	4.79	4.91	4.78	4.78
M2	4.14	4.96	4.75	5.08	4.64	4.70	4.91	4.67	4.73
M3	5.00	4.66	5.06	5.14	4.73	5.74	4.68	4.73	4.97
M4	4.89	4.88	4.84	4.80	4.73	4.95	5.06	4.66	4.85
Mean	4.69	4.88	4.74	5.01	4.68	5.04	4.89	4.71	
CD (0.05) M/Z/F - 0.1523					CD (0.05) MZ/FZ/FM - 0.3045				

Table 50. Effect of treatments on exchangeable magnesium (cmol kg^{-1}) in soil

Treatments	I cut					II cut				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
F0Z0	3.90	1.00	0.40	1.10	1.60	3.55	1.05	0.30	0.90	1.45
F0Z1	3.90	0.95	0.45	1.25	1.64	3.65	1.00	0.90	0.90	1.61
F0Z2	3.55	0.90	2.15	2.00	2.15	3.65	0.85	2.20	1.65	2.09
F0Z3	4.70	1.15	1.60	2.05	2.38	4.80	1.10	1.95	2.00	2.46
F1Z0	4.35	1.70	1.70	1.45	2.30	4.20	1.30	1.75	1.40	2.16
F1Z1	4.15	0.37	1.40	0.90	1.70	3.95	0.60	1.25	1.10	1.72
F1Z2	2.05	0.60	1.55	1.25	1.36	1.75	0.70	1.50	1.00	1.24
F1Z3	4.20	1.65	1.75	1.20	2.20	3.70	1.15	1.60	1.15	1.90
F2Z0	1.15	1.65	1.60	1.00	1.35	1.35	1.15	1.45	1.00	1.24
F2Z1	4.95	0.60	0.85	1.15	1.89	4.70	0.75	1.10	0.90	1.86
F2Z2	4.55	0.95	1.25	0.95	1.93	4.30	0.80	1.25	0.85	1.80
F2Z3	3.70	0.55	1.45	1.75	1.86	3.75	1.00	1.35	1.15	1.81
F3Z0	3.70	1.45	1.10	1.10	1.84	3.15	1.10	0.70	1.55	1.63
F3Z1	4.85	1.00	0.35	1.00	1.80	4.15	1.00	0.20	1.35	1.68
F3Z2	4.15	1.00	1.00	1.20	1.84	4.20	0.65	0.80	0.80	1.61
F3Z3	4.70	1.50	1.15	1.45	2.20	4.15	0.93	0.80	0.95	1.71
CD(0.05) FZM - 0.7840						CD(0.05) FZM - 0.8752				

Two way Table

I cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	MEAN
M1	4.01	3.69	3.59	4.35	3.27	4.46	3.58	4.33	3.91
M2	1.00	1.08	0.94	1.24	1.45	0.73	0.86	1.21	1.06
M3	1.15	1.60	1.29	0.90	1.20	0.76	1.49	1.49	1.23
M4	1.60	1.20	1.21	1.19	1.16	1.08	1.35	1.61	1.30
Mean	1.94	1.89	1.76	1.92	1.77	1.76	1.82	2.16	
CD (0.05) M/Z - 0.1960					CD (0.05) MZ/FZ/FM - 0.3920				
II cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	MEAN
M1	3.91	3.40	3.53	3.91	3.06	4.11	3.48	4.10	3.69
M2	1.00	0.94	0.93	0.92	1.15	0.84	0.75	1.04	0.95
M3	1.34	1.53	1.29	0.63	1.05	0.86	1.44	1.43	1.19
M4	1.36	1.16	0.98	1.16	1.21	1.06	1.08	1.31	1.17
Mean	1.90	1.76	1.68	1.66	1.62	1.72	1.68	1.97	
CD (0.05) M/Z - 0.2188					CD (0.05) MZ/FZ/FM - 0.4376				

Table 51. Effect of treatments on available sulphur (kg ha⁻¹) in soil

Treatments	I cut					II cut				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
F0Z0	7.59	5.91	5.12	3.99	5.65	6.84	5.48	4.25	3.62	5.05
F0Z1	9.68	7.73	7.57	4.89	7.47	7.08	6.82	6.35	4.24	6.12
F0Z2	11.02	8.32	8.50	6.21	8.51	10.02	8.12	7.75	5.55	7.86
F0Z3	8.32	6.59	6.23	4.71	6.46	7.98	5.72	5.01	4.01	5.68
F1Z0	15.11	8.74	6.55	5.46	8.97	12.19	8.00	5.90	4.80	7.72
F1Z1	18.63	11.00	10.37	6.53	11.63	15.69	11.43	6.84	5.48	9.86
F1Z2	25.17	17.58	12.95	7.91	15.90	22.34	16.74	11.83	7.18	14.52
F1Z3	16.99	9.62	7.97	5.86	10.11	16.15	9.85	70.99	4.98	25.49
F2Z0	18.75	15.82	8.61	6.64	12.46	16.74	14.99	8.65	6.21	11.64
F2Z1	22.71	17.46	10.75	7.55	14.61	11.49	16.15	10.31	7.15	11.28
F2Z2	29.18	21.59	17.46	8.43	19.16	22.63	19.30	14.28	7.98	16.05
F2Z3	19.34	16.17	9.87	7.03	13.10	18.49	14.82	9.32	6.56	12.30
F3Z0	9.97	13.94	5.98	4.49	8.60	9.04	12.94	5.29	4.32	7.90
F3Z1	13.42	16.35	7.36	5.71	10.71	16.39	15.22	6.29	5.00	10.72
F3Z2	17.29	17.81	9.73	6.33	12.79	13.82	16.44	9.39	5.73	11.35
F3Z3	15.53	15.76	7.08	5.17	10.89	5.06	14.99	6.63	4.75	7.86
CD(0.05) FZM - 1.0041						CD(0.05) FZM - 4.0419				

Two way Table

I cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	MEAN
M1	9.15	18.97	22.49	14.05	12.86	16.11	20.66	15.04	16.17
M2	7.14	11.73	17.76	15.97	11.10	13.14	16.33	12.03	13.15
M3	6.86	9.46	11.67	7.54	6.56	9.01	12.16	7.79	8.88
M4	4.95	6.44	7.41	5.43	5.15	6.17	7.22	5.69	6.06
Mean	7.02	11.65	14.83	10.75	8.92	11.11	14.09	10.14	
CD (0.05) M/Z/F - 0.2510					CD (0.05) MZ/FZ/FM -0.5021				
II cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	MEAN
M1	7.98	16.59	17.34	11.08	11.20	12.66	17.20	11.92	13.25
M2	6.54	11.50	16.31	14.90	10.35	12.40	15.15	11.34	12.31
M3	5.84	23.89	10.64	6.90	6.02	7.45	10.81	22.98	11.82
M4	4.36	5.61	6.97	4.95	4.74	5.47	6.61	5.08	5.47
Mean	6.18	14.40	12.82	9.46	8.08	9.50	12.44	12.83	
CD (0.05) M/Z/F - 1.0105					CD (0.05) MZ/FZ/FM -2.0210				

4.8.4 Effect of treatments on soil available iron, manganese, zinc and copper

The data on soil available iron, manganese, zinc and copper contents are presented in Tables 52 to 55 respectively.

Available iron content in the soil (Table 52) was significantly influenced by the treatments and their interactions for both the harvests. The highest value was recorded by M1F1Z3 (AMF x Fe @ 5 mg kg⁻¹ x Zn @ foliar) and the same trend was observed for the second harvest also.

Considering the individual microbial effect for both the harvests, AMF application (M1) recorded the highest value and was significantly superior to others.

For both the harvests, the available manganese content in the soil (Table 53) was significant only for bioaugmentation methods. The highest value for available Mn content was recorded by AMF (M1), which was on par with P solubilizer (M2) and POP (M4) for the first harvest. Evaluating the second harvest, the highest value was recorded by POP (M4) which was on par with AMF (M1) and P solubilizer (M2).

Table 52. Effect of treatments on available iron in soil (mg kg⁻¹)

Treat-ments	I cut					II cut				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
F0Z0	5.68	5.44	0.71	3.16	3.75	5.48	5.24	1.52	2.97	3.80
F0Z1	5.21	3.21	0.92	3.04	3.09	5.01	3.01	1.72	2.85	3.15
F0Z2	6.40	3.43	0.91	2.91	3.41	6.21	3.23	1.71	2.72	3.47
F0Z3	6.73	3.26	5.54	2.32	4.46	6.53	3.06	5.34	2.13	4.27
F1Z0	8.04	3.18	1.20	2.77	3.79	7.84	2.98	1.16	2.58	3.64
F1Z1	5.01	3.28	1.02	2.27	2.90	4.82	1.95	1.14	2.08	2.50
F1Z2	7.04	5.22	0.91	5.57	4.68	6.85	5.02	1.10	5.37	4.58
F1Z3	8.42	2.91	1.26	2.44	3.76	8.23	2.71	1.22	2.25	3.60
F2Z0	7.43	2.95	1.22	3.76	3.84	7.24	2.76	1.16	3.57	3.68
F2Z1	6.82	5.22	1.18	3.85	4.27	6.62	5.02	1.11	3.66	4.10
F2Z2	8.23	5.08	1.26	3.92	4.62	8.04	4.88	1.15	3.72	4.45
F2Z3	5.08	4.43	0.76	3.77	3.51	4.88	4.24	1.57	3.58	3.57
F3Z0	6.55	3.88	1.25	3.30	3.75	6.36	3.69	1.07	3.11	3.56
F3Z1	6.59	3.23	1.61	3.34	3.69	6.39	3.03	1.45	3.15	3.50
F3Z2	7.05	3.36	1.86	3.13	3.85	6.85	3.16	1.71	2.93	3.66
F3Z3	3.85	0.99	2.42	3.19	2.61	3.66	1.02	2.23	3.09	2.50
CD(0.05) FZM - 1.2421						CD(0.05) FZM - 1.2373				

Two way Table

I cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	MEAN
M1	6.00	7.13	6.89	6.01	6.93	5.91	7.18	6.02	6.51
M2	3.83	3.64	4.42	2.86	3.86	3.73	4.27	2.90	3.69
M3	2.02	1.10	1.11	1.78	1.09	1.18	1.24	2.50	1.50
M4	2.86	3.26	3.83	3.24	3.25	3.13	3.88	2.93	3.30
Mean	3.68	3.78	4.06	3.48	3.78	3.49	4.14	3.59	
CD (0.05) M/Z/F - 0.3105					CD (0.05) MZ/FZ/FM - 0.6211				
II cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	MEAN
M1	5.81	6.93	6.70	5.81	6.73	5.71	6.99	5.82	6.31
M2	3.64	3.17	4.22	2.72	3.67	3.25	4.07	2.76	3.44
M3	2.57	1.15	1.25	1.61	1.23	1.35	1.42	2.59	1.65
M4	2.67	3.07	3.63	3.07	3.05	2.93	3.69	2.76	3.11
Mean	3.67	3.58	3.95	3.31	3.67	3.31	4.04	3.48	
CD (0.05) M/Z/F - 0.3093					CD (0.05) MZ/FZ/FM - 0.6187				

Table53. Effect of treatments on available manganese in soil (mg kg⁻¹)

Treat-ments	I cut					II cut				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
F0Z0	6.20	8.03	2.31	7.30	5.96	6.07	7.90	2.17	37.67	13.45
F0Z1	8.18	7.72	3.38	7.68	6.74	8.05	7.59	3.25	7.55	6.61
F0Z2	6.74	7.10	2.79	8.48	6.28	6.61	6.97	2.65	8.35	6.15
F0Z3	7.62	7.59	3.72	7.37	6.57	7.49	7.46	3.59	7.23	6.44
F1Z0	8.23	7.52	4.33	8.09	7.04	8.10	7.39	4.20	7.96	6.91
F1Z1	7.03	7.30	3.80	8.70	6.71	6.89	7.17	3.67	8.57	6.57
F1Z2	7.60	8.33	3.68	5.69	6.33	7.47	8.20	3.54	5.56	6.19
F1Z3	8.11	7.37	2.54	7.82	6.46	7.98	7.24	2.41	7.69	6.33
F2Z0	6.74	7.78	3.41	7.73	6.41	6.60	7.65	3.28	7.60	6.28
F2Z1	36.97	9.06	3.36	8.72	14.53	36.84	8.93	3.23	8.58	14.40
F2Z2	7.11	8.48	3.08	8.47	6.79	6.98	8.35	2.95	8.34	6.65
F2Z3	7.31	8.34	3.13	7.25	6.51	7.18	8.21	3.00	7.12	6.38
F3Z0	6.26	7.73	3.32	7.71	6.25	6.13	7.59	3.19	7.58	6.12
F3Z1	8.80	8.15	3.60	7.51	7.02	8.67	8.02	3.47	7.38	6.89
F3Z2	7.13	8.15	3.71	8.23	6.81	7.00	8.02	3.58	8.10	6.67
F3Z3	8.22	3.28	7.75	7.91	6.79	8.08	3.15	7.62	8.30	6.79
CD(0.05) FZM - NS						CD(0.05) FZM - NS				

Two way Table

I cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	MEAN
M1	7.19	7.74	14.53	7.60	6.86	15.25	7.15	7.82	9.27
M2	7.61	7.63	8.42	6.83	7.76	8.06	8.02	6.65	7.62
M3	3.05	3.59	3.25	4.59	3.34	3.54	3.31	4.29	3.62
M4	7.71	7.58	8.04	7.84	7.71	8.15	7.72	7.59	7.79
Mean	6.39	6.63	8.56	6.72	6.42	8.75	6.55	6.58	
CD (0.05) M/Z/F-					CD (0.05) MZ/FZ/FM-				
II cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	MEAN
M1	7.05	7.61	14.40	7.47	6.73	15.11	7.01	7.68	9.13
M2	7.48	7.50	8.28	6.69	7.63	7.93	7.88	6.52	7.49
M3	2.92	3.46	3.11	4.46	3.21	3.40	3.18	4.15	3.49
M4	15.20	7.44	7.91	7.84	15.20	8.02	7.59	7.59	9.60
Mean	8.16	6.50	8.43	6.62	8.19	8.62	6.42	6.48	
CD (0.05) M - 3.7849					CD (0.05) MZ/FZ/FM - NS				

Available zinc content in the soil (Table 54) was significantly influenced by the treatments and their interactions. Highest value for available Zn content in the soil was recorded by M1F1Z2 (AMF x Fe @ 5 mg kg⁻¹ x Zn @ 10 mg kg⁻¹) for the first harvest. Evaluating the second harvest, the highest value was recorded by M1F0Z2 (AMF x Fe @ zero x Zn @ 10 mg kg⁻¹). Considering the individual effect of bioaugmentation methods, AMF (M1) recorded the highest value for both the harvests and was significantly superior to others. An increase in available Zn content was noted with increasing levels of Zn at both harvests.

Available copper content in the soil (Table 55) was significantly affected by the treatments and their interactions for both the harvests. Considering the individual effect of bioaugmentation methods, POP (M4) recorded highest value. The highest available Cu content in the soil was recorded by M4F1Z2 (POP x Fe @ 5 mg kg⁻¹ x Zn @ 10 mg kg⁻¹) for the first harvest. At the second harvest, a drastic reduction was observed for soil available Cu, except for AMF treatment.

4.8.5 Effect of treatments on soil microbial properties

The data on AMF colonization, bacterial population, fungal population and actinomycetes population in soil are presented in Tables 56 to 59 respectively.

AMF colonization (Table 56) was significantly influenced only by the individual effect of bioaugmentation methods (M) and its combination with levels of Fe (M x F). The highest colonization was observed for the treatments receiving AMF (M1) treatment and was significantly superior to other three treatments.

Soil bacterial count (Table 57) was significantly influenced by the treatments and their interactions. The highest count was observed for M1F0Z1 (AMF x Fe @ zero x Zn @ 5 mg kg⁻¹), which was also on par with several other treatments. Evaluating the individual effect of bioaugmentation methods, M3 (K solubilizer) recorded the highest value and was also on par with M1 (AMF).

. Table 54. Effect of treatments on available zinc in soil (mg kg⁻¹)

Treatments	I cut					II cut				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
F0Z0	1.74	1.36	0.70	0.68	1.12	1.82	1.36	0.67	0.59	1.11
F0Z1	2.29	2.36	1.50	1.74	1.97	2.24	2.20	1.75	1.72	1.98
F0Z2	3.26	2.50	2.36	1.94	2.51	3.07	2.72	2.26	1.84	2.47
F0Z3	2.07	2.21	0.84	1.66	1.69	1.99	2.12	0.94	1.59	1.66
F1Z0	1.63	0.71	0.60	1.40	1.08	1.55	0.56	0.54	1.62	1.07
F1Z1	2.57	2.21	2.21	1.89	2.22	2.07	2.11	2.15	2.04	2.09
F1Z2	3.60	2.21	2.60	2.24	2.66	2.92	2.57	2.52	2.39	2.60
F1Z3	1.70	1.37	0.78	1.40	1.31	1.68	1.36	0.81	1.62	1.36
F2Z0	1.28	1.09	0.67	0.82	0.96	1.20	1.05	0.63	0.73	0.90
F2Z1	2.01	1.39	1.42	2.46	1.82	1.79	1.69	1.29	2.35	1.78
F2Z2	3.01	2.20	2.11	2.68	2.50	2.91	2.11	2.01	2.59	2.41
F2Z3	1.40	1.23	1.24	1.90	1.44	1.36	1.05	1.14	1.81	1.34
F3Z0	0.92	1.34	0.74	1.19	1.05	0.88	1.24	0.67	1.10	0.97
F3Z1	3.06	2.14	0.86	1.32	1.84	2.87	2.20	0.78	1.23	1.77
F3Z2	3.26	2.22	0.90	1.34	1.93	3.07	2.16	0.81	1.25	1.82
F3Z3	1.64	1.68	0.77	1.25	1.33	1.58	1.53	0.66	1.19	1.24
CD(0.05) FZM - 0.1530						CD(0.05) FZM - 0.1459				

Two way Table

I cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	MEAN
M1	2.34	2.37	1.93	2.22	1.39	2.48	3.28	1.70	2.21
M2	2.11	1.62	1.48	1.84	1.13	2.02	2.28	1.62	1.76
M3	1.35	1.55	1.36	0.82	0.68	1.50	1.99	0.91	1.27
M4	1.50	1.73	1.96	1.28	1.02	1.85	2.05	1.55	1.62
Mean	1.82	1.82	1.68	1.54	1.05	1.96	2.40	1.44	
CD (0.05) M/Z/F - 0.0382					CD (0.05) MZ/FZ/FM -0.0765				
II cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	MEAN
M1	2.28	2.05	1.81	2.10	1.36	2.24	2.99	1.65	2.06
M2	2.10	1.65	1.48	1.78	1.05	2.05	2.39	1.51	1.75
M3	1.40	1.50	1.27	0.73	0.63	1.49	1.90	0.89	1.23
M4	1.43	1.91	1.87	1.19	1.01	1.83	2.02	1.55	1.60
Mean	1.80	1.78	1.61	1.45	1.01	1.90	2.32	1.40	
CD (0.05) M/Z/F - 0.0365					CD (0.05) MZ/FZ/FM - 0.0729				

Table 55. Effect of treatments on available copper in soil (mg kg⁻¹)

Treatments	I cut					II cut				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
F0Z0	1.18	3.03	1.41	2.24	1.97	1.12	0.24	0.08	0.16	0.40
F0Z1	1.32	1.41	1.86	2.55	1.78	1.26	0.08	0.13	0.19	0.42
F0Z2	1.10	1.70	3.87	1.35	2.01	1.04	0.11	0.33	0.08	0.39
F0Z3	1.43	1.60	1.40	2.79	1.81	1.37	0.10	0.08	0.22	0.44
F1Z0	1.61	1.60	1.25	1.98	1.61	1.55	0.10	0.06	0.14	0.46
F1Z1	1.02	1.59	1.11	1.16	1.22	0.96	0.10	0.05	0.06	0.29
F1Z2	1.12	1.89	1.58	6.52	2.78	1.06	0.13	0.10	0.59	0.47
F1Z3	1.66	1.41	1.83	2.43	1.83	1.60	0.08	0.12	0.18	0.50
F2Z0	1.52	2.45	2.04	3.21	2.31	1.46	0.19	0.14	0.26	0.51
F2Z1	1.46	2.53	2.05	2.37	2.10	1.40	0.19	0.15	0.18	0.48
F2Z2	1.10	2.45	1.83	3.95	2.33	1.04	0.19	0.12	0.33	0.42
F2Z3	0.99	2.72	1.08	2.60	1.85	0.93	0.21	0.05	0.20	0.35
F3Z0	1.72	1.43	1.33	2.05	1.63	1.66	0.08	0.07	0.15	0.49
F3Z1	1.40	1.65	1.69	2.37	1.78	1.34	0.11	0.11	0.18	0.43
F3Z2	1.21	2.74	1.71	2.14	1.95	1.15	0.21	0.11	0.15	0.41
F3Z3	0.64	3.33	3.06	2.06	2.27	0.58	0.27	0.25	0.18	0.32
CD(0.05) FZM - 0.6975						CD(0.05) FZM - 0.1843				

Two way Table

I cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	MEAN
M1	1.26	1.35	1.27	1.24	1.51	1.30	1.13	1.18	1.28
M2	1.94	1.62	2.54	2.29	2.13	1.80	2.20	2.26	2.10
M3	2.14	1.44	1.75	1.95	1.51	1.68	2.25	1.84	1.82
M4	2.23	3.02	3.03	2.16	2.37	2.11	3.49	2.47	2.61
Mean	1.89	1.86	2.15	1.91	1.88	1.72	2.27	1.94	
CD (0.05) M/Z/F - 0.1744					CD (0.05) MZ/FZ/FM - 0.3487				
II cut	F0	F1	F2	F3	Z0	Z1	Z2	Z3	MEAN
M1	1.20	1.29	1.21	1.18	1.45	1.24	1.07	1.12	1.22
M2	0.13	0.10	0.19	0.17	0.15	0.12	0.16	0.17	0.15
M3	0.15	0.08	0.11	0.13	0.09	0.11	0.16	0.12	0.12
M4	0.16	0.24	0.24	0.16	0.18	0.15	0.29	0.20	0.20
Mean	0.41	0.43	0.44	0.41	0.47	0.40	0.42	0.40	
CD (0.05) M/Z/F-					CD (0.05) MZ/FZ/FM-				

Table 56. Effect of treatments on AMF colonisation (%) in soil

Treatments	M1	M2	M3	M4	Mean				
F0Z0	67.50	32.50	30.00	17.50	36.88				
F0Z1	70.00	30.00	32.50	17.50	37.50				
F0Z2	62.50	37.50	32.50	15.00	36.88				
F0Z3	62.50	32.50	27.50	20.00	35.63				
F1Z0	70.00	32.50	27.50	20.00	37.50				
F1Z1	67.50	32.50	27.50	17.50	36.25				
F1Z2	62.50	32.50	27.50	17.50	35.00				
F1Z3	70.00	27.50	30.00	15.00	35.63				
F2Z0	67.50	32.50	32.50	17.50	37.50				
F2Z1	62.50	37.50	35.00	17.50	38.13				
F2Z2	72.50	32.50	37.50	15.00	39.38				
F2Z3	62.50	35.00	32.50	15.00	36.25				
F3Z0	67.50	32.50	27.50	15.00	35.63				
F3Z1	70.00	35.00	32.50	12.50	37.50				
F3Z2	70.00	32.50	37.50	15.00	38.75				
F3Z3	67.50	32.50	37.50	15.00	38.13				
	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	65.63	67.50	66.25	68.75	68.13	67.50	66.88	65.63	67.03
M2	33.13	31.25	34.38	33.13	32.50	33.75	33.75	31.88	32.97
M3	30.63	28.13	34.38	33.75	29.38	31.88	33.75	31.88	31.72
M4	17.50	17.50	16.25	14.38	17.50	16.25	15.63	16.25	16.41
Mean	36.72	36.09	37.81	37.50	36.88	37.34	37.50	36.41	
CD(0.05) M- 1.8222					CD(0.05)FM- 3.6443				

Table 57. Effect of treatments on bacterial population ($\times 10^6$ cfu g^{-1}) in soil

Treatments	M1	M2	M3	M4	Mean				
F0Z0	23.50	12.00	13.00	20.00	17.13				
F0Z1	26.50	16.50	16.00	18.00	19.25				
F0Z2	24.00	19.50	23.00	24.50	22.75				
F0Z3	26.00	15.50	16.00	23.00	20.13				
F1Z0	19.50	16.50	16.50	18.00	17.63				
F1Z1	18.00	14.00	22.00	16.00	17.50				
F1Z2	26.00	12.00	24.00	17.50	19.88				
F1Z3	17.50	14.00	19.00	17.00	16.88				
F2Z0	23.50	13.00	21.50	21.00	19.75				
F2Z1	20.00	12.00	24.00	15.00	17.75				
F2Z2	21.50	16.00	27.50	15.00	20.00				
F2Z3	17.00	11.00	24.00	14.00	16.50				
F3Z0	14.00	11.00	17.00	16.50	14.63				
F3Z1	15.50	11.00	25.00	16.50	17.00				
F3Z2	14.50	10.50	23.50	16.00	16.13				
F3Z3	15.50	12.00	23.00	16.00	16.63				
	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M0	25.00	20.25	20.50	14.88	20.13	20.00	21.50	19.00	20.16
M1	15.88	14.13	13.00	11.13	13.13	13.38	14.50	13.13	13.53
M2	17.00	20.38	24.25	22.13	17.00	21.75	24.50	20.50	20.94
M3	21.38	17.13	16.25	16.25	18.88	16.38	18.25	17.50	17.75
Mean	19.81	17.97	18.50	16.09	17.28	17.88	19.69	17.53	
CD(0.05) M/F- 1.0807			CD(0.05) FZ/FM/MZ - 2.1615				CD(0.05)FZM4.3229		

Considering the individual effect of Fe a definite pattern was not observed where as for the levels of Zn, an increase in count was observed with increasing levels of soil application of Zn.

Fungal population (Table 58) was significantly influenced by the individual effects and Fe and Zn interaction with bioaugmentation methods (M x F ; M x Z) Considering the individual effect of bioaugmentation methods, M1 (AMF) recorded the highest value and was significantly superior to others. For the levels of Fe and Zn a definite pattern was not observed for the fungal population.

Actinomycetes population (Table 59) was significantly influenced by the bioaugmentation method alone (M) and the highest value were recorded by M1 (AMF) which was significantly superior to other three treatments.

4.9 Economics of cultivation

The B:C ratio (Table 60) was significantly influenced by the individual factors as well as their interactions except the individual effect of Fe on B:C ratio. The highest B:C ratio of 4.19 was recorded by the treatment combination M4F0Z2 (POP x Fe @ zero x Zn @ 10 mg kg⁻¹) which was on par with several other treatment combinations. The B: C ratio was lowest for M1F2Z0 (AMF x Fe @ 10 mg kg⁻¹ x Zn @ zero). Evaluating the individual effects of bioaugmentation methods, M4 (POP) was significantly superior to other three treatments. In the case of Fe, the increasing levels for soil application and the treatment with foliar application decreased the B:C ratio. The levels of Zn showed a positive relation with B:C ratio up to Z1(Zn @ 5 mg kg⁻¹) and with Z3 (Zn as foliar).

Table 58 . Effect of treatments on fungal population ($\times 10^4$ cfu g⁻¹) in soil

	M1	M2	M3	M4	Mean				
F0Z0	15.50	12.00	10.50	10.50	12.13				
F0Z1	13.50	9.00	11.00	9.00	10.63				
F0Z2	15.50	10.00	13.50	10.50	12.38				
F0Z3	11.50	10.50	11.50	8.00	10.38				
F1Z0	16.00	10.50	10.50	12.00	12.25				
F1Z1	16.00	11.00	11.50	10.50	12.25				
F1Z2	13.00	14.00	14.00	11.50	13.13				
F1Z3	13.00	12.00	13.50	13.00	12.88				
F2Z0	16.50	11.00	12.50	10.50	12.63				
F2Z1	12.00	11.00	10.50	9.50	10.75				
F2Z2	11.00	11.00	9.00	9.00	10.00				
F2Z3	13.00	12.00	10.00	9.50	11.13				
F3Z0	15.00	10.50	11.50	12.50	12.38				
F3Z1	12.50	8.50	9.00	9.50	9.88				
F3Z2	13.00	11.50	9.50	11.50	11.38				
F3Z3	11.50	10.50	11.50	12.50	11.50				
	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	14.00	14.50	13.13	13.00	15.75	13.50	13.13	12.25	13.66
M2	10.38	11.88	11.25	10.25	11.00	9.88	11.63	11.25	10.94
M3	11.63	12.38	10.50	10.38	11.25	10.50	11.50	11.63	11.22
M4	9.50	11.75	9.63	11.50	11.38	9.63	10.63	10.75	10.59
Mean	11.38	12.63	11.13	11.28	12.34	10.88	11.72	11.47	
CD(0.05)F/Z/M- 0.8185					CD(ZM)- 1.6370				

Table 59. Effect of treatments on actinomycetes population ($\times 10^2$ cfu g^{-1}) in soil

Treatments	M1	M2	M3	M4	Mean				
F0Z0	3.50	1.00	2.00	1.50	2.00				
F0Z1	3.50	2.00	2.00	3.00	2.63				
F0Z2	4.50	2.00	2.00	4.00	3.13				
F0Z3	3.50	2.00	2.00	3.00	2.63				
F1Z0	4.50	1.00	1.50	3.00	2.50				
F1Z1	4.50	2.00	2.00	1.50	2.50				
F1Z2	4.50	2.00	1.50	2.50	2.63				
F1Z3	4.00	1.50	2.50	3.00	2.75				
F2Z0	4.00	1.00	1.00	2.00	2.00				
F2Z1	5.00	2.50	2.50	1.50	2.88				
F2Z2	3.50	1.50	2.00	3.00	2.50				
F2Z3	4.00	2.50	1.50	1.00	2.25				
F3Z0	5.00	1.00	1.50	2.00	2.38				
F3Z1	3.00	2.50	2.50	1.50	2.38				
F3Z2	3.00	2.00	1.50	2.00	2.13				
F3Z3	3.50	2.00	1.50	1.00	2.00				
	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	3.75	4.38	4.13	3.63	4.25	4.00	3.88	3.75	3.97
M2	1.75	1.63	1.88	1.88	1.00	2.25	1.88	2.00	1.78
M3	2.00	1.88	1.75	1.75	1.50	2.25	1.75	1.88	1.84
M4	2.88	2.50	1.88	1.63	2.13	1.88	2.88	2.00	2.22
Mean	2.59	2.59	2.41	2.22	2.22	2.59	2.59	2.41	
CD(0.05) M - 0.3644			CD(0.05) ZM - 0.7289			CD(0.05)FZM- NS			

Table 60. Effect of treatments on B:C ratio of amaranthus

Treatments	M1	M2	M3	M4	Mean				
F0Z0	2.42	2.54	2.40	3.25	2.65				
F0Z1	2.37	2.74	2.57	3.58	2.82				
F0Z2	2.52	3.58	3.12	4.19	3.35				
F0Z3	2.37	2.82	3.78	3.56	3.13				
F1Z0	2.21	2.36	3.05	4.01	2.91				
F1Z1	2.71	3.94	2.48	3.75	3.22				
F1Z2	2.33	2.69	2.79	3.11	2.73				
F1Z3	2.45	2.65	3.47	2.60	2.79				
F2Z0	1.73	2.12	2.63	3.74	2.55				
F2Z1	2.58	3.44	2.30	3.22	2.88				
F2Z2	2.57	3.82	2.28	2.96	2.91				
F2Z3	1.78	3.75	2.18	3.47	2.80				
F3Z0	2.67	2.53	2.15	2.98	2.58				
F3Z1	2.68	3.58	2.60	3.10	2.99				
F3Z2	2.08	2.30	2.77	3.65	2.70				
F3Z3	1.98	2.86	2.88	3.85	2.89				
	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	2.42	2.42	2.16	2.35	2.26	2.58	2.37	2.14	2.34
M2	2.92	2.91	3.28	2.82	2.39	3.42	3.10	3.02	2.98
M3	2.97	2.95	2.35	2.60	2.56	2.49	2.74	3.08	2.72
M4	3.65	3.37	3.35	3.39	3.50	3.41	3.48	3.37	3.44
Mean	2.99	2.91	2.79	2.79	2.67	2.98	2.92	2.90	
CD(0.05) M/Z - 0.1792			CD(0.05) FZ/FM/MZ - 0.3584			CD(0.05)FZM=-0.7168			

Discussion

5. DISCUSSION

An investigation entitled “Iron and Zinc fortification in amaranthus (*Amaranthus tricolor*) through bioaugmentation” has been carried out at the Department of Soil Science and Agricultural Chemistry, College of Agriculture, Vellayani during May to August 2012. The main objective was to study the effect of Fe and Zn application and bioaugmentation of soil with microbial additives on yield and nutrient composition of amaranthus, particularly with that of Fe and Zn. This was accomplished through a pot culture experiment using laterite soil.

5.1 Effect of bioaugmentation and iron and zinc fortification on yield and growth characteristics of amaranthus

The experiment results revealed that bioaugmentation methods and iron and zinc fortification had significantly influenced the economic yield. The treatment combination M4F0Z2 (POP x Fe@ zerox Zn@ 10 mg kg⁻¹) recorded the highest economic yield and B:C ratio. The above treatment was on par with several treatment combinations of M2 ie. , P solubiliser based (M2F0Z2, M2F1Z1, M2F2Z2, M2F2Z3, M2F3Z1) and M4 ie., POP based (M4F2Z0, M4F1Z1, M4F3Z2, M4F2Z0, M4F3Z3) and with M3F0Z3 (K solubiliser x Fe @ zerox Zn foliar). However, the effect of bioaugmentation and levels of zinc was more dominant (Fig.2) while iron played a less important role though several treatments with different levels of iron where also on par with highest yielding treatment. Evaluating the individual as well as interaction effects of different factors, the individual effect of levels of Fe alone was not significant, clearly indicating the lesser important role of Fe treatments on shoot yield (Table 14). In general, response to iron application to crops with either through soil or foliar means was low compared to other micronutrients. Despite the ubiquitous presence of iron in the earth crust, low solubility of iron in many soils prevents plant uptake and induces deficiency symptoms or hidden hunger and adversely affect the yield

(Lindsay, 1984). Fe fertilizers have very little effect on yield and the amount of iron accumulated in edible portions of crops, when they are soil applied or foliar applied due to limited phloem sap mobility of Fe (Welch, 1999). Even soils high in total Fe reported to be low in available form. Mathew and Aparna (2012) reported 8.9% deficiency in laterite soils of Thiruvananthapuram district. Mainly under such conditions plants develop root strategies to cope with Fe insolubility based on acidification, excretion of reductants or chelates and having increased root reductase activity (Rogers and Geierinot, 2002).

Iron was shown to be transported from the root to aerial plant organs in the xylem as ferric citrate complex and it has very little phloem mobility. In most of the cases major portion of the absorbed Fe get deposited at the root itself and its further translocation is prevented (Reichman, 2002). This peculiar behavior of Fe might be the major reason for lesser response of soil applied Fe on shoot biomass. Even the foliar application of Fe could not produce a positive effect as observed in the case of Zn. The process of foliar penetration of leaf applied Fe solution is quite complex and depends upon an array of environmental and plant factors. Its entry is mainly through cuticle, stomata, leaf hairs and specialized epidermal cells. Liquids having surface tension approaching that to pure water will fail spontaneously to penetrate stomata unless some external pressure is applied. In many cases, foliar application of Fe results in defoliation (Eichert *et al.*, 2002). The complicated and unpredicted behavior of Fe always poses a major threat in correction of Fe deficiency.

Among the 16 POP based treatments (M4), seven treatment combinations were on par with each other in shoot yield and B: C ratio with that of highest yielding treatment (Fig.1). The micro nutrients supplied to the crop might have favorably influenced the nutrient uptake, translocation and utilization of this for

Fig.1 Effect of bioaugmentation on shoot yield of amaranthus (g plant⁻¹)

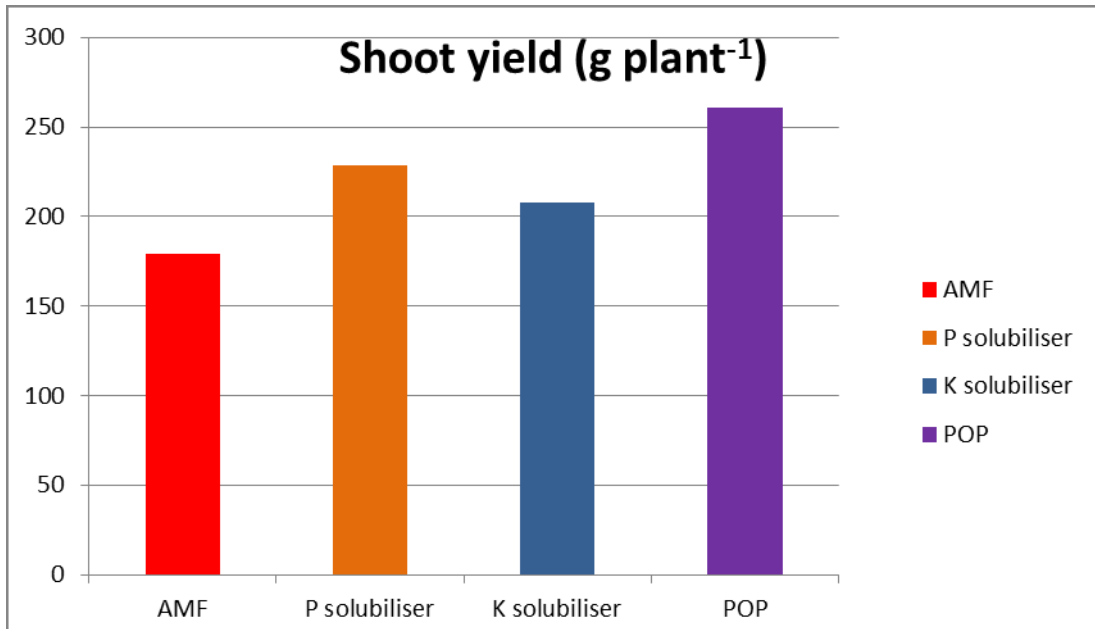
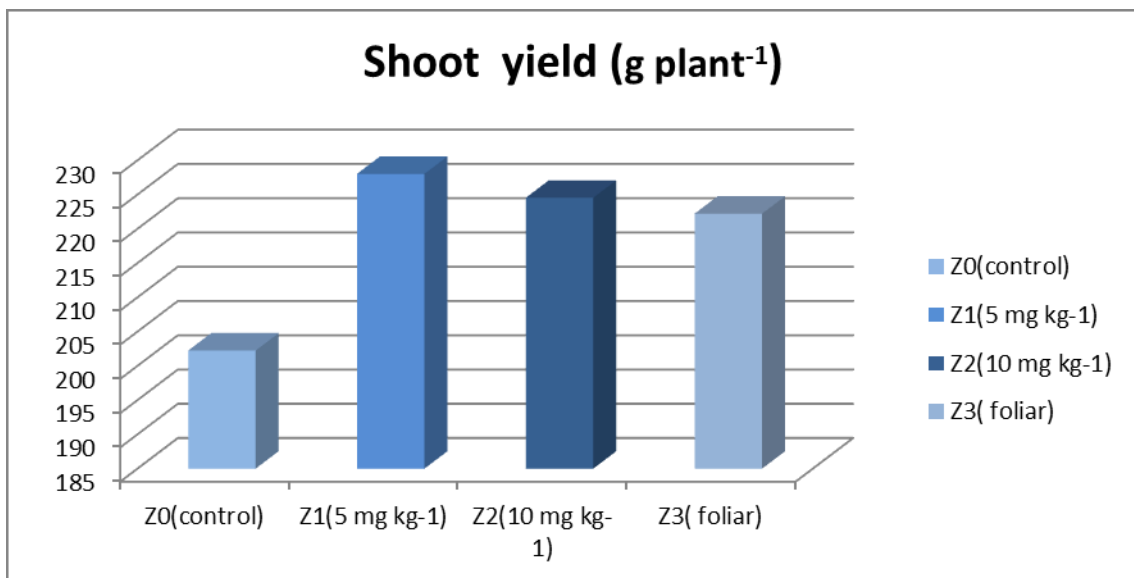


Fig.2 Effect of zinc levels on shoot yield of amaranthus (g plant⁻¹)



production of photosynthates as evidenced by the production of more no. of branches and leaves per plant and plant height (Tables 8, 5). The root characteristics like root length and root weight (Tables 9, 10) were also high for the above treatments. The better nutrition especially that of Fe and Zn, might have favorably influenced the root production and development.

Among the 16 P solubiliser (M2) based treatments, five treatments were on par with the highest yielding treatment, indicating that the P solubilisers are highly effective in producing higher shoot yield. The P solubiliser, apart from making soil P more available, it had increased the availability of soil organic carbon and available nitrogen (Tables 45, 46). The paramount importance of nitrogen on shoot yield of amaranthus has already been reported (Mwamba *et al.*, 1992). The enhanced yield might be the reflection of better availability of available N and organic carbon which had an overall influence on plant vegetative growth. Glick (2003) reported that soil bioaugmentation with microorganisms is a promising alternate for synergizing the effect of plants and animals. This technique was widely used for remediation of polluted soils while recently it had taken a turn as a tool for addressing the micronutrient malnutrition by enhancing the nutrient density of edible portion of crop plants. Mulligan *et al.*, (2001) reported that microorganisms produce surfactants, which facilitates better nutrient absorption from soil and production of enhanced biomass of plants. Availability and uptake of P and other nutrients were enhanced by the use of P solubiliser along with rock phosphate and produce a yield increase of 10 per cent (Khan *et al.*, 2010). The P solubilisers isolated from Kerala soils were highly efficient in releasing the native soil P (Meenakumari *et al.*, 2008).

Among the 16 K solubiliser based treatments, only one treatment combination (M3F0Z3) was on par with the highest yielding treatment. In general the treatment combination that received higher level of Fe (10 mg kg^{-1}) produced low shoot biomass (Table 14) and this was mainly responsible for a lower mean value for M3 (K solubiliser) compared to M2 (P solubiliser) and M4 (POP).

The plants were not able to utilize the higher quantity of Fe and this might have showed a negative impact on shoot biomass. Soil microorganisms play an important role in influencing the availability of native and applied micronutrients. In several cases under nutrient sufficient conditions their performance are not so promising resulting reduced yield (Sharma, 2008; Welch and Graham, 2005; Dinesh and Punkaj, 2011). The same might have taken place with higher level of Fe (10 mg kg^{-1}) also.

The lowest shoot yield was produced by AMF based (M1) treatments (Fig.1). In general AMF application was associated with better root ramification, nutrient uptake and biomass production. In the present experiment the influence of AMF on root biomass and other root characteristics were not positive (Table 10). The same treatment again recorded the highest population of AMF and fungi and comparatively higher population of bacteria also (Tables 56, 58, 57). The microbes might have utilized and fixed the nutrients for a short period, temporality locking the nutrient availability to the plant. Lynch (2007) reported a reduction in crop yield in well fertilized soils on inoculation with AMF. The reason attributed was that the symbiotic relationship between the plants and the micorrhizal fungi is fuelled by the photosynthates from the plants resulting in yield reduction.

Root biomass was significantly influenced by all the three factors and their interactions and followed the same trend as that of shoot biomass. The highest root biomass was also for the same treatment combination M4F0Z2 (POP x Fe @ zero mg kg^{-1} x Zn @ 10 mg kg^{-1}) Application of Fe and Zn and microbial additives have favorably influenced the root biomass also as in the case if shoot biomass.

5.2 Effect of bioaugmentation and iron and zinc fortification on nutrient density of amaranthus

5.2.1 Iron

Iron content in shoot was significantly influenced by the bioaugmentation methods. Iron content in the shoot (Table 23) was significantly influenced by the bioaugmentation methods and its interaction with Fe alone (M x F) and with Fe and Zn (M x F x Z). Fe content was highest for M1F3Z0 (AMF x Fe as foliar x Zn @ zero). Soil application of Fe had shown a favourable effect on Fe content of amaranthus (Fig. 4) only upto 5 mg kg⁻¹ (F1), though the soil availability increased with the levels of Fe. Foliar application of Fe also showed only a negligible increase in Fe content of shoot. The behaviour of Fe in both soils as well as in plant is a complex phenomenon. In general ferrous compounds are applied to soil for correcting Fe deficiency. Once they react with the soil air, get oxidised to ferric form and render Fe unavailable to plants (Silver, 1993; Cotton *et al.*, 1999). In the case of foliar application also Fe²⁺ on contact with air get oxidised and only a very minute portion of applied Fe find its way to plant tissue (Abadia *et al.*, 2002; Fernandez, 2004). Again for smooth penetration of Fe to foliage through stomata requires the help of surfactants or external pressure (Fernandez *et al.*, 2005). The available of Fe above sufficiency and a soil pH near neutrality (Table 43) might have adversely affected the Fe dynamics and its uptake by roots and translocation to shoot (Fernandez and Ebert, 2005).

The data on root Fe (Table 39) also followed the same pattern of shoot with highest value for F1 (5 mg kg⁻¹) which was significantly superior to others. The root Fe content was almost double than that of shoot Fe except in the case of AMF (M1) treatments. This clearly indicates that some phenomenon had prevented the translocation of Fe from root to shoot and major quantity of Fe gets deposited at the root itself. In most of the studies related to Fe nutrition, the accumulation of Fe at root itself has been found as the major problem for correcting Fe deficiency (Alloway, 2005; Fernandez and Ebert, 2005).

Fig. 3 Effect of bioaugmentation on iron concentration of the shoot (mg kg^{-1})

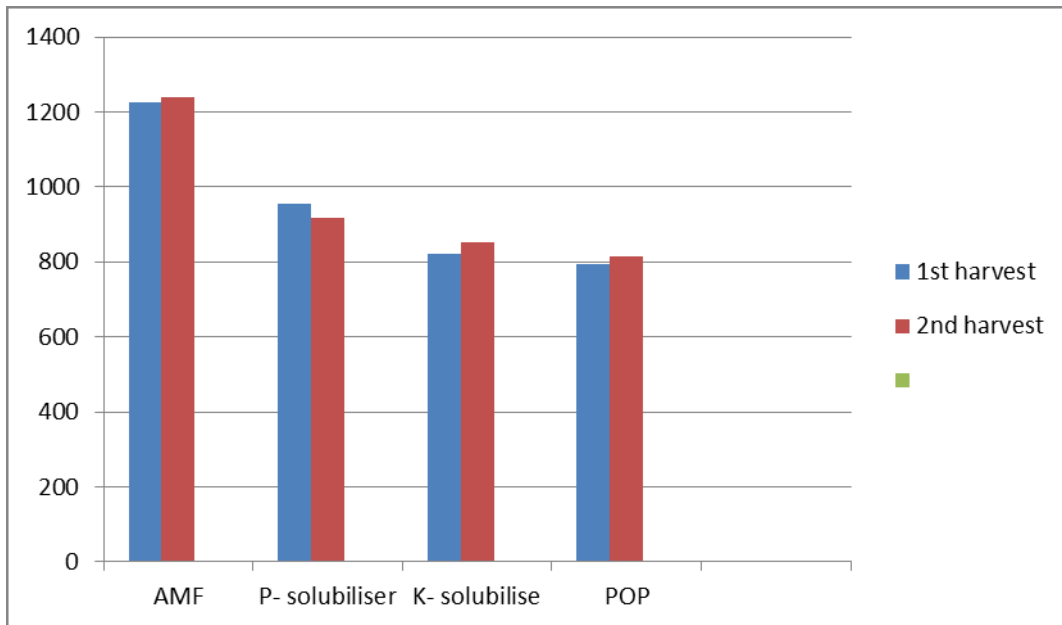
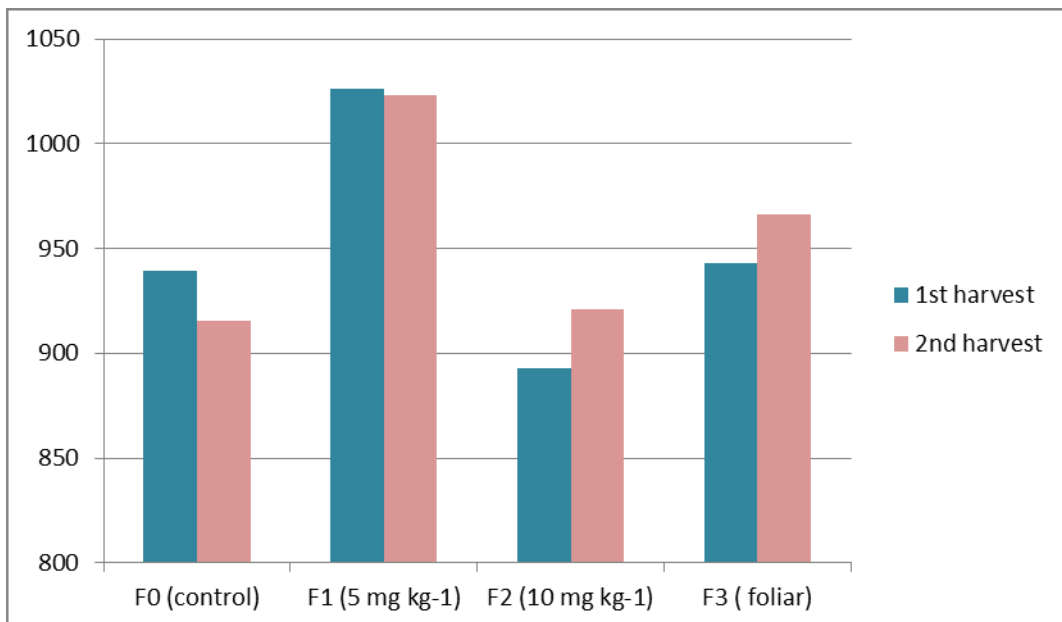


Fig.4 Effect of levels of Fe on Fe concentration of amaranthus shoot (mg kg^{-1})



Among the bioaugmentation methods, AMF (Fig.3) was significantly superior to K solubilizer (M3) and POP (M4). AMF treatment had recorded the highest shoot Fe content and lowest root Fe content, indicating better translocation efficiency. The data on soil available Fe content also supports the above information since the AMF treatments has the highest available Fe content (Table 52). The organic secretions by AMF and better contact of AMF assisted root with soil pores have helped better Fe availability in soil and its further uptake by plant roots (Clark and Zeto, 2008). The exudates from plant roots and AMF can provide carbon for other soil microbes that affects the phytoavailability of Fe and Zn and increases their acquisition by root and translocation to tissues. (Rengal, 2001; Barea *et al.*, 2005., Clark and Zeto, 2008).

The effect of Zn on Fe content of amaranthus was also found to be positive as evidenced by increase in Fe content with levels of Zn in contrast with the antagonistic behaviour of Fe and Zn (Fig.5). Better Zn contents of plants have favourably influenced the plant metabolism, uptake and translocation of Fe to shoot (Welch, 2005). However, the foliar application of Zn recorded the lowest value for shoot Fe. The direct entry of Zn to the leaves might have prevented the accumulation of Fe in leaves.

5.2.2 Zinc

Individual as well as interaction effects were significant for zinc content of shoot (Table 25). M2F0Z3 (P solubilizer x Fe @ control x Zn @ foliar) recorded the highest Zn content. It was observed that P solubiliser and foliar application of Zn showed better translocation of Zn to tissues. Foliar applied Zn can be absorbed by leaf epidermis and then transported to other plant parts via xylem and phloem and thus enriches the edible portion with Zn (Zhang *et al.*, 2008).

Considering the individual effects of microbial treatments, P solubilizer (M2) significantly increased the Zn content compared to other treatments (Fig.6). The P solubilisers might have solubilised the Zn phosphate present in soil and along with P, Zn availability also increases in contrast to their antagonistic

Fig. 5 Effect of zinc application on iron concentration of the shoot (mg kg^{-1})

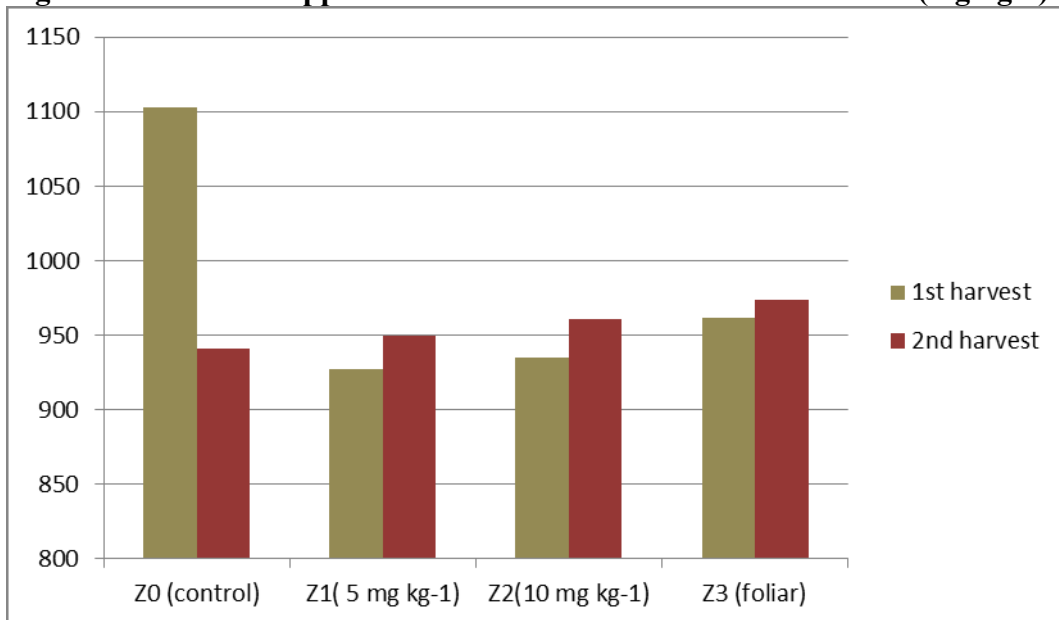
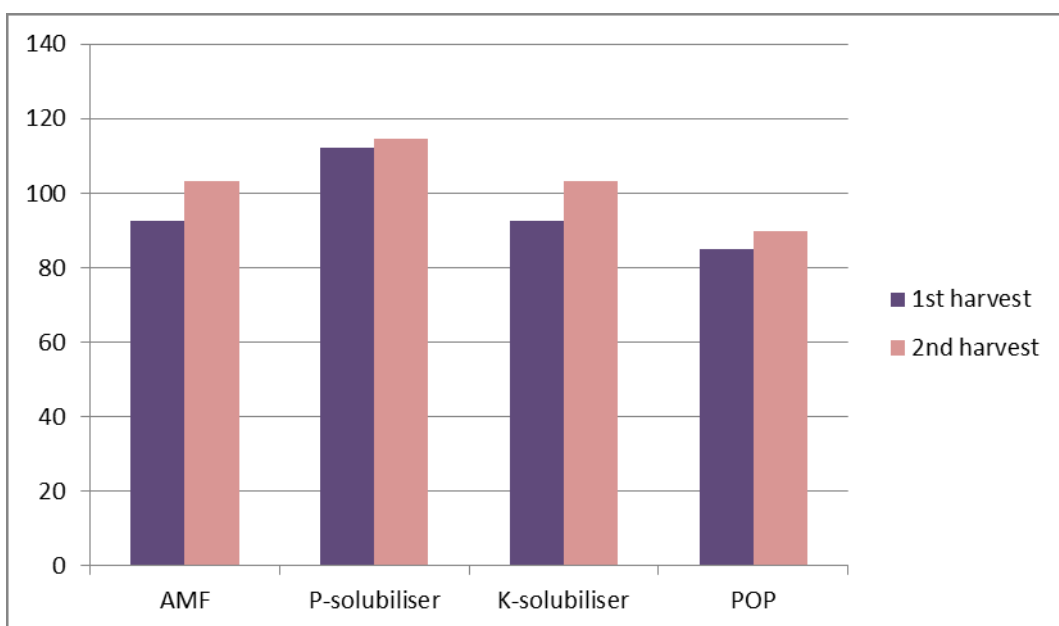


Fig. 6 Effect of bioaugmentation on zinc concentration of the shoot (mg kg^{-1})



behaviour. Organic acids and root exudates in the rhizosphere might have enhanced the Zn availability and uptake. This was supported by the Zn content of root (Table 41). M2 (P solubiliser) recorded the lowest value for root Zn content evidently due to better translocation from root to shoot. Highest value was recorded by M3(K solubiliser), this might be due to the interference of cationic elements on Zn nutrition.

Increasing levels of Fe decreased both root and shoot Zn content since both ions will compete for the same sites (Fig.8). True antagonistic effect was evident here. Dominance of Fe in soil had reduced Zn absorption (Table 25) by plants (Singh, 2009). The antagonistic effect of Fe on available zinc was not as evident as in the case of plant though it decreased with levels of Fe.

Levels of Zn had favourably influenced the Zn content of shoot as evidenced by the increase in Zn content with levels of Zn (Fig.7). However, the highest value was observed for the foliar application of Zn. The same trend was observed for the second harvest also. Zn can be easily taken by the plant foliage on foliar application, but the soil application also increased Zn contents with levels of Zn. Foliar application of Zn at grain filling stage results in Zn enrichments of Zn (Zhang *et al.*, 2008). Root Zn followed a reverse trend for soil application. For improving plant growth under Zn deficient condition, it can be deprived directly on to leaves (Zhang *et al.*, 2008).

5.2.3 Other nutrients

Shoot nitrogen content was significantly influenced by the treatments and their interactions (Table 17). Nitrogen content was highest for the combination M2F0Z1 (K solubilizer x Fe @ zero x Zn @ 5mg kg⁻¹). Considering the individual effect of bioaugmentation methods, it was observed that the treatments receiving microbial treatments (M1, M2, and M3) were significantly superior to M4 (POP). The application of microbial additives has favourably influenced the N content of shoot. The added microbes might have utilized the carbon sources

Fig. 7 Effect of levels of Zn on zinc concentration of the shoot (mg kg^{-1})

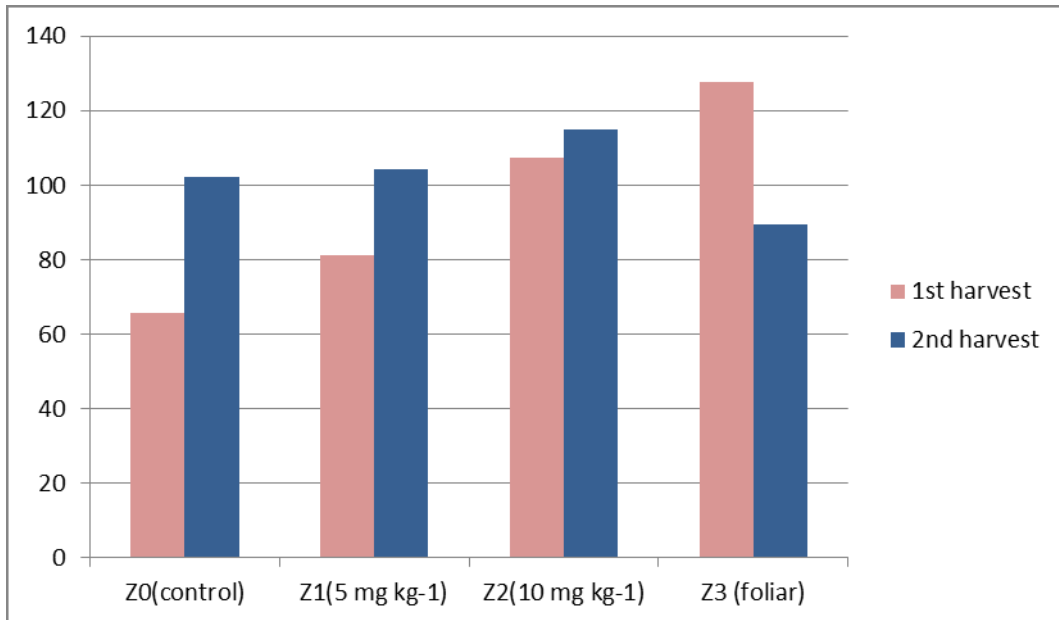
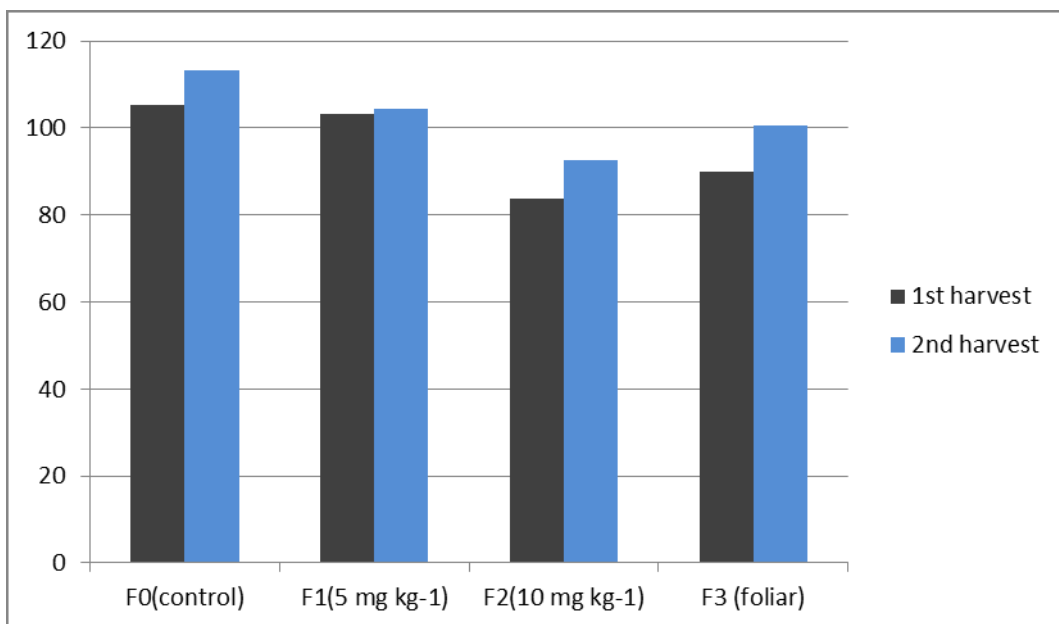


Fig. 8 Effect of levels of Fe on Zn concentration of the shoot (mg kg^{-1})



secreted by the plant roots, proliferated at faster rates, adding microbial biomass to the soil and their by contributing more available N in soil (Table 46) and its absorption by roots and further transfer to shoot resulted high shoot N content. Favourable influence of microorganisms on plant N content has been reported by several workers (Glick, 2003; Kuiper *et al.*, 200; Khan *et al.*, 2012). The root N (Table 33) did not follow the trend of the shoot. The difference in translocation of N from root to shoot might be the reason for this variable expression. With regard to the levels of Fe and Zn, an increase in N content was observed only up to 5 mg kg⁻¹ of Fe/Zn indicating this might be ideal nutrient requirement for the growth of microbially assisted plants.

Shoot phosphorous content was significantly influenced by the treatments and their interactions (Table 18). But a prominent effect was noticed only for methods of bioaugmentation. Application of P solubiliser had maintained significantly superior P content in shoot and in soil (Tables 18, 47). The P solubiliser had solubilised the native soil P present either as Fe or Al phosphates in laterite soils where P fixation is the major fertility problem. Meenakumari *et al.*, (2008) reported that the strains isolated from acid laterite soils of Kerala have high P solubilising capacity and their application maintained higher amount of soluble P in the rhizosphere which is readily available to the plants.

Shoot K content was significantly influenced by the treatments and their interactions with M3F0Z3 (K solubiliser with Fe @ zero and Zn as foliar) recording the highest value. The favourable effect of K solubiliser was clearly evident as it maintained high concentration of shoot K as well as soil available K (Tables 19, 48). Better availability of K in soil promoted the higher absorption of K from soil and its translocation to plant parts. The behaviour of K content of the root was slightly different from that of shoot with AMF (M1) recording the highest value. The K solubiliser assisted plants might have experienced a better translocation of K from root to shoot (Glick, 2003). The mucilage secreted by K solubiliser degrade the alumina silicates clays and release the non exchangeable

K present in the soil, facilitating better K availability and further uptake by plants. Along with the release of K elements like Fe and Al are also released to soil solution (Groudev, 1987; Ehrlich, 2002).

The treatments and their interactions were significant for shoot calcium and magnesium contents (Table 20 and 21). But a noticeable influence was observed only for methods of bioaugmentation. K solubiliser (M3) recorded the highest value for shoot Ca and was also on par with treatments receiving AMF or P solubiliser. Whereas for shoot Mg, P solubiliser recorded the highest value and was significantly superior only to POP (M4). The mechanisms associated with the K solubiliser is by the production of mucilage/ slime which may break up the aluminosilicate clay minerals and release various minerals present in it (Lin *et al.*, 2002). Potential microbial agents like AMF, P solubiliser, K solubiliser etc. mobilise the nutrients from hard and complex mineral materials by the production of metabolites containing organic acid (Lin *et al.*, 2002). Root Ca (Table 36) was highest for M1 (AMF) and lowest for M3 (K solubiliser) while root Mg (Table 37) was highest for M4 (POP) and lowest for M2 (P solubiliser). A different behaviour of root Ca and Mg from that of shoot might be due to the difference in translocation of above nutrients from root to shoot, which might have been affected by several plant factors. The effects of levels of Fe and Zn on the shoot contents of Ca and Mg did not maintain a definite and consistent relationship. The behaviour of metals in soil and their uptake by plants is a complicated phenomenon, making many of the reactions beyond proper explanation. The behaviour of S was very much similar to that of P.

Individual as well as interactional effect of microbial treatments, were significant for both manganese and copper contents of shoot (Table 24, 26). Evaluating the individual effects, a definite relationship was observed only for methods of bioaugmentation. The effect of levels of Fe and Zn are highly inconsistent with regard to Cu and Mn contents of shoot. P solubiliser had increased Mn and Cu content of shoot, while the effect of AMF was restricted to

Cu alone. The microbial secretions of P solubilisers might have facilitated better nutrient removal from soil, which have been further translocated to shoot.

5.3 Effect of bioaugmentation, iron and zinc fortification on nutritional quality of amaranthus

The methods of bioaugmentation and levels of Fe and Zn had significantly influenced the nutritional parameters like beta carotene, vitamin-C and crude protein. Among the variables, most conspicuous effect was that of K solubiliser, which had increased beta carotene, vitamin-C and crude protein. The highest value for crude protein (Table 29) was recorded by AMF, which was on par with K solubiliser. Perusal of the data on soil available nutrients, it was observed that microbial additives especially K solubiliser enhanced the nutrient availability in soil. This might have helped to maintain a nutritionally balanced condition and there by assures better nutritional quality to crop produces.

Anti nutritional parameters like nitrate, phenol and oxalate contents (Tables 30, 31, 32) were mainly influenced by the bioaugmentation methods and levels of Fe. The AMF treatment showed lesser quantity of anti nutritional parameters like oxalate and phenol while the nitrate content was higher for it. Better assimilation of nutrients might have reduced the content of anti-nutritional factors in microbially assisted treatments. Levels of Fe increased phenol and oxalate content of shoot. Increase in Fe content in soil had influenced availability of other nutrients adversely and this might have enhanced the anti nutritional factors.

5.4 Effect of bioaugmentation, iron and zinc fortification on soil available Fe and Zn and their phytoavailability

Available iron content in the soil (Table 52) was significantly influenced by the treatments and their interactions and the highest value was recorded by M1F1Z3 (AMF x Fe @ 5 mg kg⁻¹ x Zn @ foliar). AMF application had increased

available Fe content of the soil significantly. The AMF might have assisted in releasing Fe from insoluble soil compounds by the production of organic acids and maintain intimate contact with more volume of soil (Rengal, 2001; Clark and Zeto, 2008). The Fe availability of soil increased with levels of Fe, while Zn did not maintained a definite pattern with regard to available Fe. Soils often contain large amounts of Fe, but little of this is phytoavailable (Fernandez and Ebert, 2005). The application of inorganic Fe fertilizers to such soils is usually ineffective as it rapidly becomes unavailable to plant roots through adsorption, precipitation and oxidation reactions. For this reason, Fe-chelates are often used as soil Fe fertilizers (Shuman, 1998; Rengal *et al.*, 1999, Fernandez and Ebert, 2005).

AMF treatment had increased the shoot Fe content significantly and recorded the highest value for it. The same treatment recorded lowest Fe content in root, indicating better translocation efficiency for Fe in presence of AMF (Table 61, 63).

AMF (M1) recorded the highest value for shoot Zn and was significantly superior to others with K solubiliser (M3) recording the lowest value. As in the case of Fe, the effect of AMF on maintaining more available Zn in soil was observed here also. However the highest value for shoot Zn content was recorded by P solubiliser. Both in soil and plant the Zn content decreased with increasing levels of Fe, evidently because of the antagonistic effect between Fe and Zn. The levels of Zn had favorably influenced both available Zn in soil and shoot Zn content. Similarly, soil and/or foliar applications of Zn fertilizers can increase leaf, tuber and fruit Zn concentrations of edible crops (Shuman, 1998; Rengel *et al.*, 1999; Broadley *et al.*, 2007). In some soils, the residual effects of a single application of Zn fertilizer can be appreciated over several years. The concentration factor of Zn was found to decrease with increasing levels of Zn while translocation factor was of reverse trend. P solubiliser recorded the highest values for both (Tables 62, 64).

Table 61. Effect of treatments on concentration factor of iron

Treatments	M1	M2	M3	M4	Mean				
F0Z0	139.60	597.90	207.20	75.25	254.99				
F0Z1	139.30	119.10	210.25	94.55	140.80				
F0Z2	139.00	148.05	208.30	92.50	146.96				
F0Z3	140.00	167.60	208.35	95.00	152.74				
F1Z0	130.25	90.80	79.15	81.65	95.46				
F1Z1	110.20	78.85	75.00	80.10	86.04				
F1Z2	117.05	76.75	80.05	82.30	89.04				
F1Z3	134.20	113.70	67.60	78.75	98.56				
F2Z0	79.40	54.80	30.05	54.85	54.78				
F2Z1	78.45	56.35	28.95	53.10	54.21				
F2Z2	78.25	57.15	28.65	52.40	54.11				
F2Z3	79.85	59.55	40.75	53.95	58.53				
F3Z0	264.50	111.85	75.35	139.35	147.76				
F3Z1	237.15	140.10	141.75	145.95	166.24				
F3Z2	212.30	143.30	139.65	143.80	159.76				
F3Z3	207.30	150.45	73.90	139.00	142.66				
	F0	F1	F2	F3	Z0	Z1	Z2	Z3	Mean
M1	139.48	122.93	78.99	230.31	153.44	141.28	136.65	140.34	142.93
M2	258.16	90.03	56.96	136.43	213.84	98.60	106.31	122.83	135.39
M3	208.53	75.45	32.10	107.66	97.94	113.99	114.16	97.65	105.93
M4	89.33	80.70	53.58	42.03	87.78	93.43	92.75	1.68	91.41
Mean	173.9	92.3	55.4	129.1	138.3	111.8	112.5	90.6	
CD (0.05) FM - 86.6413					CD(0.05) F - 43.3206				

Table 63. Effect of treatments on translocation factor of iron

Treatments	M1	M2	M3	M4	mean				
F0Z0	0.52	2.86	1.50	0.42	1.32				
F0Z1	0.68	0.85	0.63	0.83	0.74				
F0Z2	0.68	0.55	0.61	0.39	0.56				
F0Z3	0.42	0.82	0.52	0.53	0.57				
F1Z0	1.73	0.31	0.64	0.23	0.73				
F1Z1	1.44	0.43	0.65	0.47	0.74				
F1Z2	1.47	0.26	0.67	0.30	0.67				
F1Z3	1.13	0.61	0.34	0.46	0.63				
F2Z0	0.91	0.53	0.30	0.60	0.58				
F2Z1	0.75	0.37	0.14	0.37	0.41				
F2Z2	0.70	0.72	0.19	0.75	0.59				
F2Z3	1.03	0.58	0.32	0.58	0.62				
F3Z0	1.25	0.52	0.28	0.74	0.69				
F3Z1	0.70	0.67	0.54	0.81	0.68				
F3Z2	0.63	0.60	0.67	0.68	0.64				
F3Z3	1.02	0.53	0.51	0.53	0.65				
	F0	F1	F2	F3	Z0	Z1	Z2	Z3	MEAN
M1	0.57	1.44	0.85	0.90	1.10	0.89	0.87	0.90	0.94
M2	1.27	0.40	0.55	0.58	1.05	0.58	0.53	0.63	0.70
M3	0.81	0.57	0.24	0.50	0.68	0.49	0.53	0.42	0.53
M4	0.54	0.36	0.57	0.69	0.49	0.62	0.53	0.53	0.54
MEAN	0.80	0.69	0.55	0.67	0.83	0.64	0.61	0.62	
	CD(FM)- 0.4147				CD(M)- 0.2073				

Table 62. Effect of treatments on concentration factor of Zinc

	M1	M2	M3	M4	mean				
F0Z0	92.46	69.08	50.56	38.47	62.64				
F0Z1	17.54	18.22	10.48	14.23	15.12				
F0Z2	12.48	17.10	6.15	8.27	11.00				
F0Z3	136.42	172.95	96.42	86.15	122.98				
F1Z0	78.39	67.53	51.27	53.09	62.57				
F1Z1	16.65	15.92	10.69	14.37	14.41				
F1Z2	9.17	10.16	10.38	14.29	11.00				
F1Z3	96.43	113.10	114.61	156.52	120.16				
F2Z0	56.97	61.12	44.82	47.76	52.67				
F2Z1	10.45	14.18	13.61	10.31	12.13				
F2Z2	6.00	9.12	8.64	8.66	8.10				
F2Z3	65.75	131.35	106.47	101.34	101.22				
F3Z0	41.32	60.94	86.16	45.62	58.51				
F3Z1	8.41	11.74	17.51	8.73	11.60				
F3Z2	8.59	10.02	9.71	5.70	8.51				
F3Z3	110.71	144.22	145.99	63.87	116.19				
	F0	F1	F2	F3	Z0	Z1	Z2	Z3	MEAN
M1	64.73	50.16	34.79	42.26	67.28	13.26	9.06	102.32	47.98
M2	69.34	51.67	53.94	56.73	64.67	15.01	11.60	140.40	57.92
M3	40.90	46.74	43.38	64.84	58.20	13.07	8.72	115.87	48.96
M4	36.78	59.57	42.01	30.98	46.23	11.91	9.23	101.97	42.33
MEAN									
	CD(0.05) FM/ ZM- 11.2581				CD(0.05) M- 5.6290				
	CD(FZM)- 22.5161								

Table 64. Effect of treatments on translocation factor of zinc

Treatments	M1	M2	M3	M4	mean				
F0Z0	0.44	0.86	0.27	0.19	0.44				
F0Z1	0.58	0.54	0.41	0.50	0.51				
F0Z2	0.73	1.51	0.41	0.52	0.79				
F0Z3	1.02	1.19	0.43	0.69	0.83				
F1Z0	0.62	0.31	0.22	0.45	0.40				
F1Z1	0.68	0.59	0.24	0.63	0.53				
F1Z2	0.64	1.16	0.42	1.06	0.82				
F1Z3	0.75	0.75	0.77	1.32	0.89				
F2Z0	0.45	0.28	0.36	0.40	0.37				
F2Z1	0.45	0.86	0.36	0.48	0.53				
F2Z2	3.66	0.73	0.99	12.73	4.53				
F2Z3	0.44	0.71	0.50	0.72	0.59				
F3Z0	0.27	0.56	0.58	0.32	0.43				
F3Z1	0.34	5.59	0.69	0.38	1.75				
F3Z2	0.63	0.57	0.66	0.45	0.58				
F3Z3	0.82	1.63	0.78	0.51	0.93				
	F0	F1	F2	F3	Z0	Z1	Z2	Z3	MEAN
M1	0.69	0.67	1.25	0.51	0.44	0.51	1.41	0.76	0.78
M2	1.02	0.70	0.65	2.08	0.50	1.89	0.99	1.07	1.11
M3	0.38	0.41	0.55	0.68	0.36	0.42	0.62	0.62	0.50
M4	0.48	0.86	3.58	0.41	0.34	0.50	3.69	0.81	1.33
MEAN	0.6425	0.66	1.5075	0.92	0.41	0.83	1.6775	0.815	
	CD(0.05)- FM/ ZM/ FZ 0.4553				CD(0.05)M/F/Z- 0.2276				
	CD(0.05) FZM - 0.9106								

Evaluating the performance of methods of bioaugmentation and levels of iron and Zn on the growth and nutrient quality of amaranthus, it was observed that the highest economic yield was recorded by the treatment combination M4F0Z2 (POP x Fe0 x Zn10). However, considering the iron and zinc contents and nutritional parameters, the treatment combinations M2F0Z2 (P solubiliser x Fe@ zero x Zn10 mg kg⁻¹) and M3F0Z3 (K solubiliser x Fe@ zero x Zn as foliar) showed better performance.

Summary

6. SUMMARY

An experiment entitled “Iron and Zinc fortification in amaranthus (*Amaranthus tricolor*) through bioaugmentation” has been carried out at the Department of Soil Science and Agricultural Chemistry, College of Agriculture, Vellayani. A pot culture experiment was conducted to study the effect of Fe and Zn application and bioaugmentation of soil with microbial additives on yield and nutrient composition of amaranthus particularly with Fe and Zn. The findings of the experiment are summarised in this section.

Plant height, number of branches per plant and number of leaves per plant were significantly influenced by the treatments and the individual effect of methods of bioaugmentation was most dominant. The highest values for all the above parameters were recorded by M4 (POP). Better nutrition provided to the POP treatment through bioaugmentation and additional application of iron and zinc had favourably influenced the above biometric characters.

M4 (POP) showed better performance for root characteristics also by showing highest values for root length and root weight. P solubilizer (M2) had shown the highest value for root volume. Levels of Fe showed a negative relation whereas Zn had shown a positive relation towards root characteristics.

The treatment combination M4F0Z2 (POP x Fe @ zero x Zn @ 10 mg kg⁻¹) showed the highest value for leaf weight per plant. While stem weight was highest for the combination M2F2Z2 (P solubilizer x Fe @ 10 mg kg⁻¹ x Zn @ 10 mg kg⁻¹). The combination M4F0Z2 (POP x Fe @ zero x Zn @ 10 mg kg⁻¹) recorded the highest shoot biomass and total biomass. Considering the individual effects, the bioaugmentation method M4 (POP) was significantly superior to others and levels of Zn had responded positively only up to @ 5mg Zn kg⁻¹ of soil.

Nitrogen content in the shoot was highest for the combination M2F0Z1 (K solubilizer x Fe @ zero x Zn @ 5 mg kg⁻¹). The treatments receiving microbial

treatments (M1, M2, and M3) were significantly superior to M4 (POP) definitely due to better nutrient availability by the microbial action. Fe and Zn, both up to @ 5 mg kg⁻¹ increased the shoot N content.

P solubiliser had significantly increased the shoot P content. The levels of Fe showed a positive trend for shoot P only up to Fe @ 5 mg kg⁻¹ and for the levels of Zn, soil application showed a negative relation with P content of shoot evidently due to the antagonistic effect of Zn on P.

The highest value for shoot K content was recorded by M3F0Z3 (K solubilizer with Fe @ zero and Zn as foliar). Addition of K solubilizer had significantly increased the K content of shoot clearly revealing the favourable effect of K solubiliser on K availability and nutrition of plants. For Fe and Zn, definite pattern of relationship with shoot K were not observed.

Application of microbial additives had significantly increased shoot Ca. K solubiliser recorded the highest value which was also on par with AMF or P solubiliser. The microbial exudates have solubilised the minerals and favoured the release of nutrient elements. A definite relation with levels of Fe and Zn were not noticed. M3F3Z3 (K solubilizer with Fe and Zn as foliar application) has recorded the highest value for shoot magnesium content. P solubilizer (M2) was significantly superior to others. Application of Fe @ 5 mg kg⁻¹ enhanced the Mg content. The behaviour of shoot S content was very much similar to that of shoot P. P solubilizer (M2) had increased the S content of shoot.

Fe content in the shoot was highest for M1F3Z0 (AMF x Fe as foliar x Zn @ zero). Among the bioaugmentation methods, AMF was significantly superior. Soil application of Fe, only up to @ 5 mg Fe kg⁻¹ of soil showed a positive and significant response to Fe application. In general only very little amount of soil or foliar applied Fe find its way to plant tissues due to the complex chemistry of iron.

For shoot manganese content the highest value was observed for M1F3Z1 (AMF x Fe @ foliar x Zn @ 5 mg kg⁻¹). P solubilizer (M2) and POP (M4) were on par with each and were significantly superior to others.

M2F0Z3 (P solubilizer x Fe @ control x Zn @ foliar) recorded the highest Zn content. P solubilizer (M2) significantly increased the Zn content compared to other treatments. This might be due to the solubilising effect of P solubiliser on zinc phosphate. With increasing levels of Fe, the shoot Zn content decreased clearly revealing the antagonistic effect. Zn content increased with the levels of Zn and the highest value was observed for foliar application. Foliar application of zinc was found to be highly effective since it had good phloem mobility.

For shoot copper content, M2F3Z3 (K solubilizer with Fe and Zn given as foliar application) recorded the highest value. AMF (M1) and P solubilizer (M2) were significantly superior to others. Levels of Fe and Zn had not shown any definite pattern.

The methods of bioaugmentation and levels of Fe and Zn had significantly influenced the nutritional parameters like beta carotene, vitamin-C and crude protein. Among the variables, most conspicuous effect was that of K solubiliser, which had increased beta carotene, vitamin-C and crude protein. The K solubiliser was able maintain better nutrient availability in soil and higher content in shoot also compared to other treatments. This might have enhanced the quality of the amaranthus shoot.. The highest value for crude protein (Table 29) was recorded by AMF, which was on par with K solubiliser.

Anti nutritional parameters like nitrate, phenol and oxalate contents were mainly influenced by the bioaugmentation methods and levels of Fe. The AMF treatment showed lesser quantity of anti nutritional parameters like oxalate and phenol while the nitrate content was higher for it. Better assimilation of nutrients might have reduced the content of anti nutritional factors in microbially assisted treatments. Levels of Fe increased phenol and oxalate content of shoot. Increase

in Fe content in soil had influenced availability of other nutrients adversely and this might have enhanced the anti nutritional factors.

N, P and K contents of root were significantly affected by the treatments. Evaluating the individual effects, the application of AMF had maintained the highest nitrogen content, P solubiliser the phosphorus content and K solubiliser the potassium content of root. Effects of levels of Fe and Zn were not that much consistent as in the case of methods of bioaugmentation.

Soil application of Fe decreased both the root calcium and magnesium contents while Zn had enhanced their contents. Levels of Fe increased root S content up to 5 mg kg⁻¹ and Zn showed significant and positive relation with its highest level.

The treatment combination M4F1Z0 (AMF x Fe @ 5 mg kg⁻¹ x Zn @ zero) recorded the highest value for iron content of root. Considering the individual effect, P solubiliser (M2) which was on par with K solubilizer (M3) recorded the highest value, definitely due to the release of soluble Fe by the microbial action. Soil application of Fe enhanced root Fe content. The enhanced availability of iron though facilitated the absorption by roots; its translocation to shoot was prevented because of low mobility of Fe. Manganese content of root was significantly increased by the P solubiliser and copper content by K solubiliser.

M3F1Z1 (K solubilizer x Fe @ 5 mg kg⁻¹ x Zn @ 5 mg kg⁻¹) recorded the highest value for root zinc content. With regard to the individual effect, significantly highest value was observed for K solubiliser (M3). Fe levels showed a negative relationship with root Zn content.

The available N, P and K contents of soil were significantly influenced by the treatments. Soil available N was significantly increased by AMF, available P by P solubiliser and available K by K solubiliser.

Considering the individual effect of microbial treatments, application of K solubilizer (M3) maintained highest exchangeable calcium and P solubiliser maintained highest exchangeable magnesium contents in soil. Levels of Fe and Zn had not shown a definite pattern with regard to exchangeable Ca and Mg.

Available iron content of soil was significantly influenced by the treatments and their interactions and the highest value was recorded by M1F1Z3 (AMF x Fe @ 5 mg kg⁻¹ x Zn @ foliar). AMF application had increased available Fe content of the soil significantly. The AMF might have assisted in releasing Fe from insoluble soil compounds by the production of organic acids and maintain intimate contact with more volume of soil.

Highest value for available Zn content in the soil was recorded by M1F1Z2 (AMF x Fe @ 5 mg kg⁻¹ x Zn @ 10 mg kg⁻¹) for the first harvest. Considering the individual effect of bioaugmentation methods, AMF (M1) recorded the highest value for both the harvests and was significantly superior to others. An increase in available Zn content was noted with increasing levels of Zn at both harvests.

AMF colonization was significantly influenced only by the individual effect of bioaugmentation methods (M) and its combination with levels of Fe (M x F). The highest colonization was observed for the treatments receiving AMF (M1) treatment and was significantly superior to other three treatments.

Evaluating the individual effect of bioaugmentation methods on bacterial population, M3 (K solubiliser) recorded the highest value and was also on par with M1 (AMF). Considering the individual effect of Fe a definite pattern was not observed whereas for the levels of Zn, an increase in count was observed with increasing levels of soil application of Zn.

M1 (AMF) recorded the highest count for soil fung and was significantly superior to others. For the levels of Fe and Zn a definite pattern was not observed for the fungal population.

Actinomycetes population was significantly influenced by the bioaugmentation method alone (M) and the highest value were recorded by M1 (AMF) which was significantly superior to other three treatments.

The highest B:C ratio of 4.19 was recorded by the treatment combination M4F0Z2 (POP x Fe @ zero x Zn @ 10 mg kg⁻¹) which was on par with several other treatment combinations. The B: C ratio was lowest for M1F2Z0 (AMF x Fe @ 10 mg kg⁻¹ x Zn @ zero). Evaluating the individual effects of bioaugmentation methods, M4 (POP) was significantly superior to other three treatments. In the case of Fe, the increasing levels for soil application and the treatment with foliar application decreased the B:C ratio. The levels of Zn showed a positive relation with B:C ratio up to Z1(Zn @ 5 mg kg⁻¹) and with Z3 (Zn as foliar).

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*Originals not seen

**IRON AND ZINC FORTIFICATION IN
AMARANTHUS (*Amaranthus tricolor*) THROUGH
BIOAUGMENTATION**

by

AMLA SAKTHIDHARAN

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ABSTRACT

An investigation entitled “Iron and Zinc fortification in amaranthus (*Amaranthus tricolor*) through bioaugmentation” has been carried out at the College of Agriculture, Vellayani during May to August 2012. The main objective was to study the effect of Fe and Zn application and bioaugmentation of soil with microbial additives on yield and nutrient composition of amaranthus, particularly with that of Fe and Zn. This was accomplished through a pot culture experiment using laterite soil.

The design was Factorial Completely Randomized Design (4 x 4 x 4). The treatments include four methods of bioaugmentation (POP + AMF; POP + P solubilizer; POP + K solubilizer and POP alone), Four levels of iron (Zero; 5 mg kg⁻¹ of soil, 10 mg kg⁻¹ of soil and Foliar application of 1.0 mg kg⁻¹ FeSO₄) and Four levels of zinc (Zero; 5 mg kg⁻¹ of soil; 10 mg kg⁻¹ of soil and Foliar application of 0.5 mg kg⁻¹ ZnSO₄).

The experiment results revealed that the treatment combination **M4F0Z2** (POP x Fe₀ x Zn @ 10 mg kg⁻¹) recorded the highest shoot biomass and B:C ratio. The above treatment was on par with several treatment combinations of **M2** (P solubilizer) and **M4** (POP) and with **M3F0Z3**. Root biomass was also highest for **M4F0Z2**. The biometric characters like plant height, number of branches per plant, number of leaves per plant, leaf weight, stem weight, leaf: stem ratio, root length and root volume were significantly influenced by the treatments while girth of stem was not significant. Interaction effects of treatment on most of the variable were also significant. Considering the individual effects, among the bioaugmentation methods, M4 (POP) showed highest values for all the above observations except root length and the lowest by M1 (AMF). Levels of iron had shown a negative effect on most of the plant characteristics while zinc maintained a positive significant effect.

Treatment effect was significant on concentration of nutrients viz., N, P, K, Ca, Mg, S, Fe, Mn, Zn, and Cu in shoot and root at each harvest. Evaluating the individual effects, it was observed that the microbial additives in general significantly increased N, Ca, Mg, Fe and Zn compared to POP. P solubilizer had significantly increased P and Zn content of the shoot and K solubilizer the K content. Fe @ 5 mg kg⁻¹ alone recorded an increase in Fe content of shoot. Zn maintained a significant positive relation with shoot Zn content and foliar application recorded the significantly highest value. Nutritional parameters like crude protein, β -carotene, nitrate, vitamin C, oxalate and phenol contents were significantly influenced by the treatments. Bioconcentration factor and translocation index were significant only for Zn.

Soil characteristics like pH, EC, organic carbon, available N, P, K, Ca, Mg, S, Fe, Mn, Zn and Cu at the time of each harvest were significantly influenced by the treatments. Levels of Zn showed a positive influence on available Zn content. Treatment with microbial additives had favorably influenced the biological parameters like AMF colonization and rhizosphere microbial count.

From the above experiment, it can be concluded that the treatment combination **POP x Fe @ zero x Zn @ 10 mg kg⁻¹ (M4F0Z2)** recorded the highest economic yield and B:C ratio. Considering the nutritional quality and iron and zinc content of economic plant part, the treatment combination **K solubilizer x Fe @ zero x Zn as foliar, (M3F0Z3)** is the best treatment since it is on par with the above treatment in yield and B:C ratio and at the same time recorded better nutritional quality and iron and zinc content.