EFFECT OF ZINC FERTILIZATION ON MAJOR PLANT AND SOIL ENZYMES IN SOUTHERN LATERITES

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DECLARATION

I, hereby declare that this thesis entitled **"EFFECT OF ZINC FERTILIZATION ON MAJOR PLANT AND SOIL ENZYMES IN SOUTHERN LATERITES"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associate ship, fellowship or other similar title, of any other University or Society.

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LIST OF ABREVIATIONS

°C	-	Degree celsius
%	-	Per cent
β	-	Beta
<	-	Less than
@	-	At the rate of
µg	-	Microgram
µg TPF hr^{-1} g^{-1}	-	Microgram TPF per hour per gram
AAS	-	Atomic Absorption Spectrophotometer
B	-	Boron
$BaCl_2$	-	Barium chloride
C D	-	Critical Difference
CA	-	Carbonic anhydrase
Ca	-	Calcium
CEC	-	Cation exchange capacity
Chl h^{-1}	-	Chlorophyll per hour
cm	-	Centimetre
CRD	-	Completely Randomized Design
Cu	-	Copper
DAP	-	Days after planting
DMP	-	Dry matter production
DMSO	-	Dimethyl Sulfoxide
dS m^{-1}	-	deci Siemens per metre
DTT	-	Dithiothreitol
EC	-	Electrical conductivity
EDTA	-	Ethylene diamine tetra acetic acid
et al	-	Co- authors/ co- workers
EU g^{-1}	-	Enzyme Unit per gram
Fe	-	Iron

Fig.	-	Figure
FYM	-	Farm yard manure
g	-	Gram
g^{-1}	-	Per gram
HEPES	-	Gibco HEPES (4-(2-hydroxyethy)-1-piperazine ethane sulfonic acid)
IAA	-	Indole 3- Acetic Acid
K	-	Potassium
KAU	-	Kerala Agricultural University
Kg ha^{-1}	-	Kilogram per hectare
KH_2PO_4	-	Potassium dihydrogen phosphate
K_m	-	Substrate affinity
m	-	Metre
M	-	Molar
m^3	-	Cubic metre
MBC	-	Microbial Biomass Carbon
mg	-	Milligram
Mg	-	Magnesium
mg^{-1}	-	Per milligram
min	-	minutes
ml L^{-1}	-	Milliliter per litre
mM	-	Millimolar
MOP	-	Muriate of Potash
MSL	-	Mean Sea Level
MUB	-	Modified Universal Buffer
N	-	Nitrogen
nm	-	Nanometer
P	-	Phosphorus
PEP	-	Phospho enol pyruvate
PGPR	-	Plant Growth Promoting Rhizobacteria

pH	-	Negative logarithm of H^+ ion concentration
pnp	-	Para nitro phenyl phosphate
POP	-	Package of Practices
ppm	-	Parts per million
RF	-	Revolving Fund
RP	-	Rock phosphate
RUBP	-	Ribulose 1,5 Bisphosphate
S	-	Sulphur
TPF	-	2,3,5- triphenyl formazan
V	-	velocity
Var	-	Variety
Viz.,	-	Namely
V_{max}	-	Maximum velocity
Zn	-	Zinc
$ZnSO_4$	-	Zinc sulphate

INTRODUCTION

1. INTRODUCTION

Soil, called as the skin of the earth, is the most vital and precious resource meant for the existence of mankind. It is the key component of agriculture because of its ability to store water and nutrient composition. Nutrient elements are those required by the plants for various metabolic processes, which are needed to maintain life and growth. Plant nutrients can be divided into macro nutrients and micro nutrients, where micronutrient malnutrition is a growing concern in the developing world. Micronutrients can improve the quality and quantity of plant products, thereby considered as the pillars of agriculture in developed countries. Zinc is an exceptional micro nutrient, needed by the plant in small concentration yet essential for its growth and development. In recent years the Zinc (Zn) deficiency problem has received increasing attention and appears to be the most serious micronutrient problem.

The functions of Zn in plants are largely associated with enzymatic activities. It has an important role in plant metabolism, can strongly act on the photochemical reactions of the photosystems, the activation of the Calvin cycle key enzyme ribulose 1, 5 bisphosphate carboxylase/oxygenase, and the equilibrium between CO_2 and O_2 binding by this protein (Loneragan and Webb, 1993) and on the activities of hydrogenase and carbonic anhydrase, stabilization of ribosomal fractions and synthesis of cytochrome (Tisdale *et al.*, 1984).

Plant enzymes activated by Zn are involved in maintenance of the integrity of cellular membranes, pollen formation, protein synthesis, carbohydrate metabolism and regulate the synthesis of auxin (Marschner, 1995). The Zn-finger transcription factors are involved in the development and function of floral tissues such as anthers, tapetum, pollen and pistil secretory tissues in many plant species, there by plays an important role in both flower and normal fruit development (Kobayashi *et al.*, 1998; Sharma *et al.*, 1987). The gene expression required for the tolerance of environmental stresses in plants are Zn dependent (Cakmak, 2000).

Zn is directly involved in the synthesis of tryptophan which is a precursor of IAA, it also has an active role in the production of an essential growth hormone auxin which produces more plant cells and more dry matter and stored in seeds as a sink (Alloway, 2004; Brennan, 2005). It maintains the integrity of cellular membranes, to preserve the structural orientation of macromolecules and ion transport systems, interaction with phospholipids and sulphydryl groups of membrane proteins contributes for the maintenance of membranes.

Zn deficiency results in the formation of abnormalities in plants which become visible as deficiency symptoms. The deficiency symptoms appear on the young leaves of plants first, the areas between the veins becomes yellow because zinc cannot be transferred to younger tissues from older tissue (zinc isn't a mobile element). In dicot plants, internode distance and leaf size will be short while in monocot plants, especially corn, bands appear on the main veins of leaves (Boardman and McGuire, 1990; Gokhan, 2002; Mousavi, 2011).

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 24.6 mg kg^{-1}, respectively with an average deficiency of 12 to 87 per cent. Crops grown in these soils have low Zn content in shoot and seed (Singh, 2009). It is estimated that India need 324 t ha^{-1} per year of fertilizer zinc to correct zinc deficiency by the year 2025 (Singh, 2009). The total concentration of zinc in soils depends on the composition of the parent material and soil mineralogy, especially the concentration of quartz, which tends to dilute most elements. Only a small fraction of the total zinc is exchangeable or soluble and about one-half of the dissolved zinc exists as the free hydrated cation.

Most of the soils are showing fatigue for sustaining higher production due to the depletion of native micronutrients by continuous cultivation of high yielding varieties (Singh, 2001). Lesser use of micronutrients in the states of Tamil Nadu, Karnataka, Kerala, Chattisgarh and Maharashtra and increased cropping intensity in marginal lands further elevated the magnitude of zinc deficiency. More and more marginal areas are brought under intensive cultivation without necessary

micronutrient supplementation will lead to increase in overall zinc deficiency from 48 per cent found in the year 1970 to 63 per cent by the year 2025 (Singh, 2009).

Kerala soils in general have high levels of iron and manganese. The laterite and associated soils which constitute more than 70 per cent of cultivated area in Kerala are reported to have low or medium status in terms of available micronutrients. For proper growth and development of plants, supply of specific nutrients is necessary at appropriate time and in readily available form.

About 34 per cent of Kerala Soils are deficient in Zn, 31 per cent in Cu and less than 1 per cent in Fe and no deficiency for Mn (Singh, 2009). Zn deficiency is expected to increase from 49 to 63 per cent by 2025. Deficiency of zinc ranges from 2.3 to 50 per cent in ten districts of Kerala (Mathew and Aparna, 2012). Available Zn less than 1mg kg^{-1} in acid soils is rated as deficient condition. The status of total zinc content in soils of Kerala ranged from 25-55 mg kg^{-1}. The Zn deficiency in Kerala was due to excessive quantity of phosphatic fertilizers applied and excessive levels of Fe, Mn and Al due to ion competition (Kumar *et al.*, 2013).

Application of Zn fertilizers to Zn deficient soils is a general strategy to attain both food security and to conquer Zn malnutrition. Selection of appropriate Zn sources can also improve plant availability of Zn. Zn fertilizers with good solubility can result in greater Zn transport to the roots. Even in soils with adequate Zn, application of Zn either as foliar or soil application was found to increase crop yield and zinc content in plant parts (Singh, 2009). Hence, the present study is carried out to observe the effect of zinc fertilization in a Zn deficient soil and to assess the various sources of Zn nutrition supplementation on plant and soil enzymes and other biochemical parameters in a laterite soil and envisaged with the following objective.

- To assess the effect of various sources and methods of application of zinc on the activities of major plant enzymes, soil enzymes, biochemical and microbial parameters in laterite soils using tomato (*Solanum lycopersicum*) as a test crop.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 IMPORTANCE OF ZINC IN CROP PRODUCTION

Zinc, a relatively rare element having a central role in life of Earth. Essentiality of Zn as a micronutrient for higher plants was first proven by Sommer and Lipman (1926). It is an essential micronutrient needed for the growth and development of all living organisms. It is an exceptional micronutrient because it is the only trace metal involved in all classes of enzymes (Broadley *et al.*, 2007). Nearly 2,800 proteins in biological system require Zn for their structural stability and their activity (Andreini *et al.*, 2009).

Zn plays an important role in regulating the nitrogen metabolism, cell multiplication, photosynthesis and auxin synthesis in plants and helps in the consumption of phosphorous and nitrogen during seed formation. Zn is essential for protecting the cells from oxidation damage and maintaining regeneration (Cakmak, 2000). Alloway (2009) claimed that Zn plays an important role in enzyme activation or catalysis, carbohydrate metabolism, chlorophyll production, energy dissipation, cytochrome and nucleotide synthesis. Shuman *et al.* (1995) explained the involvement of Zn in stomatal opening, as a component of carbonic anhydrase maintaining the bicarbonate in the guard cell and also in the K^+ uptake in to the guard cells.

Zinc is the main component of ribosome and is required for their development. Due to Zn deficit, amino acids get accumulated in plant tissues and results in reduction of protein synthesis. An important site of protein synthesis is pollen tube, the quantity of zinc in there tip is 150 micrograms per gram of dry matter. In addition zinc also has a role in pollen tube formation there by contributing to pollination (Marschner, 1995; Outten and O'Halloran, 2001; Pandey *et al.*, 2006). Zinc can protect proteins and lipids from oxidation damage; by linking to membrane phospholipids or forming tetragonal compounds with cysteine polypeptide chain residues or becoming a component of sulfhydryl (Salami and

Kene, 1970; Domingo *et al.*, 1992; Marschner, 1995). Deficiency or toxicity of Zn may cause damage in the metabolic functions of vascular plants.

2.2 ROLE OF ZINC IN ENZYMES AND BIOCHEMISTRY

Zn is required for functioning of more than 300 enzymes. It functions as a cofactor for 6 classes of enzymes like transferases, lyases, oxidoreductases, isomerase, hydrolases and ligases (Broadley *et al.*, 2007). Marschner (1986) asserted that Zn is a structural part of enzymes such as alcohol dehydrogenase, superoxide dismutase, carbonic anhydrase and RNA polymerase. Zinc is the main structural part of many enzymes and is required for the plant enzymes formation; furthermore, many enzymatic reactions activated by zinc (Vitosh *et al.*, 1994; Pedler *et al.*, 2000; Akay, 2011).

There are a number of enzymes in which Zn is a crucial component and four different types of union sites of Zn have been identified such as catalytic, structural, co-catalytic and protein interface. In Zinc, site classification is based on its coordinating properties. For carbonic anhydrase and carboxipeptidases, Zn has a catalytic function therefore it is coordinated to four ligands. Three of which are amino acids such as histidine (His), Glu and Asp, a water molecule is the fourth ligand in all the catalytic centers. The structural sites of Zn are united to four proteins in which the metal is attached to the water (Auld, 2005; Patel *et al.*, 2007).

The alkaline phosphatase family holds three metals in the active site where as the rest of the co-catalytic zinc sites contain two metals. These sites contains metals such as copper, iron, and magnesium along with zinc. Cu /Zn combinations are seen in the superoxide dismutase (SOD) family. The ligand nature of the catalytic zinc site varies for the total grouping of the dehydrogenases but is quite constant for the sub families .The dimeric mammalian ADHs are NADH-dependent and have two Cys and one His as ligands to a catalytic zinc site, where as the fourth ligand will be water (Auld, 2005).

Alcohol dehydrogenase is an enzyme molecule having two atoms of zinc, one of the atoms has a catalytic function while the other has a building role. This

enzyme helps in the conversion of acetaldehyde to ethanol. In plants, ethanol is produced in the root tip meristematic tissue under aerobic conditions, the alcohol dehydrogenase enzyme activity declined, there by resulted in diminished root development due to Zn deficiency (Marschner, 1995; Gokhan *et al.*, 2003).

In Cu/Zn-Superoxide dismutase enzyme zinc is linked to copper where zinc has catalytic and copper has building role. Super oxide is a toxic substance and it mediates lipids peroxidation of membrane and results in increase in permeability in higher plants. Superoxide dismutase activity is reduced in zinc deficiency conditions and is accompanied with increased free radical oxygen (super oxide).

Carbonic anhydrase has a zinc atom that catalyzes CO_2 hydration. The main site of activity is in chloroplasts and cytoplasm and is dependent to zinc content in the plant. The important functions of this enzyme are increasing photosynthesis and biomass production, dehydration of CO_2, increasing absorption of carbon dioxide per leaf area unit. The activity of this enzyme is reduced in plants that are met with Zn deficiency (Ohki, 1976; Dell and Wilson, 1985; Marschner, 1995).

Most enzymes that play a role in carbohydrates metabolism are activated by zinc. Furthermore Fructose-1, 6 bisphosphate and Aldolase enzymes are activated by zinc and these enzymes are active in the chloroplasts and cytoplasm. The main function of Fructose 1, 6-bisphosphate is separation of six-carbon sugar molecule between chloroplasts and cytoplasm. The transportation of three-carbon sugars molecule in photosynthesis from cytoplasm to chloroplasts are carried out by Aldolase. In zinc deficiency condition, the activity of these enzymes declined leading to subsequent carbohydrate accumulation in leaves (Marschner and Cakmak, 1989, Mousavi, 2011; Taheri *et al.*, 2011). In addition, Zn also activates tryptophan synthetase which is essential for tryptophan synthesis, which is necessary for auxin formation. Metabolism of plant hormones such as auxin (IAA) and tryptophan declines in zinc deficiency condition, consequently leaf growth stops (Marschner, 1995; Pedler *et al.*, 2000).

2.3 ZINC DEFICIENCY IN SOIL

Almost all crops respond positively to Zn application and Zn deficiency can be found in every part of the world (Welch, 2002). Soils take Zn mainly from the rocks through geochemical and pedochemical weathering processes. The total amount of Zn present in the soil depends on the type, intensity of weathering, climate and numerous other factors during the process of soil formation besides mineralogical composition of the parent material (Saeed and Fox, 1977). High pH and high contents of $CaCO_3$, organic matter, clay and phosphate can fix Zn in the soil and causes reduction of available Zn (Imtiaz, 1999).

About 30% of the cultivable soils of the world contain low levels of plant available Zn as reported by Food and Agriculture Organization (FAO) (Sillanpaa, 1990). The higher levels of Zn are seen in fluvisols (60 $\mu g\ g^{-1}$) and histosols (58$\mu g\ g^{-1}$) while the lowest Zn concentrations are found in Spodosols (28 $\mu g\ g^{-1}$) and luvisols (35 $\mu g\ g^{-1}$) (Kiekens, 1995).

Zn deficiency is expected generally more in calcareous soils, sandy soils, peat soils, and soils with high phosphorus, silicon (Alloway, 2008) and also in submerged soils. Submerged soils are renowned for their lack of Zn availability to the plants; particularly due to the reaction of Zn with free sulphide (Mikkelsen and Shiou, 1977) and also due to the changes in pH value with the formation of insoluble Zn compounds. The insoluble Zn compounds formed are more likely with Mn and Fe hydroxides due to the breakdown of oxides and adsorption on carbonates, particularly magnesium carbonate.

2.4 FACTORS ASSOCIATED WITH ZINC DEFICIENCY

In general, soils of arid and semiarid regions and the slightly acidic, leached soils of warm and tropical climates are most liable to Zn deficiency. Soils which are inclined to Zn deficiency should possess certain characters like strongly alkaline in reaction, high phosphorus status, leached sandy soils, acid soils of low total Zn status developed on highly weathered parent material, calcareous soil, peat and

muck soils, submerged soils (water logged) and soils with high bicarbonate and magnesium (Benton, 2003).

Major Zn deficiency factors include: soils of low Zn content (Parent material), soils with restricted zones, pH, soils low in organic matter, microbially inactivated Zn, cool soil temperature, plant species and genotypes, high level of available phosphorus and effects of nitrogen (Lindsay, 1972; Pendias and Pendias, 1992; Alloway, 2008).

2.4.1 Parent Material

One of the major factors affecting the concentration of Zn in soils is the soil parent material. Total Zn is low in soils derived from gneiss and granite (Krauskopf, 1972) and also in highly leached acid sandy soils found in many coastal areas. Those originating from sandstone and limestone also have lower Zn contents (Pendias and Pendias, 1992). The reported concentrations of Zn in quartz (sand) are very low, ranging from 1.0 $\mu g\ g^{-1}$ to < 5 to 8 $\mu g\ g^{-1}$ as a result quartz dilutes Zn in soil (Helmke *et al*, 1977). The total Zn content ranges from 10 to 300 $\mu g\ g^{-1}$ with an average of 50 $\mu g\ g^{-1}$ (Lindsay, 1972). The remaining of the total Zn is fixed in an insoluble or unexchangeable form in the soil and is not available to plants (Stahl and James, 1991).

2.4.2 Soil Organic matter

Soil organic matter originates from decomposition of animal and plant products. The most stable organic compounds are humic and fulvic acids, which are generally termed as humic substances. The peculiarity of these substances is that they contain functional groups like OH, COOH, SH which shows a high affinity for metal ions like Zn^{2+}.Over a wide range of pH, fulvic acid can form chelates with Zn and thereby increases the solubility and mobility in soil (Kiekens, 1995). Simple organic compounds (amino acids, hydroxy acids and phosphoric acids) can form complexes with Zn and thus increases the solubility and mobility of Zn in soils (Pendias and Pendias, 1992). It was observed that available Zn increases with increase in organic matter in soil. However, if the organic matter content in soil is

too high (peat and muck soils) it will leads to Zn deficiency particularly due to the linkage of Zn on solid state humic substances (Katyal and Randhawa, 1983).

2.4.3 Soil Texture

Light textured soils (sands) contain low levels of Zn because of low CEC values. Heavy textured soils like clay have higher CEC values and have highly reactive sites which can retain more Zn as a result of these they have high capacity for Zn absorption than light textured soils (Shukla and Mittal, 1979). This adsorption of Zn is also favoured by pH along with CEC (Ellis and Knezek, 1972). It was observed that a portion of Zn adsorbed on clay was not exchangeable and was not available to the plants. Clays such as bentonite and illite with higher CECs have a lead role in fixing of Zn more strongly than Kaolonite, thus making it unavailable to plants (Reddy and Perkin, 1974).

2.4.4 Phosphate Fertilizers

Soils with higher phosphate levels arising due to native P or application of phosphate fertilizers, can cause Zn deficiency in crops (Alloway, 2008). Prolonged use or heavy application of phosphatic fertilizers can reduce Zn uptake (Olsen, 1972). This effect is termed as "P-induced Zn deficiency" (Singh *et al*, 1968).

2.4.5 Soil Temperature

Soil temperature affects Zn uptake mainly in the rate of Zn mineralization (Takkar and Walker, 1993).Temperatures below 16°C during growth caused decreased Zn uptake in maize tops (Ellis *et al*, 1965). Zn uptake was higher in rice in warm and moist soils than in maize (*Zea mays L.*) (Bauer and Lindsay, 1965). It seems that Zn deficiency was associated with cool and wet seasons. In plants, high light intensity and long day-lengths are the other factors which can cause Zn deficiency (Marschner and Cakmak, 1989) Soil management practices carried out by man can often cause Zn deficiency. Plants can also suffer from Zn deficiency under conditions of drought or compaction (Alloway, 2008)

2.4.6 Soil Flooding

Zn deficiency is associated mainly with flooded soil than dry soil. In alkaline soils, Zn is precipitated as $Zn(OH)_2$ and as ZnS in sulfur-rich acidic soils. Under submerged condition, the oxides of Mn and Zn along with $CaCO_3$ or $MgCO_3$ are strongly absorbed by Zn. With increase in pH, the solubility and availability of Zn decreases.

In calcareous soils, HCO_3^- is the predominant anion, which reduces Zn transport from root to shoot, but shows meagre effect in Zn uptake by roots. Zn uptake reduced under submerged conditions, when the organic acid concentration increasesd. Under anaerobic condition, Zn forms insoluble Zn phosphate as a result plant roots cannot take up Zn from the Zn solution. The Zn uptake is also reduced under acidic rhizosphere condition, due to the release of H^+ ions from the roots and by the uptake of more amount of cations over anions. Zn released from acid-soluble fractions (e.g., absorbed Zn, organic matter or Fe $(OH)_3$ are available for plant uptake under acidic rhizosphere conditions. Rice plants absorb Zn mainly from the solubilization in the rhizosphere probably due to the low availability of Zn under flooded condition (Dobermann and Fairhurst, 2000).

2.4.7 Zn on microbial activity

Microorganism needs nutrients for their growth and metabolism. Among the nutrients, Zn is an important one as it is present in the enzyme system as co-factor and act as a metal activator of several enzymes (Vankatakrishnan *et al.*, 2003). Zinc affects the growth of bacteria at higher levels (>13.60 mg kg^{-1}). Under high levels of Zn, cell growth as well as microbial populations and their activity on soil are badly affected (Baath, 1992; Doelman and Haanstra, 1984).

2.5 ZINC AND SOIL CHEMICAL PARAMETERS

Nutrients may interrelate with Zn by affecting its availability from soils and its concentration in the plant through the processes of Zn absorption, distribution or utilization. In doing so, they may increase or decrease the response of plant growth to Zn. On the contrary, Zn may affect other nutrients in the same way. When there

is an interaction, where the nutrient is added as a salt, one of the three main factors other than the nutrient itself could be responsible for affecting a Zn response and they are presence of another ion in the salt, Zn as an impurity in the nutrient salt and a change in the root environment. Addition of nutrient may cause changes in the rhizosphere pH and is also considered.

2.5.1 Soil pH

Zinc availability is highly sensitive to changes in pH. The availability of Zn is usually very low when the pH is above 6, due to the lower solubility of the soil Zn. Increasing pH generally depresses Zn absorption and increases Zn deficiency mainly because of its ability to enhance Zn absorption on to soil constituents and thereby increasing the formation of Zn-organic complexes that control the process of Zn absorption by roots (Wear, 1956). The amount of Zn in the soil solution reduces from 10^{-4} (6.5 $\mu g\ g^{-1}$) to 10^{-10} M (0.007 $\mu g\ L^{-1}$) with a rise from pH 5 to pH 8 (Kiekens, 1995). Likely there is more chance that, Zn deficiency will occur in alkaline soil rather than in acidic. Soils characterized by high quantity of hydroxyl (OH^-) ions, suggest that the solubility constant values of $ZnCO_3$ and hydroxides contains only a small amount of available Zn. It is mainly ascribed to the precipitation of Zn as $Zn(OH)_2$ or $ZnCO_3$ (Shukla and Mittal, 1979; Saeed and Fox, 1977). The higher amount of carbonate in these soils also absorb Zn and hold it is in unexchangeable form (Udo *et al.*, 1970).

Liming can reduce the Zn uptake (Shukla and Moris, 1967) and can initiate Zn deficiency (Viets, 1966). Liming of acidic soils increases the pH and the Zn fixing capacity, mainly in soils with high P levels (Alloway, 2004). The availability of Zn in limed soils is significantly lower than that in acidic soils and as a result the absorption of Zn by the crop may be low. However, response to soil pH varies with plant species. While increasing soil acidity having without altering plant growth enhanced Zn concentrations in the tops of subterranean clover plants but had no effect on those of oats grown on the same soil (Williams, 1977).

2.5.2 Nitrogen – Zinc Interactions

Nitrogen fertilizers can severely increase or ameliorate Zn deficiency in plants. The N-Zn interactions formed by the application of N fertilizers, promoted plant growth and to a smaller extent, changes the pH of the root environment. In view of the fact that application of N promotes the growth of plants, it is likely to find affirmative interactions between increasing levels of Zn and N fertilizers (Alloway, 2004). Chaudhry and Loneragan (1970) stated that wheat when grown on N deficient soil with sufficient quantity of all nutrients other than N and Zn, did not respond to Zn application in the absence of NH_4NO_3 fertilizer, yet on the other hand a strong response was observed in the presence of N fertilizer.

N fertilizers have ameliorated Zn deficiency by promoting Zn absorption through changing pH in soils with low Zn and high fertility (Viets *et al.*, 1957). Concurrent dressings of N with $ZnSO_4$ were found to be effective in ameliorating Zn deficiency where $ZnSO_4$ alone had no influence, which might be due to the acidifying effects of ammonium ions (Viets *et al.*, 1953). NH_4^+ salts repressed Zn absorption from low concentration of Zn^{2+} in wheat seedlings (Chaudhry and Loneragan, 1972). Ammonium ions, inhibited Zn^{2+} absorption much stronger than alkali and alkaline earth cations, but were competitive with them. So its effect would be reduced by relatively high concentrations of competing ions in the soil. Direct effect of ammonium ions on Zn absorption would disappear with nitrification and subsequent soil acidification (Chaudhry and Loneragan, 1972)

2.5.3 Phosphorus- Zinc Interaction

The interaction between P and Zn is termed as 'P induced Zn deficiency'. This condition is mainly associated with elevated levels of soil available P or by the application of phosphatic fertilizers to soil. The interaction between P and Zn has been studied in 1936 (Barnette *et al.*, 1936) and until now, it is still under investigation. Generally four possible reasons were identified as responsible for 'P induced Zn deficiency' (Olsen, 1972). They are

(i) P enhances the sorption of Zn to soil components

(ii) a slower rate of translocation of Zn from the roots to shoot,

(iii) cations added with P salts inhibit Zn absorption from solution

(iv) a metabolic disorder within plant cells related to an imbalance between P and Zn.

Of these mechanisms, Lambert *et al.* (1979) has asserted that only the first has been shown clearly to induce Zn deficiency in plants. Applied P heightened the Zn deficiency in plants (Loneragan *et al.*, 1979; Sharma *et al.,* 1968). Under high Zn supply, P may restrain Zn in roots by the formation of Zn phytate (Van Steveninck *et al.*, 1993). Some studies proposed that even though P reduced the zinc concentrations in the shoot, the total zinc concentration increased or rather remained the same (Boawn and Leggett, 1968; Adriano *et al.*, 1971). The Zn deficiency symptoms can be managed by the application of Zn fertilizers.

2.5.4 Macronutrient Cations-Zinc Interaction

Ca, Mg and K are the macronutrient cations that inhibit the absorption of Zn by plants from solution. These cations seems to be less effective in soil in the inhibition of Zn absorption compared to their salts on soil pH. Bell *et al.* (1989) identified that Zn concentration was highest in legume shoots at the lowest calcium level, when grown in solution culture at constant pH and continuously reduced with rising Ca concentrations in solutions.

A short term study conducted by Chaudhry and Loneragan (1972) have found that elevated concentrations of Ca $(NO_3)_2$ from 0 mM to 40 mM repressed the rate of Zn absorption in wheat seedlings by a non-competitive manner, then again higher Ca concentrations (100 mM) which had no extra effect on Zn absorption. This inhibition contributed by the variable anions and had diminutive effect on Zn absorption.

Macronutrient cations K, NH_4 and Mg all inhibited the rate of Zn absorption in solutions of low Ca concentrations whereas with increasing Ca concentrations (2.5-10 mM) the inhibitory effects eventually vanished, proclaiming that

they activate through the identical mechanism of that of Ca (Chaudhry and Loneragan, 1972).

2.5.5 Micronutrient- Zinc Interaction

2.5.5.1 Copper- Zinc Interaction

The interaction between Cu and Zn is mainly by 3 ways as opined by (i) Zn strongly reduces grain yield by lowering Cu absorption, (ii) Cu competitively hinders Zn absorption and (iii) Cu nutrition disturbs the reallocation of Zn within plants. In the first one, Cu-Zn interaction has been detected in the grain yield of wheat crops which were grown in both Cu and Zn deficient soils (Chaudhry and Loneragan, 1970; Kausar *et al.*, 1976). In the second one, Zn increased Cu deficiency by reducing Cu uptake. This effect may have been resulted from the competitive inhibition of Zn on Cu absorption proved by Bowen (1969) with excised leaf discs.

None of the studies reported about the effects of Cu on Zn, on the plant growth or yield reductions. In soils, larger proportion of Cu is complexed when compared with that of Zn (Hodgson *et al.*, 1965, 1966; Geering and Hodgson, 1969). As a result, the Zn activity will be greater than Cu activity and making it a competitor mainly at the absorbing sites in Cu absorption and thus rendering it less sensitive. When Cu and Zn were present in chelated forms and in adequate supply, increase in Cu^{2+} activity have meagre effect on Zn concentrations in roots as well as in shoots of maize (Bell *et al.*, 1991).

2.5.5.2 Iron- Zinc Interaction

The increased application of Fe usually have a depressive effect on Zn in plant tissues (Watanabe *et al.*, 1965; Zhang *et al.*, 1991), whereas it has been seen to increase (Giordano *et al.*, 1974), have no effect (Chaudhry and Loneragan, 1972) or to reduce (Giordano *et al.*, 1974; Rashid *et al.*, 1976; Zhang *et al.*, 1991) the rate of Zn absorption by roots. At the same time, the application of higher levels of Zn had little effect (Norvell and Welch, 1993), increased (Watanabe *et al.*, 1965; Jolley and Brown, 1991), or decreased (Safaya, 1976; Jolley and Brown, 1991)

Fe concentration in plant shoots. This contradiction is mainly due to the differences in experimental aspects, most probably in plant species and the quantity, and finally the ionic state and complex formation ability of Fe.

From the solution containing 1 or 10 μM Zn and 50 mM $Ca(NO3)_2$ the wheat seedlings with low concentration of Fe^{2+} (10 μM) had no effect on Zn absorption rate (Chaudhry and Loneragan, 1972). Fe at a higher concentration (100 μM Fe^{2+}) and in flooded rice soil completely suppress the Zn absorption from a solution of 0.05 μM $ZnCl_2$ with no Ca (Giordano et *al.*, 1974).

In dicotyledonous plants, the reason for increasing Zn absorption is the acidification of the rhizosphere resulting from Fe deficiency (Marschner *et al.*, 1989). The release of phytosiderophores under Zn deficiency is responsible for the higher Zn absorption rate in grasses under Zn deficiency. Phyto siderophores have enhanced the mobilization of Zn from calcareous soils (Treeby *et al.*, 1989).

Under Zn deficient conditions, Fe accumulation was noticed in the shoots of navy beans and corn plants, most probably by involving mechanisms like acidification of the rhizosphere, release of reductants and phytosiderophores (Ambler and Brown, 1969; Jackson *et al.*, 1967). So in short, the interaction (antagonism) between Zn and Fe is as complex like that of P-Zn interaction.

2.5.5.3 Boron- Zinc Interaction

The B-Zn interaction is identical as that of P-Zn interaction, where Zn deficiency enhanced P toxicity in many species. Low Zn treatments enhanced B concentrations to toxic levels in solution culture of barley (Graham *et al.*, 1987). In Zn deficient soils, the B concentration of wheat increased while lowering the dry matter of wheat (Singh *et al.*, 1990).

2.5.5.4 Cobalt, Manganese- Zinc Interactions

Co and Mn may inhibit Zn absorption under certain conditions. But these effects does not affect plant growth except high concentrations of Mn in combination with Fe. This effect decreases the absorption of Zn by rice in flooded

soils. In solutions containing Ca^{2+} and Co^{2+} where the same concentration of Zn^{2+} is present, Mn had no effect on Zn absorption by sugar cane leaf discs (Bowen, 1969) or wheat seedlings (Chaudhry and Loneragan, 1972). In wheat seedlings Co^{2+} decreased the rate of Zn absorption by 10% when present in tenfold excess. Even under similar conditions or at l0-fold excess over Zn^{2+}, Mn^{2+} had no effect on Zn absorption by excised roots (Schmid *et al.*, 1965) or leaf discs (Bowen, 1969).

In complete nutrient solution where the activities of transition metals were buffered by an excess quantity of HEDTA, where the total Mn concentration and Mn^{2+} activity varied 10,000-fold from deficient to near toxic levels, there wasn't any changes in the Zn concentrations of shoots and roots of barley (Webb *et al.*, 1993). Contradictory when present at 2,000-fold excess in the absence of Ca^{2+} and Mn^{2+}, reduced the rate of absorption of Zn by about 50% suggesting that the higher concentrations of reduced Mn and Fe which were developed in paddy field may aggress Zn deficiency in rice (Giordano *et al.*, 1974).

2.6. ZN UPTAKE FROM SOIL

The quantity, concentration of ions in the soil solution and its transport to the root surface have a key role in Zn uptake. Other important factors are root growth and surface area, as they can determine the distances between "source" (quantity, e.g. exchangeable fraction) and "sink" in the root surface. Zn in soil mainly occurs in three primary fractions: (i) Water soluble Zn (Zn^{2+} and soluble organic fractions), (ii) Adsorbed or exchangeable Zn in the colloidal fraction (associated with Al and Fe hydroxides, with clay particles and humic compounds), and (iii) Insoluble Zn complexes and minerals.

The first two fractions are easily available (Alloway, 1995). The soluble Zn present in the soil solution, co-precipitated as secondary minerals, adsorbed or exchange sites and associated with sesquioxides and organic matter can control solubility and availability of Zn to the plants (Almendros *et al.*, 2008).

Since Zn is bound to the soil matrix, the concentration of it in soil solution is very low and therefore the supply by mass flow to the roots is also limited. The

concentration of Zn in soil solution is pH dependent and decreases to a very low level at high soil pH (Jeffery and Uren, 1983; Brummer *et al.*, 1986). Most of the zinc is present as free metal ion and as labile complex in soil solutions (Jeffery and Uren, 1983). Zinc supply to the roots is mainly confined to diffusion and to a zone which does not extend beyond the root hair cylinder. Wilkinson *et al.,* (1968) conducted a field experiment in wheat using ^{65}Zn and auto radiographic methods and observed that there was depletion of zinc around roots, as this was typically used for supply by diffusion using auto radiographic method.

2.6.1 Distribution and transport of Zn in Plants

Zinc can move from senescing tissue to growing vegetative tissue and reproductive organs and seeds. The prime pool of Zn is seed and it is mobilized to the growing seedling. Zinc taken up by roots can be stored in stems and later be mobilized to growing tissue.

Zn is transported symplastically from root cells to the xylem. Tiffin (1967) concluded that Zn is transported as a cation in xylem sap and it can be taken as Zn^{2+} or as Zn-phytosiderophore complex across the plasma membrane (Broadley *et al.*, 2007). In plasma membrane Ca^{2+} channels are also permeable to Zn^{2+} (White *et al.*, 2002). ZIPS (ZIP 1, ZIP 3 and ZIP 4) mediates Zn^{2+} influx to the cytoplasm (Palmgren *et al.*, 2008). Zn is restored in the vacuole as an organic acid complex. Zn may be transported as Zn^{2+} or as complexed with histidine, nicotinamine or organic acids. It was predicted that Zn is mainly transported as Zn-citrate complex and Zn-citrate or malate complexes in the xylem of soyabean and tomato. Zinc transporter proteins are involved in the phloem transport of Zn in to seeds, these are located specifically on root cell membranes and the plasma membrane of phloem tissue (Curie *et al.*, 2009).

2.7. ZINC FERTILIZERS

Extensive research has been done on the role of Zn fertilizers to correct Zn deficiencies in crops. These fertilizers vary considerably in Zn content, chemical reactivity, cost, and effectiveness for crops. Methods of Zn application varies,

depending on the crop type, farming system and tools available. Therefore it is important to know about Zn sources, their methods of production and application, and their performance in soil, along with chemical evaluation and regulations affecting Zn fertilizers before applying them in the soil (Robson, 1993). Mainly there are four classes of Zn sources: inorganic, synthetic chelates, natural organic complexes, and inorganic complexes. Inorganic sources include ZnO, $ZnCO_3$, $ZnSO_4$, $Zn(NO_3)_2$ and $ZnCl_2$ (Mortvedt, 1992). When metallic salts react with some organic by-products of the wood pulp industry natural organic complexes are formed. Several classes of these complexes include lignosulfonates, phenols, and polyflavonoids. One inorganic complex of Zn is ammoniated $ZnS0_4$ solution. This Commercial product generally contain 10-15% N, 10% Zn, and 5%S, the main use of this complex is in liquid starter fertilizers containing ammonium polyphosphate (Mortvedt, 1991).

2.7.1 $ZnSO_4$

$ZnS0_4$ is the most common source, and it is sold in both crystalline and granular form. Generally it has good solubility resulting in greater Zn transport to roots when compared with insoluble ZnO (Giordano and Mortvedt, 1972). It was observed that split application of $ZnSO_4$ was better when compared with basal application (Naik and Das, 2007). Stomph *et al.* (2011) asserted that foliar application of $ZnSO_4$ can be used to correct Zn deficiency and to improve grain Zn concentration. This increase in Zn concentration in the grain was mainly attributed to the enhanced leaf remobilization of Zn.

The critical level for soil Zn deficiency to occur is established based on the standard DTPA method (Dobermann and Fairhurst, 2000). It was observed that application of $ZnS0_4$ at the rates of 5-10 kg Zn ha^{-1} can be used to correct Zn deficiency (Qadar, 2002). In a highly Zn deficient region like Konya, Central Anatolia field trials were conducted using duram wheat by applying $ZnSO_4$ and it was affirmed that the application of $ZnSO_4$ to soil increased both yield and grain Zn concentration (Ekiz *et al.*, 1998).

2.7.2 Zn EDTA

Synthetic chelates are formed by linking a chelating agent with a metal ion through a coordinate bond. The availability of the chelated metal to the plant is decided by the stability of the metal-chelate bond. Zn EDTA is the most commonly used Zn chelate, probably because of the low rate of substitution of chelated metal ion for other cations in the soil. Its stability constant is 17.5, which is much higher than that of Ca EDTA (11.6) (Norvell, 1991), thus maintaining the metal in chelated form. Only a little amount of chelated Zn can be substituted by Ca in neutral and calcareous soils therefore Zn EDTA remains more effective for plants in these soils. Karak and Das (2006) noticed significant increase in grain Zn content and yield through the foliar application of Zn as Zn-EDTA. Singh *et al.* (2005) confirmed that the most efficient sources of Zn for low land rice production was Zn-EDTA. The residual effect of chelated Zn (Zn-EDTA) in maintaining Zn in soil was more than that of $ZnSO_4$ (Karak and Das, 2006). The foliar-applied Zn EDTA is more effective than $ZnSO_4$ if applied before tillering stage for enhancing grain yield (Brennan, 1991).

2.7.3 Zn Solubilizing Microorganisms

Microorganisms play a key role in Zn solubilisation. Some species of rhizobacteria are capable of mobilizing Zn in accessible form in soils. Zn solubilizing bacteria are capable of solubilizing ZnO, $ZnCO_3$ and Zn phosphate through production and excretion of organic acids (Shruthi, 2013). Hutchins *et al.* (1986) confirmed that *Thiobacillus thioxidans*, *Thiobacillus ferroxidans* and some facultative thermophilic iron oxidizers can solubilize zinc from sulphide ore. If the cultures cannot tolerate higher level of zinc, its solubilization may not continue. A field experiment was conducted by Kumar *et al.* (2004) to study the effect of Zn enriched organic manures and Zn solubilizers on turmeric plant and observed that FYM + zinc solubilizing bacteria showed the highest turmeric rhizome yield. Nomen *et al.* (2015) recommended the dosage of 39 kg S and 4.5 kg Zn ha^{-1} with Zn solubilizer in sandy loam soils low in S and Zn for improving the productivity and profitability of groundnut. Naz *et al.* (2016) used *Azospirillum*, *Pseudomonas*

and *Rhizobium* on wheat and reported that these organisms can significantly increase zinc contents in different growth stages at different parts of the plant.

2.7.4 Zn Humate

Humic acids can enhance plant growth and development and their effects may be maximized if they were combined with micronutrients. They form the largest portion of soil organic matter. They are colloid-sized, polymeric substances having dark colours. They are involved in uptake of other nutrients as well as can increase root and shoot growth and can provide resistance under conditions of different stress factors (Quaggiotti *et al.* 2004). Zinc humate was equal or superior to $ZnSO_4$ at increasing shoot growth of wheat and soybean under Zn-deficiency conditions. Addition of Zn humate removed Zn-deficiency symptoms and improved the dry matter production by 50% in soybean and 120% in wheat (Ozkutlu *et al.,* 2006). It was observed that in wheat under $ZnSO_4$ and Zn Humate were found to be equally effective in increasing shoot growth and raising shoot Zn concentration (Cakmak *et al.* 1996; Reuter and Robinson 1997; Alloway 2004).

Recently it has been observed that in alfalfa and wheat plants, Zn humic complexes significantly increased the bioavailability of Zn in soil and the concentration of Zn concentration in the plants. These changes were mainly associated with increase in shoot and root dry matter production (Garcia-Mina *et al.*, 2004). Besides improving solubility and uptake of Zn and other nutrients, Zn humates can also stimulate root and shoot growth in different ways (Wang *et al.*, 1995; Kelting *et al.*, 1998; Adani *et al.* 1998).

2.8 Kinetics of Zn

Root Zn^{2+} absorption may be studied using time dependent or concentration-dependent kinetics of Zn^{2+} uptake. A biphasic uptake characterised by an initial rapid entry followed by a slower linear phase of transport across the plasma membrane was observed when it was studied as a function of time (Santa *et al.*, 1988; Schmid *et al.*, 1965; Veltrup, 1978).

Based on the application of enzyme kinetics analysis a lot of studies on concentration dependence of Zn^{2+} uptake has been carried out (Epstein and Hagen, 1952). Michaelis-Menten kinetics proved that the transport of Zn is facilitated by a transport protein with specific transport characteristics (K_m and V_{max}). There are reports of saturation kinetics for Zn^{2+} absorption over a wide range of concentration, with K_m values that are high as approximately 50 mmol m^{-3} (Bowen, 1981, 1986; Ramani and Kannan, 1978).

Chaudhry and Loneragan (1972) conducted a short term study on wheat roots using $^{65}Zn^{2+}$ flux techniques and stated that the uptake followed Michaelis-Menten kinetics with a K_m of about 3 mmol m^{-3}. Kinetic parameters for maize root Zn^{2+} was carried out by Mullins and Sommers (1986) using the solution depletion method of Claassen and Barber (1974) and observed a saturation kinetics with a K_m of approximately 1.5 mmol m^{-3} while using a realistic concentration range for Zn^{2+}uptake (0 to 10 mmol m^{-3}).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present study entitled "Effect of zinc fertilization on major plant and soil enzymes in southern laterites" has been carried out in the Department of Soil Science and Agricultural Chemistry, College of Agriculture, Vellayani during June, 2017 to September, 2019. The study was envisioned to assess the effect of various sources and methods of application of zinc on the activities of major plant enzymes, soil enzymes, biochemical and microbial parameters in laterite soils using tomato (*Solanum lycopersicum*) as a test crop. The details of the experimental site, season and weather conditions, materials and methods followed are outlined in this chapter.

3.1 EXPERIMENTAL SITE

3.1.1 Location

The experiment was conducted in farmer's field in Kakamoola near Vavvamoola kayal, Trivandrum District located at 8° 24' North latitude and 76° 59' East longitude, at an altitude of 29 m above mean MSL.

3.1.2 Weather parameters

A warm humid tropical climate was prevailant in the study area during the entire period. The mean air temperature of the location ranged from 31.4°C to 24.5°C and the relative humidity from 91.8 to 78.6 per cent during the crop growth period. A total rainfall of 546.6 mm was received during the growing period. Weather parameters prevailed during the cropping season were monitored and are given in Fig 1 and Appendix I

3.1.3. Soil

A soil test database has been developed and maintained by the Department of Soil Science & Agricultural Chemistry under the project RF- Soil Testing Lab. From the database, laterite soil in Neyyatinkkara series with Zn deficiency was located. Then soil samples were collected and subjected to analysis of major and micro nutrients to confirm the deficiency of Zn. Based on the analytical results a convenient experimental site was selected for the field study. The soil of the

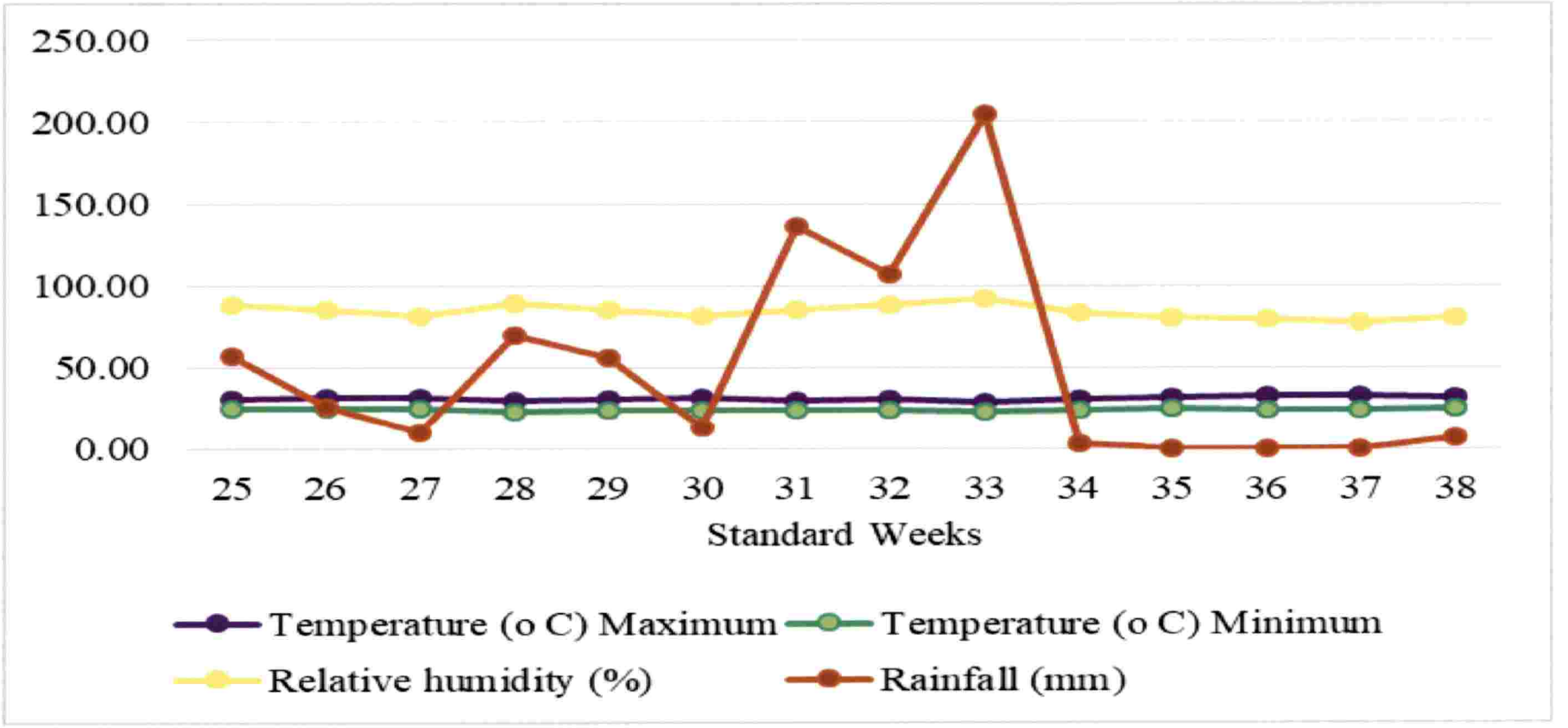

Fig.1 Weather data during the cropping period (June 2018– September 2018)

experimental site was loamy, kaolinitic, isohyperthermic, typic haplustalf and belongs to vellayani series. The initial samples collected were air dried and sieved using 2 mm sieve and analysed for various chemical properties and enzymes *viz.*, dehydrogenase and beta glucosidase. Fresh soil samples stored at room temperature were used to analyse soil respiration rate and microbial biomass carbon. The initial data as per standard procedures are presented in Table 1.

Table 1. Initial chemical and biological properties of soil

Sl No.	Soil parameter	Unit	Status
Chemical			
1	pH		5.0
2	EC	dS m^{-1}	0.117
3	Available N	kg ha^{-1}	196.52
4	Available P	kg ha^{-1}	10.08
5	Available K	kg ha^{-1}	197.90
6	Exchangeable Ca	ppm	236.67
7	Exchangeable Mg	ppm	114.0
8	Available S	ppm	17.50
9	Available B	ppm	0.04
10	Available Fe	ppm	17.7
11	Available Zn	ppm	0.506
12	Available Cu	ppm	0.463
Biological			
1	Soil respiration rate	mg CO_2 100 g^{-1} d^{-1}	167.62
2	Dehydrogenase	µg TPF g^{-1} 24 h^{-1}	18.04
3	β- glucosidase	mg PNP kg^{-1} h^{-1}	46.24
4	Microbial biomass carbon	µg g^{-1}	133.33

3.1.4. Cropping season

The experiment was conducted during June to September 2018.

3.1.5 Cropping history of the field

The experimental site was under the cultivation of Cassava for about three years.

3.2 EXPERIMENTAL MATERIALS

3.2.1 Crop and variety

The experiment was carried out with Tomato variety Anagha, released from College of Horticulture, Vellanikkara. Seeds were procured from the Farm office, College of Horticulture, Vellanikkara. Tomato (*var*. Anagha) which is early maturing with a crop duration of 4 months. It has semi determinate habitat, resistant to bacterial wilt, fruit cracking and tolerant to leaf curl and mosaic.

3.2.2 Fertilizers and Manures

Urea (46% N), Rajphos (20% P_2O_5) and Muriate of Potash (60% K_2O) were used as the inorganic nutrient sources. Lime (500 kg ha^{-1}) and Farm yard manure (FYM) was applied @ 20 t ha^{-1} as per Package of Practices Recommendation of KAU (KAU POP, 2016). Foliar spray of calcium nitrate was given during the cropping period to avoid cupping of leaves resulting from calcium deficiency.

3.2.3 Design and Layout of experiment

Crop	:	Tomato
Variety	:	Anagha
Design	:	Randomized Block Design (RBD)
Treatments	:	8
Replications	:	3
Plot size	:	3.0 m x 3.0 m
Spacing	:	60 cm x 60 cm

3.2.4 Treatments

Treatment combinations of the field experiment is depicted in Table 2 and layout of the experiment field is depicted in Fig. 2

Table 2. Treatment details

T_1	Absolute control
T_2	N, P, K as per POP (75:45:25 kg ha^{-1})
T_3	N, P, K+ Soil application of Zn as $ZnSO_4$ (10 Kg ha^{-1})
T_4	N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$
T_5	N, P, K+ Zn as Zn EDTA (18 Kg ha^{-1})
T_6	N, P, K+ Zn solubilizer (5 %)
T_7	N, P, K+ Zn Humate (44 Kg ha^{-1})
T_8	N, P, K+ K solubilizer (5%)

* FYM was applied 20 tonnes per hectare as per POP (KAU, 2016) recommendation to all the treatments except absolute control. Agricultural lime was applied 500 kg ha^{-1} to all the treatments.

* K solubilizer also have the ability to solubilize Zn (Sakthidharan, 2013).

3.2.5 Composition of Zn Sources

Table 3. Composition of Zn Sources

Sl. No	Name	Formula	Content (per cent)
1	Zinc Sulphate	$ZnSO_4.7H_2O$	21.0
2	Chelated Zn	Zn-EDTA	12.0
3	Zn-Humate	$C_9H_8O_4Zn$	10.0
4	Zn Solubilizer	-	CFU= $5x10^7$ per gram
5	K Solubilizer	-	CFU= $5x10^7$ per gram

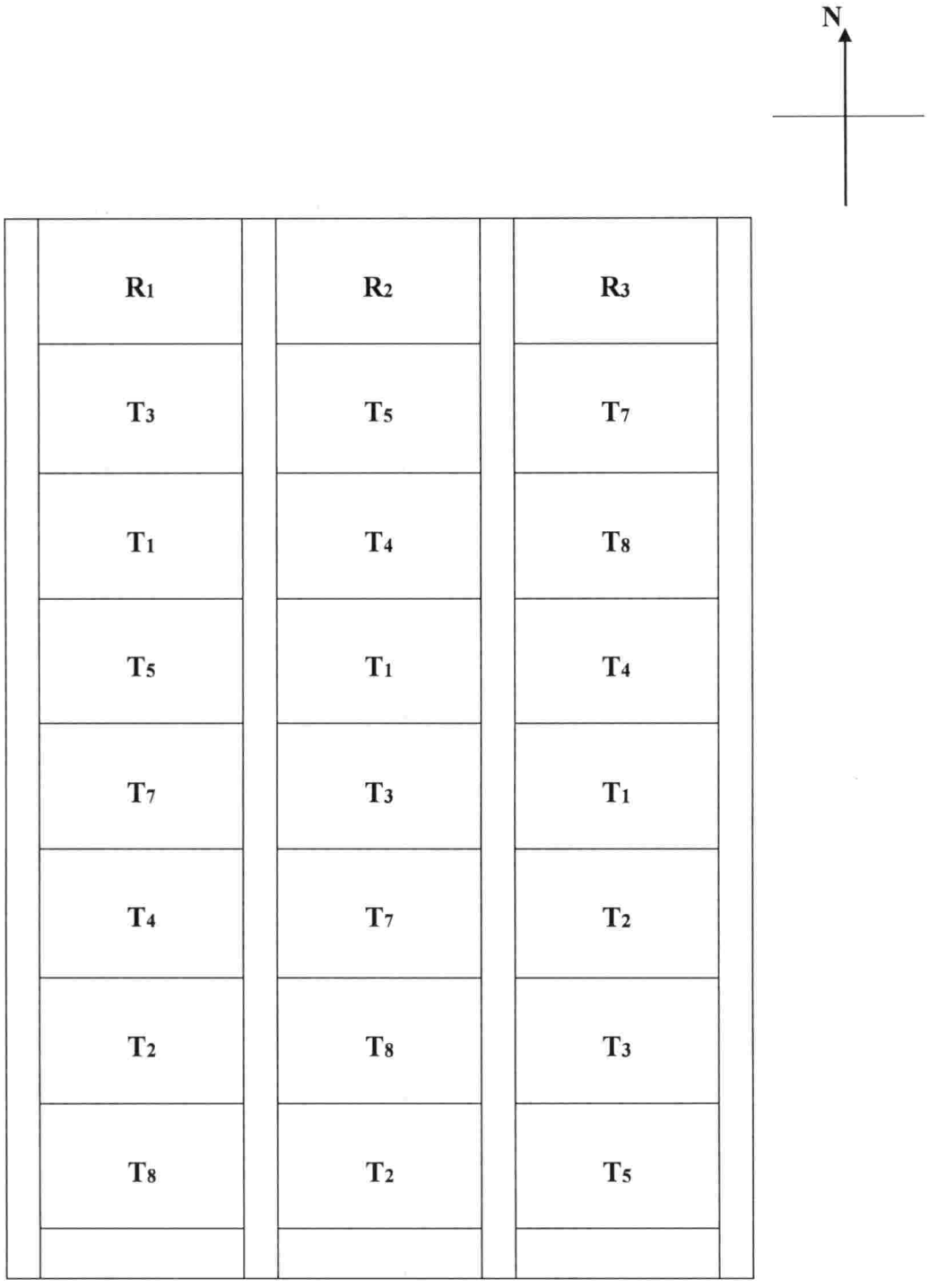

Fig 2. The layout of the experimental field

3.3 FIELD EXPERMENT

3.3.1 Seeds and seedlings

The seeds of tomato were sown in protrays and were kept in poly house provided with insect proof netting on all sides and were irrigated daily. After twenty five days, seedlings were transplanted in the main field.

3.3.2 Land preparation and transplanting

The experimental site was levelled and prepared to fine tilth by ploughing. The whole field was laid out in 3 blocks each with 8 treatments. Irrigation was withheld for one week before transplanting and irrigated the previous day. Seedlings from the protrays were transplanted at a spacing 60 cm x 60 cm after FYM and required quantity of basal dose of fertilizers as per treatment was applied. Irrigation was scheduled and given at regular intervals.

3.3.3 Manure and fertilizer application

The fertilizer recommendation for tomato was 75:40:25 kg ha^{-1} of N: P: K and 20 t ha^{-1} of FYM. As basal dose, half dose of nitrogen (urea), full dose of phosphorus (as Rajphos) and half dose of potassium (as Muriate of potash) were given. Another $1/4^{th}$ dose of nitrogen and the remaining potassium was applied 30 days after planting and the remaining quantity of nitrogen was applied two months after planting.

3.3.4 Application of Treatments

Zinc treatments *viz.*, zinc sulphate, Zn EDTA, Zn solubilizer, Zn humate and K solublizer were applied to the soil at 10 days after transplanting. While foliar application of Zn as $ZnSO_4$ were applied during the branching stage and flowering stage.

Plate 1. Layout of the field

Plate 2. A general view of the Experimental field

3.3.5 Plant protection

Two weeks before transplanting the soil was drenched with 0.3% Copper Oxychloride to avoid fungal infestation. Spraying of Ekalux (2ml L^{-1}) was done to avoid sucking pest during the flowering and peak fruiting stages. Coragen was applied at the rate of 3ml 10 L^{-1} for controlling cut worms and horn worms.

3.3.6 Weeding

Hand weeding was done at 15 days interval.

3.3.7 Staking

Wooden pieces of fine thickness were used as stakes and threads were used to tie the plants.

3.3.8 Harvesting

Periodical observations (30^{th} day, 60^{th} day and 90^{th} day) were taken on the plant characters like plant height, number of primary branches, days to flowering and number of fruits per plant. Fruits were harvested from 60 days after transplanting, weighed and yields were recorded. After the crop period, the plants were rooted out and bhusa yield was recorded. Later these plants were oven dried and dry weight was recorded. Fruits collected from tagged plants (4) were oven dried and subjected to chemical analysis.

3.4 BIOMETRIC OBSERVATIONS

Four plants were selected from each treatment unit and were tagged. Observations with respect to plant height, fruit characters, yield and other attributes were recorded.

3.4.1 Plant Height

Plant height was measured from the base of the tagged plant to the terminal leaf bud, at final harvest and the average height was taken and expressed in centimetre.

3.4.2 Branches per plant

The total number of branches arising from the main stem of each tagged plant was recorded at three months after planting and the mean value was computed.

3.4.3 Flowers per branch

The total number of flowers arising from each branch of the observational plant was recorded at the flowering period and the mean value was calculated.

3.5.4 Total dry matter production

Total dry matter production was recorded at final harvest stage. The tagged plants were uprooted and separated in to roots, stems and leaves and were oven dried at 65°C for 10 hours. The total dry weight of fruits and bhusa were added together to get the total dry matter production which was expressed in kilogram per plant.

3.5 FRUITING CHARACTERS AND YIELD

3.5.1 Days to first flowering

The number of days taken for the first flower to emerge after transplanting was recorded for each plant and the average value was recorded.

3.5.2 Number of fruits per plant

Total number of fruits produced per tagged plant till the last harvest was counted.

3.5.3 Fruit Length

Length of fruits were measured as the distance between the pedicel attachment of the fruit and its apex using twin and scale. Mean value was worked out and expressed in cm.

3.5.4 Fruit Weight

Average weight of fruits wherein the fruit length observations were taken and was expressed in g.

3.5.5 Total yield

Average fruit yield per plant was computed from the observational plant and was expressed in kg ha^{-1}

3.6 PLANT ENZYMES AND PIGMENTS

3.6.1 Carbonic anhydrase

About 400 mg of leaf material was ground with 10 ml of ice cold 10 mM tris-Hcl buffer at a pH of 8.2 containing 5 mM 2-mercapto-ethanol. The mixture was centrifuged at 12,000 rpm for 10 min. The supernatant thus received was used for enzyme analysis. CA activity was assayed during fractionation by calorimetric method (Wilbur and Anderson, 1948). It was then expressed as enzyme unit per gram of fresh leaf weight (EU g^{-1}).

3.6.2 PEP Carboxylase (PEPC)

The activity of PEPC was assayed by coupling with NAD malic dehydrogenase and monitoring NADH oxidation at 340 nm in a Shimadzu UV-Vis Spectrophotometer. The assay was performed at 30°C, the assay mixture (1 ml) contained 50 Mm TRIS- HCl, pH 7.3, 5 mM $MgCl_2$, 0.2 mM NADH, 2 mM MAD, 2.5 mM PEP, 10 mM $NaHCO_3$ and leaf extract. The extraction was done by taking 1 g of fresh leaves and washing it with deionised water. The leaf sample was ground

into a fine paste in a chilled mortar and pestle by adding the extraction buffer was later filtered and centrifuged at 30000 x g for 30 min at 4°C. It was then expressed in μmol mg^{-1} Chl h^{-1} (Parvathi *et al.*, 2000).

Table 4. Analytical procedures followed for enzyme and pigment analysis of the plant tissues

Sl No.	Enzymes and Pigments	Methods	Reference
1	Dehydrogenase	Colorimetric determination of 2,3,5-triphenyl formazan (TPF)	Casida *et al.* (1964)
2	Catalase	Extraction by 0.0067 M phosphate buffer and estimation using Spectrophotometer	Luck (1974)
3	Peroxidase	Extraction by 0.01M phosphate buffer and estimation by spectrophotometer	Srivastava and Kumar (1989)
4	Indole Acetic Acid (IAA)	Extraction by methanol and estimation using spectrophotometer	Stoessl and Veins (1970)
5	Chlorophyll a and b	Extraction by 10 ml acetone (80%): DMSO mixture (1:1 v/v) and estimation by spectrophotometer	Hiscox and Israelstam (1979)

3.6.3 Carboxy dismutase

Five gram leaf sample was ground in 20 ml ice cold extraction medium (10 mM of $MgCl_2$, DTT, $MnCl_2$, and 1.3 g sodium salt of HEPES in 75 ml distilled water) and centrifuged at 20,000 g for 10 minutes. A reaction mixture was prepared using 0.20 ml enzyme extract with 0.10 ml HEPES buffer, 0.05 ml RuBP, 0.05 ml $MgCl_2$ and 0.05 mL DTT and it was illuminated for 2 min. After illumination the reaction was initiated using 1 micro mole of $NaH^{14}CO_3$ and was incubated for 10 minutes after using 0.1 ml of 10% acetic acid. Then it was filtered and counted.

Along with this a blank was also prepared using 0.05 ml buffer instead of RUBP (Kung *et al.*, 1980).

3.6.4 Catalase

One gram plant tissue was grounded in chilled pestle and mortar using 0.0067 M phosphate buffer and the contents were centrifuged at 18000 g at 4°C for 15 min. The supernatant was used as enzyme source within 2-4 hours. 0.01ml of enzyme sample was mixed with 3 ml H_2O_2-PO_4 buffer and read against a control cuvette containing H_2O_2- free PO_4 buffer at 240 nm and the time was noted for a decrease in absorbance from 0.45 to 0.40. It was expressed in Units ml^{-1} extract (Luck, 1974).

3.6.5 Peroxidase

One gram of leaf sample was homogenised in 5 ml of 0.01M sodium phosphate buffer (pH 6.5) and it is centrifuged at 5000 rpm for 15 min for 4°C. 50 µl of enzyme extract was taken and 1ml 0.05M pyrogallol was added and to initiate the reaction, one ml of H_2O_2 was added and the concentration was measured at 420 nm and was expressed in activity min^{-1} g^{-1} of sample (Srivastava and Kumar, 1989).

3.6.6 Indole Acetic Acid (IAA)

One gram of sample was taken and ground in 10 ml methanol to a fine suspension. IAA extraction was done using 10 ml 50 mM KH_2PO_4 and the pH of the solution was maintained at pH 3 with 0.28 M phosphoric acid and was transferred to 10 ml ether. IAA was estimated using a spectrophotometer as per the procedure outlined by Stoessl and Veins (1970) and expressed as µg $g^{-1}$of sample.

3.7 FRUIT QUALITY PARAMETERS

3.7.1 Lycopene

Full ripe fruits were used for lycopene estimation. It was estimated by colorimetric method (Sadasivam and Manickam, 1992) and expressed in μg g^{-1} of fresh ripe fruits.

3.7.2 Ascorbic acid (Vitamin C)

Ascorbic acid was estimated by redox titration method using 2,6 dichlorophenol indophenol (Sadasivam and Manickam, 1992) and expressed in mg 100 g^{-1}.

3.8 CHEMICAL AND BIOLOGICAL ANALYSIS

3.8.1 Soil Analysis

Soil samples were collected before sowing and after harvesting. The samples were air dried under shade, and the sieved through 2mm sieve and used for analysis of various chemical properties like pH, EC, available N, P, K, Ca, Mg, S, Fe, Zn, Cu and biological characteristics like dehydrogenase and β- glucosidase. Fresh soil samples stored at room temperature were used to analyse soil respiration rate and microbial biomass carbon. Analysis of soil samples before and after the experiment was carried out by using the standard procedures as presented in Table 5 and 6.

3.8.2 Plant Analysis

Fruit samples collected during each harvest were mixed together and was dried under shade for 7 days and later oven dried at 65°C for 10 hours. Plant samples collected after the final harvest were dried, powdered and used for analysis. Di acid digestion (HNO_3: $HClO_4$ in 9:4 ratio) was employed in the chemical analysis (Table 7).

Table 5. Analytical procedures followed for soil analysis in the experimental field

Sl. No.	Parameter	Method	Reference
1	pH	Potentiometric method using pH meter (1:2.5 soil water ratio)	Jackson (1973)
2	EC	Conductometric method with Conductivity meter (1:2.5 soil water ratio)	Jackson (1973)
3	Available N	Alkaline potassium permanganate method	Subbiah and Asija (1956)
4	Available P	Bray No. 1 extraction and estimation using spectrophotometer	Bray and Kurtz (1945)
5	Available K	Neutral normal ammonium acetate extraction and estimation using flame photometer	Jackson (1973)
6	Exchangeable Ca and Mg	Versanate titration method	Hesse (1971)
7	Available S	$CaCl_2$ extraction and estimation using spectrophotometer.	Massoumi and Cornfield (1963)
8	Available B	Hot water extraction and estimation using spectrophotometer.	Gupta (1967)
9	Available Fe, Cu and Zn	0.1.N HCl extraction and estimation using atomic absorption spectrophotometer	Sims and Johnson (1991)

Table 6. Analytical methods followed for biological assay of soil

Sl.No.	Parameter	Method	Reference
1	Soil respiration rate	Alkali trap method.	Anderson (1982)
2	Dehydrogenase activity	Colorimetric determination of 2,3,5- triphenyl formazan (TPF)	Casida *et al.* (1964)
3	β- glucosidase	Colorimetric estimation of p-nitrophenol released	Eivazi and Tabatabai (1988)
4	Microbial biomass Carbon	Chloroform fumigation–incubation technique	Jenkinson and Ladd (1981)

Table 7. Analytical methods followed in plant analysis

Sl. No.	Properties	Methods	Reference
1	Nitrogen	Microkjedahl digestion and distillation	Jackson (1973)
2	Phosphorus	Diacid (HNO_3:$HClO_4$ in the ratio 9:4) digestion and estimation using vanado molybdo phosphoric yellow colour method	Jackson (1973)
3	Potassium	Diacid (HNO_3:$HClO_4$ in the ratio 9:4) digestion and estimation using flame photometer	Jackson(1973)
4	Calcium and Magnesium	Diacid (HNO_3:$HClO_4$ in the ratio 9:4) digestion and estimation using Versanate titration method	Hesse (1971)
5	Sulphur	Diacid (HNO_3:$HClO_4$ in the ratio 9:4) digestion and turbidimetry	Massoumi and Cornfield (1963)
6	Boron	Spectrophometry and Azomethine- H method	Roig *et al.*, 1988
7	Micronutrients Fe, Zn and Cu	Diacid (HNO_3:$HClO_4$ in the ratio 9:4) digestion and estimation using AAS	Jackson (1973)

Plate 3. Flowering

Plate 4. Fruiting

Plate 5. A general view of Incubation study

Plate 6. β- glucosidase Assay

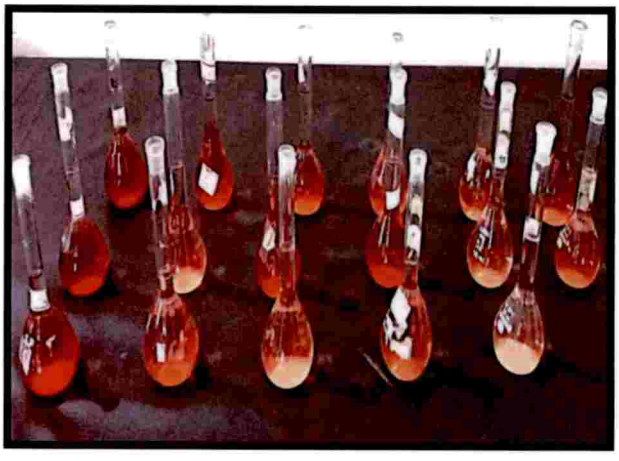

Plate 7. Soil Dehydrogenase Assay

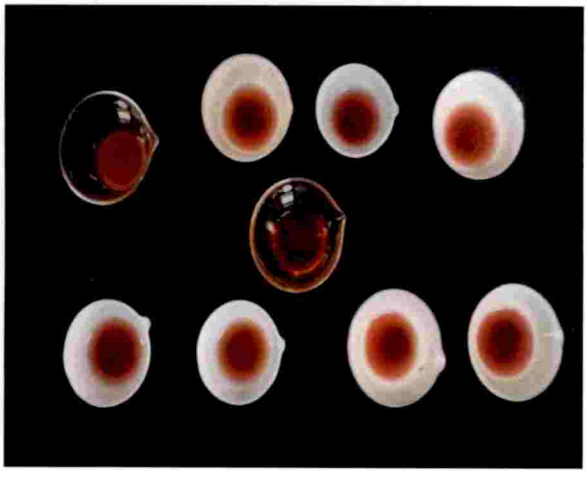

Plate 8. Carbonic anhydrase Assay

Plate 9. Peroxidase Assay

Plate 10. Soil Respiration rate study

3.8.3 Nutrient uptake

The nutrient uptake was calculated from the nutrient content and dry weight of samples after oven drying at 70°C.

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

PART II

3.9 LABORATORY INCUBATION STUDY

A laboratory incubation study was conducted for a period of 3 months from 14-12-2018 to 8-03-2019. The main objective of the study was to assess the relationship between various doses of zinc and enzymes, at periodic intervals *viz.*, 0^{th}, 14^{th}, 28^{th}, 42^{nd}, 56^{th}, 70^{th} and 84^{th} day of incubation.

3.9.1 Collection and preparation of soil sample for incubation study

The soil samples were collected from the experimental site for the incubation study and was analyzed and confirmed the deficient condition of Zn. The collected soil samples were thoroughly mixed, air dried under shade and sieved using 2 mm sieve. 5 kilogram of soil was filled in plastic buckets and the five treatments were imposed separately. N, P, K and farm yard manure were added as per the POP recommendations for tomato. Field capacity (60 %) was maintained throughout the study period. The details of experiment are presented below.

3.9.2 Design and Layout of the Experiment

Design : CRD

Treatments : 5

Replications : 4

3.9.3 Treatment Details

T_1: Soil alone

T_2: Soil + Zn as $ZnSO_4$ (0.5 ppm)

T_3: Soil + Zn as $ZnSO_4$ (1.0 ppm)

T_4: Soil + Zn as $ZnSO_4$ (1.5 ppm)

T_5: Soil + Zn as $ZnSO_4$ (2.0 ppm)

3.9.4 Soil Sampling

Samples were drawn at 0^{th}, 14^{th}, 28^{th}, 42^{nd}, 56^{th}, 70^{th} and 84^{th} day of incubation and analysis was done for the following parameters.

3.9.4.1 Analysis of the sample

Soil samples collected at the designated intervals was analysed for parameters *viz.,* dehydrogenases, peroxidases, carbonic anhydrase and enzyme kinetic parameters were studied at different intervals of incubation period.

3.9.4.2 *Enzyme Kinetics (V_{max} and K_m – Michaelis Menten Constant)*

The enzyme kinetic parameters (V_{max} and K_m) were calculated for three major enzymes, dehydrogenase, peroxidase and carbonic anhydrase for the five treatments based on the Line Weaver Burk plot. The substrate for the enzymes were Zn as $ZnSO_4$ (0.5, 1.0, 1.5 and 2.0 ppm respectively) and an absolute control (soil alone). A straight line graph is obtained in Line Weaver Burk plot method, when 1/V is plotted against 1/S (V is the velocity and S is the substrate concentration). The slope is K_m/V_{max}, where V_{max} is the maximum rate velocity and K_m is the enzyme's affinity for the substrate. The intercept is $1/V_{max}$ (Vaugham and Ord, 1991).

3.10 STATISTICAL ANALYSIS

The data generated from the experiment were subjected to statistical analysis for its significance using the analysis of variance technique for Randomized Block Design and Completely Randomized Block Design (Cochran and Cox, 1969). The F values for treatments were compared with the table values. Critical difference at 0.05% probability level was calculated using statistical tools.

RESULTS

4. RESULTS

An experiment titled "Effect of zinc fertilization on major plant and soil enzymes in southern laterites" has been carried out in the Department of Soil Science and Agricultural Chemistry, College of Agriculture, Vellayani during 2018- 2019. The study was envisioned to assess the effect of various sources and methods of application of zinc on the activities of major plant enzymes, soil enzymes, biochemical and microbial parameters in laterite soils using tomato (*Solanum lycopersicum*) as a test crop. The soil samples and plant samples were collected from the experimental plots and were analyzed in the soil lab after recording biometric, yield and quality attributes. Results based on statistically analysed data related to the experiment during the course of investigation are presented in this chapter.

4.1. EFFECT OF TREATMENTS ON BIOMETRIC CHARACTERISTICS

The biometric characteristics viz., plant height, number of branches, days to first flowering, flowers per branch were significantly influenced by the treatments.

4.1.1. Plant height

A perusal of the data presented in the Table 8 revealed that the plant height is significantly influenced by the treatments. The mean values ranged from 85.67 to 98.33 cm. The treatment T_6 with the application of N, P, K+ Zn solubilizer (5 %) recorded the highest value of 98.33 cm which was on par with T_7 with the application of N, P, K+ Zn Humate (44 kg ha^{-1}). The lowest value was recorded by T_1 (Absolute control) which was on par with T_2 with the application of N, P, K as per POP (75:45:25 kg ha^{-1}).

4.1.2. Branches per plant

It was observed from Table 8 that the treatments exerted significant effect with respect to branches per plant. The mean values ranged from 4.7 to 8.3. Treatment T_5 with the application of N, P, K+ Zn as Zn EDTA (18 kg ha^{-1}) and T_7 with the application of N, P, K+ Zn Humate (44 kg ha^{-1}) showed the highest value

Table 8. Effect of treatments on biometric characters of tomato

Treatments	Plant height (cm)	Branches per plant	Days to first flowering
T_1- Absolute control	85.67	4.70	40.00
T_2-N, P, K as per POP (75:45:25 kg ha^{-1})	86.67	7.00	34.30
T_3- N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1})	90.00	6.30	37.70
T_4-N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$	91.67	6.70	36.00
T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1})	88.33	8.30	32.70
T_6- N, P, K+ Zn solubilizer (5 %)	98.33	7.70	34.00
T_7- N, P, K+ Zn Humate (44 kg ha^{-1})	96.00	8.30	31.70
T_8- N, P, K+ K solubilizer (5%)	89.67	7.30	36.30
CD (0.05)	3.03	2.02	2.60

Table 9. Effect of treatments on fruit characters

Treatments	Flowers per branch	Fruits per plant	Fruit length (cm)
T_1- Absolute control	5.29	29.70	2.96
T_2-N, P, K as per POP (75:45:25 kg ha^{-1})	5.70	34.00	3.06
T_3- N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1})	6.15	32.70	3.09
T_4-N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$	6.69	32.7	3.18
T_5- N, P, K+ Zn as Zn EDTA (kg ha^{-1})	6.23	42.3	3.13
T_6- N, P, K+ Zn solubilizer (5 %)	5.93	38.70	3.40
T_7- N, P, K+ Zn Humate (44 kg ha^{-1})	7.12	46.70	3.50
T_8- N, P, K+ K solubilizer (5%)	7.35	41.30	3.46
CD (0.05)	0.05	3.42	0.06

of 8.33 which was on par with T_6- N, P, K+ Zn solubilizer (5 %), T_8- N, P, K+ K solubilizer (5%) and T_2- N, P, K as per POP (75:45:25 kg ha^{-1}). Treatment T_1 (Absolute control) registered the lowest value (4.7) and was on par with T_3 with the application of N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1}) and T_4 with the application of N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$.

4.1.3. Days to first flowering

Observations of results pertaining to the days to first flowering revealed that the treatments had a significant influence (Table 8). The mean values ranged from 31.70 to 40.00. The treatment with the shortest duration was T_7 (31.70) with the application of N, P, K+ Zn Humate (44 kg ha^{-1}) which was on par with T_5 with the application of N, P, K+ Zn as Zn EDTA (18 kg ha^{-1}) and T_6 with the application of N, P, K+ Zn solubilizer (5 %). The longest duration was observed for T_1 (Absolute control) and was found to be on par with treatment T_3 with the application of N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1}).

4.2. EFFECT OF TREATMENTS ON FRUIT CHARACTERS

The fruit characters viz., flowers per branch, fruits per plant and fruit length are presented in Table 9. It is observed that the treatments had significantly influenced the above characteristics.

4.2.1. Flowers per branch

The treatments has significantly influenced the flowers per branch as observed in the Table 9. The mean value ranged from 5.29 to 7.35. The highest value of 7.35 was observed for T_8 with the application of N, P, K+ K solubilizer (5%) and was found to be superior over all other treatments. Treatment T_1 (absolute control) registered the lowest value of 5.29.

4.2.2. Fruits per plant

A perusal of the data presented in Table 9 revealed that the mean values for the fruits per plant ranged from 29.70 to 46.70. The applied treatments had significantly influenced the number of fruits per plant. The highest value registered

was 46.7 by the treatment T_7 with the application of N, P, K+ Zn Humate (44 kg ha^{-1}) while the lowest value registered was 29.7 by the treatment T_1 (absolute control) which was found to be on par with treatment T_3 with the application of N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1}) and T_4 with the application of N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$.

4.2.3. Fruit length

The mean values for fruit length ranged from 2.96 to 3.50 cm. The highest value was recorded for T_7 (3.50) with the application of N, P, K+ Zn Humate (44 kg ha^{-1}) and was found to be on par with T_8 with the application of N, P, K+ K solubilizer (5%). The treatment T_1 (Absolute control) recorded the lowest value for fruit length with a value of 2.96 cm (Table 9).

4.3. EFFECT OF TREATMENTS ON YIELD CHARACTERS

The biometric observations on yield characters viz., fruit weight, total yield and total dry matter production are presented in Table 10. The treatments had significantly influenced the above characteristics.

4.3.1. Fruit weight

The mean values for fruit weight ranged from 18.00 to 29.32 g. The highest value was recorded for the treatment T_5 (29.32) with the application of N, P, K+ Zn as Zn EDTA (18 kg ha^{-1}). The treatments T_6 with the application of N, P, K+ Zn solubilizer (5 %), T_7 with the application of N, P, K+ Zn Humate (44 kg ha^{-1}), T_3 with the application of N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1}), T_8 with the application of N, P, K+ K solubilizer (5%) and T_4 with the application of N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$ were stastically on par with T_5 (N, P, K+ Zn as Zn EDTA (18 kg ha^{-1})). However, the treatment T_2 with the application of N, P, K as per POP (75:45:25 kg ha^{-1}) recorded the lowest value (18.00 g) which was statistically on par with T_1 (Absolute control).

Table 10. Effect of treatments on yield characters

Treatments	Fruit weight (g)	Total yield (kg ha^{-1})	Total dry matter production (kg $plant^{-1}$)
T_1- Absolute control	18.45	15185.20	0.051
T_2-N, P, K as per POP (75:45:25 kg ha^{-1})	18.00	21111.13	0.058
T_3- N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1})	27.30	24722.24	0.081
T_4-N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$	26.91	24351.87	0.080
T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1})	29.32	27592.61	0.092
T_6- N, P, K+ Zn solubilizer (5 %)	28.46	30555.58	0.092
T_7- N, P, K+ Zn Humate (44 kg ha^{-1})	27.67	35833.36	0.108
T_8- N, P, K+ K solubilizer (5%)	27.09	31018.54	0.093
CD (0.05)	3.154	1511.186	0.008

Table 11. Effect of treatments on plant enzymes

Treatments	Carbonic anhydrase (EU g^{-1}).	PEP carboxylase (µmol mg^{-1})	Carboxy dismutase (Units g^{-1})
T_1- Absolute control	362.50	52.00	100.00
T_2-N, P, K as per POP (75:45:25 kg ha^{-1})	268.67	68.67	103.50
T_3- N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1})	401.67	119.67	138.00
T_4-N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$	665.67	131.33	227.00
T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1})	710.50	102.67	200.50
T_6- N, P, K+ Zn solubilizer (5 %)	575.83	73.00	110.50
T_7- N, P, K+ Zn Humate (44 kg ha^{-1})	910.00	91.67	129.00
T_8- N, P, K+ K solubilizer (5%)	404.67	78.00	107.00
CD (0.05)	21.83	8.94	16.13

4.3.2. Total fruit yield

Treatments significantly influenced the total yield. The mean values ranged from 15185.20 to 35833.36 kg ha^{-1}. Treatment T_7 with the application of N, P, K+ Zn Humate (44 kg ha^{-1}) recorded the highest yield of 35833.36 kg ha^{-1}. The lowest value was observed for the treatment T_1 (absolute control) with a value of 15185.20 kg ha^{-1} (Table 10).

4.3.3. Total dry matter production

The data presented in Table 10 revealed that, the mean values for total dry matter production ranged from 0.051 to 0.108 kg $plant^{-1}$. The treatment T_7 with the application of N, P, K+ Zn Humate (44 kg ha^{-1}) recorded the highest value of 0.108 kg $plant^{-1}$ which was significantly superior to all other treatments. The lowest value of 0.051 kg $plant^{-1}$ was observed for the treatment T_1 (Absolute control) which was significantly on par with T_2 with the application of N, P, K as per POP (75:45:25 kg ha^{-1}).

4.4. EFFECT OF TREATMENT ON PLANT ENZYMES

4.4.1. Carbonic anhydrase

Observations revealed that the treatments had significantly influenced the enzyme carbonic anhydrase. The mean values ranged from 268.67 to 910.00 EU g^{-1}. The highest value for carbonic anhydrase was noticed in the treatment T_7 (910.00 EU g^{-1}) with the application of N, P, K+ Zn Humate (44 kg ha^{-1}) which was found to be stastically superior over all other treatments. The lowest value was recorded for the treatment T_2 (268.67 EU g^{-1}) with the application of N, P, K as per POP (75:45:25 kg ha^{-1}).

4.4.2. PEP Carboxylase

There was significant difference among treatments with respect to enzyme PEP Carboxylase (Table 11). The mean values ranged from 52.00 to 131.33 µmol mg^{-1}. The highest value of 131.33 µmol mg^{-1} was recorded by the treatment T_4 with the application of N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$. The treatment T_1 (Absolute control) recorded the lowest value of 52.00 µmol mg^{-1}.

4.4.3. Carboxy dismutase

The treatments significantly influenced the enzyme carboxy dismutase (Table 11). The mean values ranged from 100.00 to 227.00 Units g^{-1}. Treatment T_4 (227.00 Units g^{-1}) with the application of N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$ recorded the highest value of 227.00 Units g^{-1}. The lowest value of 100.00 Units g^{-1} was observed for the treatment T_1 (Absolute control) which was significantly on par with treatments T_2- N, P, K as per POP (75:45:25 kg ha^{-1}), T_8- N, P, K+ K solubilizer (5%) and T_6 with the application of N, P, K+ Zn solubilizer (5 %).

4.5. EFFECT OF TREATMENTS ON PLANT OXIDO- REDUCTASES

4.5.1. Effect of treatments on dehydrogenases

It was observed from Table 12 that, the mean values of dehydrogenases ranged from 160.78 to 455.03 µg TPF g^{-1} 24 h^{-1} and was significantly influenced by the treatments. The highest value of 455.03 µg TPF g^{-1} 24 h^{-1} was recorded in T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1}) which was found to be statistically superior over all other treatments and treatment T_8 with the application of N, P, K+ K solubilizer (5%) recorded the lowest value of 160.78 µg TPF g^{-1} 24 h^{-1}.

4.5.2. Effect of treatments on peroxidase

The peroxidase (Table 12) was significantly influenced by the different treatments. The mean values ranged from 11.70 to 48.17 activity min^{-1} g^{-1}. The lowest value of 11.70 activity min^{-1} g^{-1} was reported for T_1 (Absolute control) while the treatment T_7 with the application of N, P, K+ Zn Humate (44 kg ha^{-1}) recorded the highest value of 48.17 activity min^{-1} g^{-1} which was significantly different from all other treatments.

4.5.3. Effect of treatments on catalase

The treatments had significantly influenced the catalase content (Table 12). The mean values ranged from 10461.8 to 27058.4 Units ml^{-1}. Treatment T_7-N, P, K+ Zn Humate (44 kg ha^{-1}) recorded the highest value for catalase and significantly

Table 12. Effect of treatments on Oxido reductases (Plant tissue)

Treatments	Dehydrogenase (µg TPF g^{-1} $24h^{-1}$)	Peroxidase (activity min^{-1} g^{-1})	Catalase (Units ml^{-1} extract.)
T_1- Absolute control	331.67	11.70	15134.8
T_2-N, P, K as per POP (75:45:25 kg ha^{-1})	247.99	22.17	13582.2
T_3- N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1})	365.26	34.60	13564.7
T_4-N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$	219.45	33.30	15134.4
T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1})	455.03	41.57	16972.4
T_6- N, P, K+ Zn solubilizer (5 %)	238.90	23.57	10461.8
T_7- N, P, K+ Zn Humate (44 kg ha^{-1})	338.33	48.17	27058.4
T_8- N, P, K+ K solubilizer (5%)	160.78	22.73	12334.4
CD (0.05)	22.693	0.599	300.488

Table 13. Effect of treatments on plant pigments and plant growth regulator

Treatments	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	IAA (µg g^{-1} of sample)
T_1- Absolute control	0.575	0.781	49.17
T_2-N, P, K as per POP (75:45:25 kg ha^{-1})	0.577	0.785	90.27
T_3- N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1})	0.581	0.796	151.33
T_4-N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$	0.599	0.776	145.60
T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1})	0.621	0.842	182.50
T_6- N, P, K+ Zn solubilizer (5 %)	0.575	0.746	187.27
T_7- N, P, K+ Zn Humate (44 kg ha^{-1})	0.631	0.908	217.83
T_8- N, P, K+ K solubilizer (5%)	0.573	0.772	119.33
CD (0.05)	NS	NS	0.429

AG

differed from all other treatments. The lowest value for catalase was recorded by the treatment T_5 with the application of N, P, K+ Zn as Zn EDTA (18 kg ha^{-1}).

4.6. EFFECT OF TREATMENT ON PLANT PIGMENTS AND PLANT GROWTH REGULATOR

4.6.1 Effect of treatments on chlorophyll a

The data pertains to chlorophyll a, as presented in Table 13 revealed that, the treatments did not vary significantly. However, the highest value of 0.631 mg g^{-1} was reported for the treatment of T_7 with the application of N, P, K+ Zn Humate (44 kg ha^{-1}) and the lowest value of 0.573 mg g^{-1} was observed for T_8 with the application of N, P, K+ K solubilizer (5%).

4.6.2. Effect of treatments on chlorophyll b

The treatments did not influence any significant effect on the Chlorophyll b content. The mean values ranged from 0.746 to 0.908 mg g^{-1} (Table 13). However, T_7 with the application of N, P, K+ Zn Humate (44 kg ha^{-1}) recorded the highest value of 0.908 mg g^{-1} while the lowest value was recorded by the treatment T_6- N, P, K+ Zn solubilizer (5 %).

4.6.3. Effect of treatments on IAA

Observations revealed that, the treatments were found to impose significant effects with respect to indole acetic acid (Table 13). The mean values ranged from 49.17 to 217 µg g^{-1}. The highest value for IAA was noticed for T_7 with the application of N, P, K+ Zn Humate (44 kg ha^{-1}) followed by T_6: N, P, K+ Zn solubilizer (5 %). The lowest value was recorded by T_1 (absolute control).

4.7. EFFECT OF TREATMENTS ON FRUIT QUALITY

4.7.1. Effect of treatments on lycopene

Statistical analysis of the data on lycopene content indicated a significant effect due to the application of treatments (Table 14). The mean value ranged from 17.42 to 26.11 µg g^{-1} with the highest value recorded for T_7 with the application of

Table 14. Effect of treatment on fruit quality

Treatments	Lycopene ($\mu g\ g^{-1}$)	Ascorbic acid (mg $100\ g^{-1}$)
T_1- Absolute control	17.42	25.14
T_2-N, P, K as per POP (75:45:25 kg ha^{-1})	17.84	26.22
T_3- N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1})	19.19	25.69
T_4-N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$	20.65	25.49
T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1})	25.79	26.12
T_6- N, P, K+ Zn solubilizer (5 %)	18.88	25.43
T_7- N, P, K+ Zn Humate (44 kg ha^{-1})	26.11	25.35
T_8- N, P, K+ K solubilizer (5%)	23.87	27.23
CD (0.05)	0.598	0.044

Table 15. Effect of treatments on soil pH and EC

Treatments	pH	EC (dS m^{-1})
T_1- Absolute control	5.57	0.117
T_2-N, P, K as per POP (75:45:25 kg ha^{-1})	5.93	0.119
T_3- N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1})	6.01	0.139
T_4-N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$	5.86	0.157
T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1})	6.15	0.143
T_6- N, P, K+ Zn solubilizer (5 %)	6.16	0.137
T_7- N, P, K+ Zn Humate (44 kg ha^{-1})	5.46	0.172
T_8- N, P, K+ K solubilizer (5%)	6.28	0.135
CD (0.05)	0.13	0.005

N, P, K+ Zn Humate (44 kg ha^{-1}) which was found to be on par with T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1}). The lowest value was registered by the treatment T_1 (Absolute control) which was on par with treatments T_2- N, P, K as per POP (75:45:25 kg ha^{-1}).

4.7.2. Effect of treatments on Ascorbic acid

The treatment effect was significant on the ascorbic acid content of fruit (Table 14). The mean value ranged from 25.14 to 27.23 mg 100 g^{-1}. The highest value was recorded by the treatment T_8 (27.23 mg 100 g^{-1}) with the application of N, P, K+ K solubilizer (5%). The lowest value was recorded by T_1 (absolute control).

4.8. SOIL ANALYSIS

The data on soil chemical parameters estimated at the time of final harvest are presented in Tables 15-20.

4.8.1. Soil pH

The data on soil pH shows a significant increase from the initial value of 5.00 in all the treatments and are presented in the Table 15. The mean values ranged from 5.46 to 6.28. The highest pH value of 6.28 was recorded by the treatment T_8- N, P, K+ K solubilizer (5%) which was on par with the treatment T_6 -N, P, K+ Zn solubilizer (5 %) and the lowest value of 5.46 was noticed in the treatment T_7- N, P, K+ Zn Humate (44 kg ha^{-1}).

4.8.2. Electrical Conductivity

The soluble salt content of soil as measured by electrical conductivity was significantly influenced by the treatments. From the perusal of the data presented in Table 15, it is understood that the mean values ranged from 0.117 to 0.172 dS m^{-1}. The highest value of 0.172 dS m^{-1} was recorded by treatment T_7-N, P, K+ Zn Humate (44 kg ha^{-1}) and was significantly superior to all other treatment while the lowest value of 0.117 dS m^{-1} was registered by T_1 (absolute control).

4.8.3. Available Nitrogen

From the perusal of data presented (Table 16), it was observed that the treatments had no influence on the available nitrogen content of the soil. The mean values ranged from 200.70 to 225.79 kg ha^{-1}. Treatment T_3- N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1}) and treatment T_7-N, P, K+ Zn Humate (44 kg ha^{-1}) recorded the highest value (225.79 kg ha^{-1}) while the lowest value was observed for the treatment T_8- N, P, K+ K solubilizer (5%).

4.8.4. Available Phosphorus

A perusal of the data revealed that the available P content of the soil was significantly influenced by different treatments (Table 16). The mean values ranged from 32.18 to 56.60 kg ha^{-1}. The treatment T_6- N, P, K+ Zn solubilizer (5 %) registered the highest value of 56.60 kg ha^{-1} and was significantly superior to all other treatments while the lowest value of 32.18 kg ha^{-1} was recorded by T_1 (Absolute control).

4.8.5. Available Potassium

The treatments had significantly influenced the available potassium content in the soil at final harvest as observed from Table 16. The mean values ranged from 250.13 to 425.60 kg ha^{-1}. The treatment which showed the highest value (425.60 kg ha^{-1}) was T_8-N, P, K+ K solubilizer (5%)) which was on par with T_7- N, P, K+ Zn Humate (44 kg ha^{-1}). The lowest value of 250.13 kg ha^{-1} was recorded in treatment T_6- N, P, K+ Zn solubilizer (5 %).

4.8.6. Exchangeable Calcium

The exchangeable calcium content of the soil was significantly influenced by the different treatments (Table 17) and the mean values ranged from 283.33 to 436.67 ppm. The highest value of 436.67 ppm was registered by treatment T_7- N, P, K+ Zn Humate (44 kg ha^{-1}). The treatment T_1 (Absolute control) and T_2- N, P, K as per POP (75:45:25 kg ha^{-1}) recorded the lowest value of 283.33 ppm which was

Table 16. Effect of treatments on soil primary nutrients

Treatments	Available N	Available P	Available K
	kg ha^{-1}		
T_1- Absolute control	221.62	32.18	287.47
T_2-N, P, K as per POP (75:45:25 kg ha^{-1})	213.25	34.76	358.40
T_3- N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1})	225.79	36.81	388.27
T_4-N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$	213.30	40.10	377.07
T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1})	221.70	40.10	369.60
T_6- N, P, K+ Zn solubilizer (5 %)	213.45	56.60	250.13
T_7- N, P, K+ Zn Humate (44 kg ha^{-1})	225.79	45.36	403.20
T_8- N, P, K+ K solubilizer (5%)	200.70	49.70	425.60
CD (0.05)	NS	1.466	34.805

NS: Non Significant

Table 17. Effect of treatments on soil secondary nutrients

Treatments	Exchangeable Ca (ppm)	Exchangeable Mg (ppm)	Available S (ppm)
T_1- Absolute control	283.33	118	20.83
T_2-N, P, K as per POP (75:45:25 kg ha^{-1})	283.33	124	20.33
T_3- N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1})	290.00	148	24.67
T_4-N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$	346.67	146	22.00
T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1})	393.33	152	23.17
T_6- N, P, K+ Zn solubilizer (5 %)	350.00	144	24.00
T_7- N, P, K+ Zn Humate (44 kg ha^{-1})	436.67	166	26.33
T_8- N, P, K+ K solubilizer (5%)	290.00	136	22.83
CD (0.05)	17.357	13.148	2.886

on par with treatments T_8- N, P, K+ K solubilizer (5%) and T_3- N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1}).

4.8.7. Exchangeable Magnesium

The treatments had significantly influenced the exchangeable Mg content in the soil at the time of harvest. The mean values ranged from 118 to 166 ppm recorded in Table 17. Treatment T_7: N, P, K+ Zn Humate (44 kg ha^{-1}) registered the highest value of 166 ppm. The lowest value of 118 ppm was registered by the treatment T_1 (Absolute control) which was on par with T_2- N, P, K as per POP (75:45:25 kg ha^{-1}).

4.8.8. Available Sulphur

Table 17 represented the available S content in the soil at the time of final harvest. The mean values ranged from 20.33 to 26.33 ppm. The treatments showed a significant influence on available S of the soil. The highest value of 26.33 ppm was registered by T_7- N, P, K+ Zn Humate (44 kg ha^{-1}) and was on par with T_3- N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1}) and T_6- N, P, K+ Zn solubilizer (5 %). The lowest value of 20.33 ppm was registered by T_2- N, P, K as per POP (75:45:25 kg ha^{-1}) and was on par with T_1 (Absolute control).

4.8.9 Effect of treatments on soil micro nutrients

4.8.9.1 *Available Fe*

Available Fe in soil under different treatments, at the time of final harvest is presented in Table 18. The mean values ranged from 8.50 to 16.49 ppm. The highest value of 16.49 ppm was registered by T_1 (Absolute control) and was on par with T_8 with the application of N, P, K+ K solubilizer (5%) with the mean value of 14.43 ppm. Treatment T_7- N, P, K+ Zn Humate (44 kg ha^{-1}) registered the lowest value which was on par with T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1})), T_6- N, P, K+ Zn solubilizer (5 %), T_3- N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1}), T_4- N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$ and T_2 with the application of N, P, K as per POP (75:45:25 kg ha^{-1}).

4.8.9.2 Available Zn

Treatments significantly influenced the available Zn content in the soil at the time of harvest. The mean values ranged from 2.76 to 5.12 ppm. Treatment T_7 with the application of N, P, K+ Zn Humate (44 kg ha^{-1}) registered the highest value of 5.12 ppm and was found to be on par with T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1}) with a mean value of 4.86 ppm. The lowest value of 2.76 ppm was recorded by T_1 (Absolute control) which was on par with T_2 with the application of N, P, K as per POP (75:45:25 kg ha^{-1}) having a mean value of 2.94 ppm.

4.8.9.3 Available B

Available B content in the soil was also significantly influenced by different treatments (Table 18). The mean values ranged from 0.045 to 0.221 ppm. The highest value of 0.221 ppm was recorded by treatment T_7 with the application of N, P, K+ Zn Humate (44 kg ha^{-1}) while the lowest value was recorded by T_2 with the application of N, P, K as per POP (75:45:25 kg ha^{-1}) which was found to be on par with T_6- N, P, K+ Zn solubilizer (5 %) with a mean value of 0.069.

4.8.9.4 Available Cu

The treatments had significantly influenced the available Cu content in the soil at final harvest as observed in Table 18. The mean values ranged from 0.44 to 1.18 ppm. Treatment T_5 with the application of N, P, K+ Zn as Zn EDTA (18 kg ha^{-1}) registered the highest value of 1.18 ppm. Treatment T_1 (Absolute control) registered the lowest value and was found to be on par with T_3- N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1}) and T_4 with the application of N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$ with mean values 0.44, 0.51 and 0.54 respectively.

4.9 Effect of treatments on soil biological characters

4.9.1 Soil respiration rate

The soil respiration rate observed under different treatments varied significantly. The mean values ranged from 127.62 to 177.14 mg CO_2 100 g^{-1} d^{-1} (Table 19). The treatment T_7 with the application of N, P, K+ Zn Humate

Table 18. Effect of treatments on soil micro nutrients

Treatments	Fe (ppm)	Zn (ppm)	B (ppm)	Cu (ppm)
T_1- Absolute control	16.49	2.76	0.08	0.73
T_2-N, P, K as per POP (75:45:25 kg ha^{-1})	10.42	2.95	0.05	0.64
T_3- N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1})	10.22	3.75	0.13	0.52
T_4-N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$	10.25	3.75	0.10	0.55
T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1})	9.86	4.86	0.09	1.19
T_6- N, P, K+ Zn solubilizer (5 %)	10.13	3.57	0.07	0.75
T_7- N, P, K+ Zn Humate (44 kg ha^{-1})	8.50	5.12	0.22	0.68
T_8- N, P, K+ K solubilizer (5%)	14.43	3.30	0.08	0.44
CD (0.05)	2.122	0.42	0.03	0.16

Table 19. Effect of treatment on soil biological characters

Treatments	Soil respiration rate (mg CO_2 100 g^{-1} d^{-1})	Microbial biomass carbon (µg g^{-1})
T_1- Absolute control	127.62	173.33
T_2-N, P, K as per POP (75:45:25 kg ha^{-1})	167.62	153.33
T_3- N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1})	166.67	178.67
T_4-N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$	174.29	173.33
T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1})	159.05	160.00
T_6- N, P, K+ Zn solubilizer (5 %)	170.48	166.67
T_7- N, P, K+ Zn Humate (44 kg ha^{-1})	177.14	186.67
T_8- N, P, K+ K solubilizer (5%)	165.71	169.33
CD (0.05)	7.832	15.045

(44 kg ha^{-1}) recorded the highest value of 177.14 mg CO_2 100 g^{-1} d^{-1} and T_4 with the application of N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$ was the second best one, which were found to be statistically on par with each other. The lowest value (127.62 mg CO_2 100 g^{-1} d^{-1}) was reported for the treatment T_1 (absolute control).

4.9.2 Microbial biomass carbon

The different treatments had significantly influenced the Microbial biomass carbon as seen from Table 19. The mean values ranged from 153.33 to 186.67 µg g^{-1}. The highest value of 186.67 µg g^{-1} was noticed with the treatment T_7 with the application of N, P, K+ Zn Humate (44 kg ha^{-1}) which was on par with T_3- N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1}) and T_1 (Absolute control) and T_4 (N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$) with a mean value of 173.33 µg g^{-1}. The lowest value (153.33 µg g^{-1}) was noticed in the treatment T_2- N, P, K as per POP (75:45:25 kg ha^{-1}) which was on par with T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1}) and T_6- N, P, K+ Zn solubilizer (5 %).

4.9.3 Effect of treatments on soil enzymes

4.9.3.1 Dehydrogenase

Data on dehydrogenase (Table 20) showed that the mean value ranges from 43.31 to 119.32 µg TPF g^{-1} 24 h^{-1}. The treatment T_7 with the application of N, P, K+ Zn Humate (44 kg ha^{-1}) has recorded the highest value of 119.32 µg TPF g^{-1} 24 h^{-1} while the lowest value (43.31 µg TPF g^{-1} 24 h^{-1}) was recorded by the treatment T_1 (Absolute control).

4.9.3.2 β- glucosidase

The effect of treatments on β- glucosidase was significant (Table 20). The mean values ranged from 46.90 to 68.60 mg PNP kg^{-1} h^{-1}. Treatment T_7 with the application of N, P, K+ Zn Humate (44 kg ha^{-1}) recorded the highest mean value of 68.60 mg PNP kg^{-1} h^{-1} while the lowest value of 46.90 60 mg PNP kg^{-1} h^{-1} was recorded by T_1 (Absolute control).

Table 20. Effect of treatment on soil Enzymes

Treatments	Dehydrogenase (μg TPF g^{-1} 24 h^{-1})	β- glucosidase (mg PNP kg^{-1} h^{-1})
T_1- Absolute control	43.31	48.12
T_2-N, P, K as per POP (75:45:25 kg ha^{-1})	44.98	48.03
T_3- N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1})	72.75	46.90
T_4-N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$	84.13	50.21
T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1}	90.60	59.27
T_6- N, P, K+ Zn solubilizer (5 %)	55.02	53.57
T_7- N, P, K+ Zn Humate (44 kg ha^{-1})	119.32	68.60
T_8- N, P, K+ K solubilizer (5%)	55.21	57.63
CD (0.05)	1.24	0.39

Table 21. Effect of treatments on uptake of primary nutrients

Treatments	N	P	K
	kg ha^{-1}		
T_1- Absolute control	29.67	3.77	30.48
T_2-N, P, K as per POP (75:45:25 kg ha^{-1})	38.34	5.49	25.84
T_3- N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1})	62.46	8.27	41.83
T_4-N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$	60.74	8.15	39.94
T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1}	81.95	11.72	50.71
T_6- N, P, K+ Zn solubilizer (5 %)	73.77	12.98	39.91
T_7- N, P, K+ Zn Humate (44 kg ha^{-1})	87.87	12.05	40.92
T_8- N, P, K+ K solubilizer (5%)	77.78	15.69	51.13
CD (0.05)	7.671	1.468	3.451

4.10. EFFECT OF TREATMENTS ON UPTAKE OF PRIMARY NUTRIENTS

4.10.1. Uptake of nitrogen

The data on nitrogen uptake (Table 21) revealed that, the treatment effect was significant. The mean values ranged from 29.67 to 87.87 kg ha^{-1}. The highest value was noticed for the treatment T_7 (87.87 kg ha^{-1}) with the application of N, P, K+ Zn Humate (44 kg ha^{-1}) which was found to be on par with T_5 with the application of N, P, K+ Zn as Zn EDTA (18 kg ha^{-1}) while the lowest value was recorded in treatment T_1 (Absolute control).

4.10.2. Uptake of phosphorus

The plant phosphorus uptake ranged from 3.77 to 15.69 kg ha^{-1}. The treatments had significantly influenced the P uptake. Treatment T_8 with the application of N, P, K+ K solubilizer (5%) recorded the highest mean value of 15.69 kg ha^{-1} and the lowest value of 3.77 kg ha^{-1} was recorded by T_1 (Absolute control).

4.10.3. Uptake of potassium

The treatments had significantly influenced the K uptake (Table 21). The mean values ranged from 25.84 to 51.13 kg ha^{-1}.Treatment T_8 with the application of N, P, K+ K solubilizer (5%) recorded the highest value of 51.13 kg ha^{-1} and was found to be on par with Treatment T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1}). The lowest value of 25.84 kg ha^{-1} for K uptake was recorded by the treatment T_2 with the application of N, P, K as per POP (75:45:25 kg ha^{-1}).

4.11. EFFECT OF TREATMENTS ON UPTAKE OF SECONDARY NUTRIENTS

4.11.1. Uptake of Ca

It was observed from Table 22 that, the mean values of Ca uptake ranged from 8.41 to 19.94 kg ha^{-1}. The treatments imparted a significant effect on Ca uptake. The highest value of 19.94 kg ha^{-1} was recorded for T_4 with the application of N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$ which were found to be on par

with T_7- N, P, K+ Zn Humate (44 kg ha^{-1}) while the treatment T_1 (Absolute control) recorded the lowest value of 8.41 kg ha^{-1}.

4.11.2. Uptake of Mg

There was significant difference among treatments with respect to Mg uptake. The mean values ranged from 37.09 to 75.29 kg ha^{-1} (Table 22). The highest value was recorded for treatment T_7 (75.29 kg ha^{-1}) with the application of N, P, K+ Zn Humate (44 kg ha^{-1}) and was found to be on par with T_5: N, P, K+ Zn as Zn EDTA (18 kg ha^{-1}). Treatment T_1 (Absolute control) registered the lowest value of 37.09 kg ha^{-1} which was found to be on par with T_2 with the application of N, P, K as per POP (75:45:25 kg ha^{-1}).

4.11.3. Uptake of S

The treatments significantly influenced the S uptake as seen in Table 22. The mean values ranged from 2.14 to 11.09 kg ha^{-1}. The highest value was noticed for the treatment T_7 (11.09 kg ha^{-1}) with the application of N, P, K+ Zn Humate (44 kg ha^{-1}) and the lowest value for treatment T_1 (absolute control).

4.12. EFFECT OF TREATMENTS UPTAKE OF MICRONUTRIENTS

4.12.1. Uptake of Fe

The treatments impart significant effect on the uptake. The mean values ranged from 0.669 to 1.136 kg ha^{-1}. Treatment which recorded the highest Fe uptake was T_8 (1.136 kg ha^{-1}) with the application of N, P, K+ K solubilizer (5%) and was found to be superior over all other treatments. Treatment T_1 (Absolute control) recorded the lowest value of 0.669 kg ha^{-1} and was significantly on par with T_3-N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1}) and T_5 with the application of N, P, K+ Zn as Zn EDTA (18 kg ha^{-1}).

4.12.2. Uptake of Zn

Zn uptake was significantly influenced by the different treatments (Table 23). The mean values ranged from 0.053 to 0.187 kg ha^{-1}. Treatment T_7 with the application of N, P, K+ Zn Humate (44 kg ha^{-1}) reported the highest value of

Table 22. Effect of treatments on uptake of secondary nutrients

Treatments	Ca (kg ha^{-1})	Mg (kg ha^{-1})	S (kg ha^{-1})
T_1- Absolute control	8.41	37.09	2.14
T_2-N, P, K as per POP (75:45:25 kg ha^{-1})	11.01	42.09	2.80
T_3- N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1})	8.94	59.84	6.50
T_4-N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$	19.94	50.65	6.50
T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1})	16.50	68.04	4.42
T_6- N, P, K+ Zn solubilizer (5 %)	15.22	58.11	4.29
T_7- N, P, K+ Zn Humate (44 kg ha^{-1})	19.54	75.29	11.09
T_8- N, P, K+ K solubilizer (5%)	14.65	66.68	3.82
CD (0.05)	2.106	7.858	0.604

Table 23. Effect of treatments on uptake of micronutrients

Treatments	Fe (kg ha^{-1})	Zn (kg ha^{-1})	B (kg ha^{-1})	Cu (kg ha^{-1})
T_1- Absolute control	0.67	0.05	0.12	0.10
T_2-N, P, K as per POP (75:45:25 kg ha^{-1})	1.01	0.07	0.21	0.11
T_3- N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1})	0.68	0.11	0.45	0.16
T_4-N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$	0.75	0.11	0.34	0.13
T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1})	0.74	0.18	0.54	0.74
T_6- N, P, K+ Zn solubilizer (5 %)	0.93	0.12	0.29	0.09
T_7- N, P, K+ Zn Humate (44 kg ha^{-1})	0.88	0.19	0.57	0.52
T_8- N, P, K+ K solubilizer (5%)	1.14	0.09	0.48	0.54
CD (0.05)	0.06	0.02	0.08	0.08

0.187 kg ha^{-1} and was on par with T_5 corresponds to the the application of N, P, K+ Zn as Zn EDTA (18 kg ha^{-1}). Treatment T_1 (Absolute control) recorded the lowest value of 0.053 kg ha^{-1} and was significantly on par with T_2 with the application of N, P, K as per POP (75:45:25 kg ha^{-1}).

4.12.3. Uptake of B

In the case of B uptake, the treatments showed significant influence and the mean values ranged from 0.12 to 0.57 kg ha^{-1} (Table 23). The highest value of 0.57 kg ha^{-1} was found in T_7 corresponds to the application of N, P, K+ Zn Humate (44 kg ha^{-1}) which was found to be on par with T_5 with the application of N, P, K+ Zn as Zn EDTA (18 kg ha^{-1}). The lowest value of 0.12 kg ha^{-1} was observed in treatment T_1 (Absolute control).

4.12.4. Uptake of Cu

Cu uptake was significantly influenced by the treatments (Table 23). The mean values ranged from 0.103 to 0.742 kg ha^{-1}. The highest value of 0.742 kg ha^{-1} for Cu uptake was recorded by T_5 with the application of N, P, K+ Zn as Zn EDTA (18 kg ha^{-1}) which was found to be significantly superior over all other treatments. The lowest value of 0.103 kg ha^{-1} was observed for treatment T_1 (Absolute control) which was found to be significantly on par with T_2- N, P, K as per POP (75:45:25 kg ha^{-1}), T_3- N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1}) and T_4 with the application of N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$

4.13 LABORATORY INCUBATION STUDY

The incubation study under laboratory condition for a period of 3 months was carried out to assess the effect of various dosages of zinc on enzymes, for this purpose, soil samples collected at periodic intervals viz., 0^{th}, 14^{th}, 28^{th}, 42^{nd}, 56^{th}, 70^{th} and 84^{th} day of incubation and the results were recorded and are presented in Table 23-25

4.13.1. Effect of treatments on Dehydrogenase

Significant differences in the activity of Dehydrogenase was noticed in the incubation period and the results are presented in Table 24. On 0^{th} day of incubation the highest mean value of 151.01 µg TPF g^{-1} 24 h^{-1} was registered by T_5 with the application of Soil + Zn as $ZnSO_4$ (2 ppm) and the lowest mean value of 120.87 µg TPF g^{-1} 24 h^{-1} registered by T_4 with the application of Soil + Zn as $ZnSO_4$ (1.5 ppm). The lowest mean value for all the treatments except T_1 (Soil alone) was noticed in the 14^{th} day of incubation. The peak value of 248.18 µg TPF g^{-1} 24 h^{-1} was registered on the 84^{th} day of incubation with T_5: Soil + Zn as $ZnSO_4$ (2 ppm). On the 28^{th} , 42^{nd} , 56^{th}, 70^{th} and 84^{th} day of incubation treatment T_5 with the application of Soil + Zn as $ZnSO_4$ (2 ppm) registered the highest values of 147.89, 183.45, 177.21, 190.07 and the peak value of 248.18 µg TPF g^{-1} 24 h^{-1} respectively. It was observed that there was a sudden decrease in the activity of dehydrogenase due to the treatments on the 70^{th} day of incubation.

4.13.2. Effect of treatments on Peroxidase

The treatments showed significant variation in peroxidase activity as reported in Table 25. In general there was a decreasing trend with respect to peroxidase activity with the advancement of the incubation period.

The treatment T_3 with the application of Soil + Zn as $ZnSO_4$ (1 ppm) registered the highest mean value of 13.53 activity min^{-1} g^{-1} on 0^{th} day of incubation. On the 14^{th} day there was an increase in peroxidase activity in all the treatments except T_3 with the application of Soil + Zn as $ZnSO_4$ (1 ppm). Treatment T_4 with the application of Soil + Zn as $ZnSO_4$ (1.5 ppm) recorded the highest mean value of 11.98 activity min^{-1} g^{-1} and was found to be on par with T_2: Soil + Zn as $ZnSO_4$ (0.5 ppm), T_3: Soil + Zn as $ZnSO_4$ (1 ppm) and T_5: Soil + Zn as $ZnSO_4$ (2 ppm). Treatment T_4 with the application of Soil + Zn as $ZnSO_4$ (1.5 ppm) recorded the highest mean value of 11.20, 7.97, 7.65 and 5.83 activity min^{-1} g^{-1} on the 42^{nd}, 56^{th}, 70^{th} and 84^{th} day of incubation respectively.

Table 24. Effect of treatment on Dehydrogenase

Treatments	Dehydrogenase activity (μg TPF g^{-1} 24 h^{-1})						
Incubation Period	0^{th} day	14^{th} day	28^{th} day	42^{nd} day	56^{th} day	70^{th} day	84^{th} day
T_1: Soil alone	127.16	130.86	130.90	151.01	147.89	128.70	112.28
T_2: Soil + Zn as $ZnSO_4$ (0.5 ppm)	130.90	109.07	115.45	173.61	134.36	171.64	173.42
T_3: Soil + Zn as $ZnSO_4$ (1 ppm)	130.86	89.35	128.70	141.03	136.28	166.60	176.44
T_4: Soil + Zn as $ZnSO_4$ (1.5 ppm)	120.87	83.40	127.16	176.97	153.55	167.52	192.61
T_5: Soil + Zn as $ZnSO_4$ (2 ppm)	151.01	112.28	147.89	183.45	177.21	190.07	248.18
CD (0.05)	1.700	1.535	1.515	1.226	1.551	1.266	1.183

Table 25. Effect of treatment on Peroxidase

Treatments	Peroxidase (activity min^{-1} g^{-1})						
Incubation period	0^{th} day	14^{th} day	28^{th} day	42^{nd} day	56^{th} day	70^{th} day	84^{th} day
T_1: Soil alone	5.35	6.03	7.77	5.98	5.69	5.46	4.85
T_2: Soil + Zn as $ZnSO_4$ (0.5 ppm)	10.75	11.78	11.33	8.60	7.94	7.48	5.58
T_3: Soil + Zn as $ZnSO_4$ (1 ppm)	13.53	11.43	9.43	9.03	5.98	6.03	5.44
T_4: Soil + Zn as $ZnSO_4$ (1.5 ppm)	8.28	11.98	7.79	11.20	7.97	7.65	5.83
T_5: Soil + Zn as $ZnSO_4$ (2 ppm)	9.45	11.13	7.95	6.02	7.77	6.50	5.35
CD (0.05)	1.071	1.194	1.262	1.348	1.262	0.891	0.371

4.13.3. Effect of treatments on Carbonic anhydrase

The data on carbonic anhydrase activity is presented in Table 26. In general there was an increasing trend with respect to carbonic anhydrase activity with the advancement of the incubation period. There was significant variation in carbonic anhydrase activity by the treatments during the 56th, 70th and 84th day of incubation.

The Treatment, T_4 with the application of Soil + Zn as $ZnSO_4$ (1.5 ppm) and T_5 with the application of Soil + Zn as $ZnSO_4$ (2 ppm) recorded the highest mean value of 165 EU g^{-1} on the 0th day of incubation. On the 28th day treatment T_3: Soil + Zn as $ZnSO_4$ (1 ppm) registered the highest value of 220 EU g^{-1}. Treatment T_2 with tha application of Soil + Zn as $ZnSO_4$ (0.5 ppm) and T_4 with the application of Soil + Zn as $ZnSO_4$ (1.5 ppm) registered the highest value of 275 and 385 during the 70th and 84th day of incubation respectively. These treatments were found to be on par with T_3: Soil + Zn as $ZnSO_4$ (1 ppm) and T_5 with the application of Soil + Zn as $ZnSO_4$ (2 ppm) during this period.

4.14. ENZYME KINETICS

Three enzymes (dehydrogenase, peroxidase and carbonic anhydrase) were selected for the incubation study. The data obtained from the study on V_{max} and K_m at biweekly interval for three months were recorded and reported in Tables 26-31.

4.14.1. Effect of treatments on kinetic parameters of dehydrogenase

From the data presented in Table 27 and 28, it was evident that there was a decreasing trend in V_{max} with the progress in incubation period. The peak value for V_{max} was recorded by T_1 (Soil alone) having a value of 75.23 µg TPF g^{-1} 24 h^{-1} on the 28th day. The lowest value of 1.45 µg TPF g^{-1} 24 h^{-1} was recorded on the 28th day by treatment T_3 with the application of Soil + Zn as $ZnSO_4$ (1 ppm). On the 84th day treatment T_1 (Soil alone) recorded the highest value of 18.65 µg TPF g^{-1} 24 h^{-1}.

The K_m values ranged from 0.10 to 3.12 µg TPF g^{-1} 24 h^{-1}. Generally there was a decreasing trend for K_m value with the advancement of incubation period. The peak value of 3.12 µg TPF g^{-1} 24 h^{-1} for K_m was observed for treatment T_1

Table 26. Effect of treatments on Carbonic anhydrase

Treatments	Carbonic anhydrase (EU g^{-1})						
Incubation Period	0^{th} day	14^{th} day	28^{th} day	42^{nd} day	56^{th} day	70^{th} day	84^{th} day
T_1: Soil alone	110	137.5	165	166.25	165	110	165
T_2: Soil + Zn as $ZnSO_4$ (0.5 ppm)	110	165	165	165	165	275	385
T_3: Soil + Zn as $ZnSO_4$ (1 ppm)	110	165	220	220	220	247.5	330
T_4: Soil + Zn as $ZnSO_4$ (1.5 ppm)	165	165	165	220	330	275	385
T_5: Soil + Zn as $ZnSO_4$ (2 ppm)	165	165	165	165	167.5	220	357.5
CD (0.05)	NS	NS	NS	NS	112.509	70.998	102.621

NS: Non Significant

Table 27. Effect of treatments on kinetic parameter (V_{max}) of dehydrogenase

Treatments	Dehydrogenase (µg TPF g^{-1} 24 h^{-1})						
	V_{max}						
Incubation period	0^{th} day	14^{th} day	28^{th} day	42^{nd} day	56^{th} day	70^{th} day	84^{th} day
T_1: Soil alone	18.08	23.48	75.23	3.99	14.12	11.45	18.65
T_2: Soil + Zn as $ZnSO_4$ (0.5 ppm)	75.16	18.13	19.71	23.24	14.53	11.92	4.42
T_3: Soil + Zn as $ZnSO_4$ (1 ppm)	23.49	31.15	1.45	13.89	4.13	5.77	3.74
T_4: Soil + Zn as $ZnSO_4$ (1.5 ppm)	18.08	23.88	18.07	3.80	19.74	13.10	4.16
T_5: Soil + Zn as $ZnSO_4$ (2 ppm)	3.99	18.65	14.13	3.24	12.54	3.71	2.54

Table 28. Effect of treatments on kinetic parameter (K_m) of dehydrogenase

Treatments	Dehydrogenase (µg TPF g^{-1} 24 h^{-1})						
	K_m						
Incubation period	0^{th} day	14^{th} day	28^{th} day	42^{nd} day	56^{th} day	70^{th} day	84^{th} day
T_1: Soil alone	0.75	0.97	3.13	0.16	0.58	0.47	0.77
T_2: Soil + Zn as $ZnSO_4$ (0.5 ppm)	3.12	0.75	0.81	0.96	0.60	0.49	0.18
T_3: Soil + Zn as $ZnSO_4$ (1 ppm)	0.97	1.29	0.47	0.57	0.16	0.23	0.15
T_4: Soil + Zn as $ZnSO_4$ (1.5 ppm)	0.75	0.98	0.75	0.15	0.82	0.54	0.17
T_5: Soil + Zn as $ZnSO_4$ (2 ppm)	0.16	0.77	0.58	0.13	0.52	0.15	0.10

Table 29. Effect of treatments on kinetic parameter (V_{max}) of Peroxidase

Treatments	Peroxidase (activity min^{-1} g^{-1})						
	V_{max}						
Incubation Period	0^{th} day	14^{th} day	28^{th} day	42^{nd} day	56^{th} day	70^{th} day	84^{th} day
T_1: Soil alone	4987.6	1400.9	4695.9	29608	3145.1	972.3	11.4
T_2: Soil + Zn as $ZnSO_4$ (0.5 ppm)	913.6	958.3	1340.5	11934	4136.7	9.1	1180.3
T_3: Soil + Zn as $ZnSO_4$ (1 ppm)	12691.5	492.4	59432.2	2178	29634.4	12603.3	369.9
T_4: Soil + Zn as $ZnSO_4$ (1.5 ppm)	13.3	1224.6	23045.9	5438	5328.1	6543.4	1371.3
T_5: Soil + Zn as $ZnSO_4$ (2 ppm)	15567.2	2464.0	3297.5	4938	46959.1	48688.5	4987.5

(Soil alone) on the 28th day. The lowest values for all treatments except T_1 (Soil alone) were registered on the 84th day. The highest values were recorded on 14th day of incubation for all other treatments except for T_2 with the application of Soil + Zn as $ZnSO_4$ (0.5 ppm)

4.14.2. Effect of treatments on kinetic parameters of Peroxidase

In case of Peroxidase enzyme, the values for V_{max} ranged from 9.1 to 48688.5 activity min^{-1} g^{-1}. An increasing trend in V_{max} activity was noticed in T_4 with the application of Soil + Zn as $ZnSO_4$ (1.5 ppm) and T_5: Soil + Zn as $ZnSO_4$ (2 ppm). The peak value was registered by treatment T_5 with the applicaton of Soil + Zn as $ZnSO_4$ (2 ppm) on the 70th day of incubation. There was a general increase in V_{max} value on the 28th day of incubation in all the treatments. The lowest value of 9.1 activity min^{-1} g^{-1} was registered by treatment T_2 with the application of Soil + Zn as $ZnSO_4$ (0.5 ppm) on the 70th day. A general decrease in V_{max} value was noticed in the 84th day of incubation for all treatments.

With respect to K_m a decreasing trend was noticed. The values of K_m ranged from 0.17 to 1606.7 activity min^{-1} g^{-1}. The peak value of 1606.7 activity min^{-1} g^{-1} was registered by treatment T_3 with the application of Soil + Zn as $ZnSO_4$ (1 ppm) on the 28th day of incubation. The lowest value was registered by treatment T_1 (Soil alone) on the 84th day of incubation. An increase in K_m activity was noticed in the 28th day of incubation for all the treatments.

4.14.3. Effect of treatments on kinetic parameters of Carbonic anhydrase

From the Tables 31 and 32, it is evident that the V_{max} value varied from 0.12 to 674.00 EU g^{-1}. The peak value of 674.00 EU g^{-1} was registered on the 56th day day of incubation by T_3 with the application of Soil + Zn as $ZnSO_4$ (1 ppm).The lowest value of 0.12 EU g^{-1} was registered by treatment T_3 with the application of Soil + Zn as $ZnSO_4$ (1 ppm) on the 28th day of incubation. The 0th day and 84th day of incubation registered the same value of V_{max} in all the treatments. On the 28th day of incubation all treatments recorded V_{max} value of 608.78 EU g^{-1} except T_3 with the application of Soil + Zn as $ZnSO_4$ (1 ppm).

Table 30. Effect of treatments on kinetic parameter (K_m) of Peroxidase

Treatments	Peroxidase (activity min^{-1} g^{-1})						
	K_m						
Incubation Period	0^{th} day	14^{th} day	28^{th} day	42^{nd} day	56^{th} day	70^{th} day	84^{th} day
T_1: Soil alone	166.1	46.7	1565.3	987	104.7	32.2	0.17
T_2: Soil + Zn as $ZnSO_4$ (0.5 ppm)	423.0	16.3	44.6	398	137.8	10.7	39.1
T_3: Soil + Zn as $ZnSO_4$ (1 ppm)	5.0	40.7	1606.7	73	987.6	419.9	12.1
T_4: Soil + Zn as $ZnSO_4$ (1.5 ppm)	518.8	82.1	768.0	181	177.5	217.9	45.5
T_5: Soil + Zn as $ZnSO_4$ (2 ppm)	166.1	46.7	109.8	164	156.5	166.2	166.0

Table 31. Effect of treatments on kinetic parameter (V_{max}) of Carbonic anhydrase

Treatments	Carbonic anhydrase (EU g^{-1})						
	V_{max}						
Incubation Period	0^{th} day	14^{th} day	28^{th} day	42^{nd} day	56^{th} day	70^{th} day	84^{th} day
T_1: Soil alone	608.78	497.40	608.78	601.29	608.78	200.56	608.78
T_2: Soil + Zn as $ZnSO_4$ (0.5 ppm)	6.77	529.10	608.78	608.78	608.78	371.60	6.77
T_3: Soil + Zn as $ZnSO_4$ (1 ppm)	414.19	608.78	0.12	674.00	674.00	320.90	414.19
T_4: Soil + Zn as $ZnSO_4$ (1.5 ppm)	271.12	10.48	608.78	14.89	414.19	376.42	271.12
T_5: Soil + Zn as $ZnSO_4$ (2 ppm)	236.38	608.78	608.78	608.78	589.24	35.72	236.38

In the case of Carbonic anhydrase, the K_m value ranged from 0.89 to 16.84 EU g^{-1}. The 0^{th} day and 84^{th} day of incubation registered the same value of K_m in all the treatments. The peak value of 16.84 EU g^{-1} was registered by treatment T_3 with the application of Soil + Zn as $ZnSO_4$ (1 ppm) on the 56^{th} day of incubation. On the 28^{th} day of incubation all the treatments except T_3 with the application of Soil + Zn as $ZnSO_4$ (1 ppm) registered by a value of 15.21 EU g^{-1}. The lowest value of 0.89 EU g^{-1} was registered by treatment T_5 with the application of Soil + Zn as $ZnSO_4$ (2 ppm) on the 70^{th} day of incubation.

Table 32. Effect of treatments on kinetic parameter (K_m) of Carbonic anhydrase

Treatments	Carbonic anhydrase (EU g^{-1}).						
	K_m						
Incubation Period	0^{th} day	14^{th} day	28^{th} day	42^{nd} day	56^{th} day	70^{th} day	84^{th} day
T_1: Soil alone	15.21	12.43	15.21	15.04	15.21	5.01	15.21
T_2: Soil + Zn as $ZnSO_4$ (0.5 ppm)	6.77	14.79	15.21	15.21	15.21	9.29	6.77
T_3: Soil + Zn as $ZnSO_4$ (1 ppm)	10.35	15.21	1.37	16.84	16.84	8.02	10.35
T_4: Soil + Zn as $ZnSO_4$ (1.5 ppm)	6.78	10.48	15.21	14.89	10.35	9.41	6.78
T_5: Soil + Zn as $ZnSO_4$ (2 ppm)	5.91	15.21	15.21	15.21	14.72	0.89	5.91

4.15. STATISTICAL ANALYSIS

4.15.1. Correlation between soil enzymes, plant enzymes and micronutrients

From the correlation matrix (Table 33), it was observed that a positive correlation exits between dehydrogenase and Zn (r= 0.942**), β- glucosidase and Zn (r= 0.803**), carbonic anhydrase and Zn (r= 0.899**). In case of oxido reductases, a significant positive correlation between peroxidase and

Table 33. Correlation between soil enzymes, plant enzymes and micronutrients

	Dehydrogenase	β-glucosidase	Carbonic anhydrase	PEP carboxylase	Carboxy dismutase	Dehydrogenase	Peroxidase	Catalase	Iron	Zinc	Boron	Copper
Dehydrogenase	1.000											
β- glucosidase	0.739**	1.000										
Carbonic anhydrase	0.911**	0.796**	1.000									
PEP carboxylase	0.603*	0.027	0.428	1.000								
Carboxy dismutase	0.546	0.075	0.521	0.823**	1.000							
Dehydrogenase	0.435	0.175	0.325	0.181	0.276	1.000						
Peroxidase	0.954**	0.665*	0.815**	0.674*	0.556	0.476	1.000					
Catalase	0.827**	0.742**	0.732**	0.151	0.159	0.421	0.700**	1.000				
Iron	-0.665	-0.356**	-0.592	-0.578	-0.428**	-0.263**	-0.799	-0.379**	1.000			
Zinc	0.942**	0.803**	0.899**	0.498	0.492	0.542	0.950**	0.726**	-0.686	1.000		
Boron	0.865**	0.687*	0.735**	0.369	0.141	0.321	0.761**	0.869**	-0.437**	0.744**	1.000	
Copper	0.245	0.285	0.378	-0.072**	0.322	0.734**	0.286	0.193	-0.223**	0.469	-0.072**	1.000

Values in correlation coefficients represents r values

*Significant @ 5% ** Significant @ 1% NS: Non significant

Zn (r= 0.950**) and catalase with Zn (r= 0.726**) were observed. A negative correlation is noticed between B and Fe (r= -0.437**) and Cu with Fe (r= -0.223**).

The enzymes *viz.*, plant dehydrogenase, β- glucosidase, carboxy dismutase and catalase exhibited negative correlation with Fe. It is noticed from the correlation matrix that a significant positive correlation exists between carboxy dismutase and PEPcarboxylase (r= 0.823**). Carbonic anhydrase revealed a positive correlation with peroxidase (r= 0.815**) and catalase (r= 0.732**).

4.15.2. Regression between soil enzymes, plant enzymes and micronutrients

Regression analysis carried out between soil enzymes, plant enzymes and micronutrients were presented in Table 34.

The results of the regression analysis revealed that the independent variables *viz.*, soil dehydrogenase (p= 0.001*), peroxidase (p= 0.001*) and catalase (p= 0.041*) are significantly related with the dependent variable Zn.

Out of the parameters studied, it was observed that carbonic anhydrase (p= 0.038*), peroxidase (p= 0.028*) and catalase (p= 0.012*) exhibited a significant relationship with micronutrient B.

The plant enzyme peroxidase exhibited a significant relation with Fe (p= 0.017*) while dehydrogenase (plant) enzyme revealed a significant functional relation with Cu (p= 0.038*).

Table 34. Regression coefficients between soil enzymes, plant enzymes and micronutrients

Sl. No.	Parameters	Iron	Zinc	Boron	Copper
1	Dehydrogenase (soil)	0.072	0.001*	0.006	0.558
2	β- glucosidase	0.386	0.0164	0.060	0.494
3	Carbonic anhydrase	0.122	0.495	0.038*	0.356
4	PEP carboxylase	0.133	0.209	0.369	0.865
5	Carboxy dismutase	0.290	0.215	0.738	0.436
6	Dehydrogenase	0.529	0.165	0.438	0.038*
7	Peroxidase	0.017*	0.001*	0.028*	0.493
8	Catalase	0.414	0.041*	0.012*	0.585
	R^2	1.046	1.155	1.126	1.632

Values in regression coefficients represents P values

* Significant @ 5%

DISCUSSION

5. DISCUSSION

The investigation entitled "Effect of zinc fertilization on major plant and soil enzymes in southern laterites" has been carried out in the Department of Soil Science and Agricultural Chemistry, College of Agriculture, Vellayani during June, 2017 to September, 2018. The present study was undertaken to study the effect of various sources and methods of application of zinc on the activities of major plant enzymes, soil enzymes, biochemical and microbial parameters in laterite soils using tomato (*Solanum lycopersicum*) as a test crop. A brief interpretation of results relevant to the field study conducted is presented in this chapter.

5.1. EFFECT OF TREATMENTS ON GROWTH CHARACTERISTICS OF TOMATO

The growth characteristics were significantly influenced by the treatments. The treatment T_7 corresponds with the application of N, P, K+ Zn Humate (44 kg ha^{-1}) recorded the highest values for number of branches per plant, days to first flowering, fruits per plant and fruit length (Fig 3.). The positive effects of humic substances and Zn could have resulted in the growth parameters of tomato. The humic substances released during the dissociation of Zn humate forms complex with other nutrients and enhanced the nutrient uptake. Similar results were also reported by (Yildirim, 2007; Karakurt *et al.*, 2009; Elayaraja and Singaravel, 2017).

The humic acid thus produced might have influenced the vegetative phase of tomato by stimulating the production of plant enzymes and hormones. Plant growth regulators such as indole acetic acid, gibberellins and cytokinins extracted from humic acid had significant effects on plant growth (Atiyeh *et al.*, 2002). Similar results were also reported by (Sarir *et al.*, 2005; Mart, 2007)

Application of Zn might have also contributed to the enhanced vegetative growth by improving the photosynthetic and metabolic activities. Zinc is a major component of chlorophyll, nucleotides and enzymes that are involved in various metabolic processes which had a direct effect on vegetative phase of plants (Elayaraja and Singaravel, 2017). Similar increase in vegetative growth with the

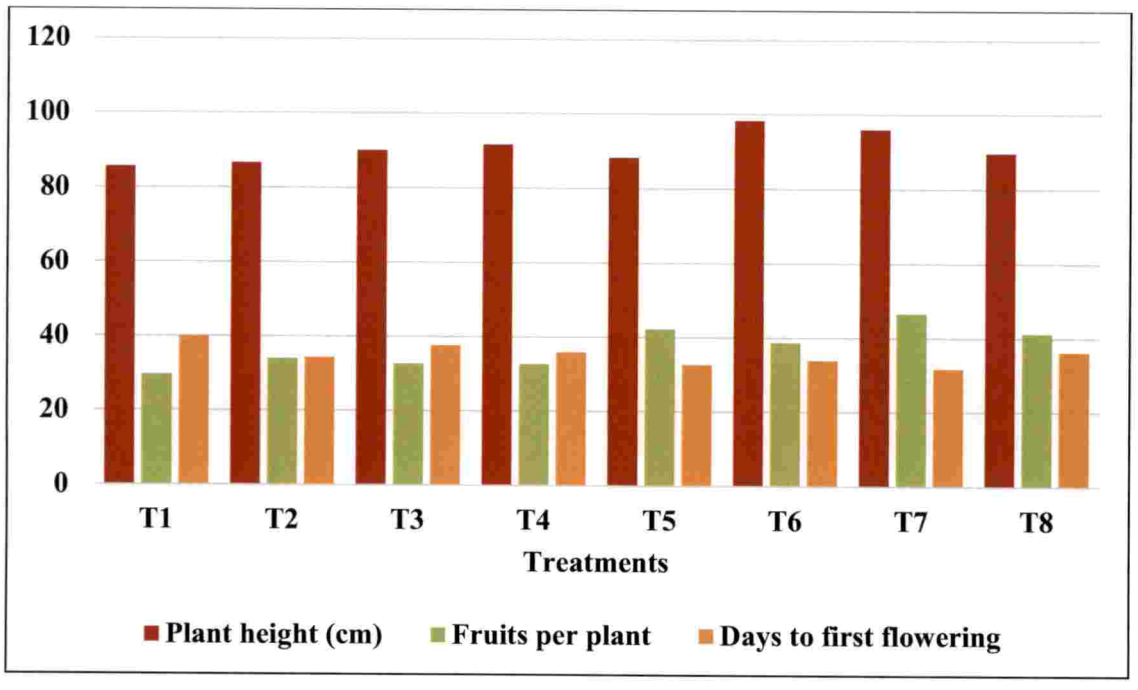

Fig 3. Effect of treatments on plant biometric characters

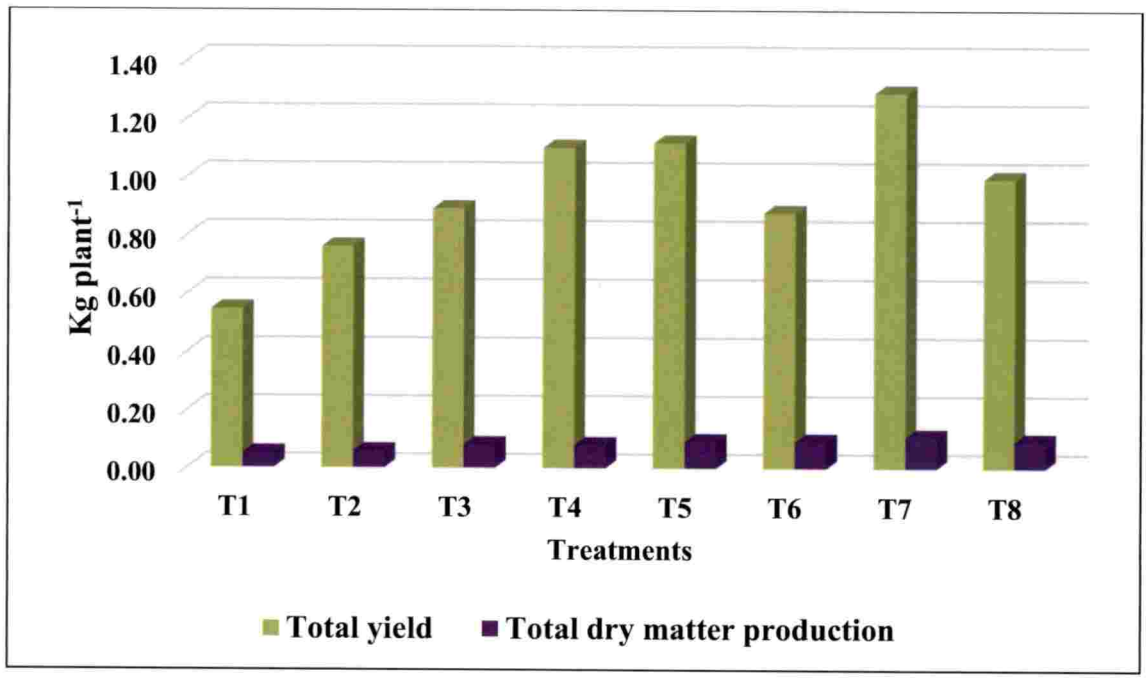

Fig 4. Effect of treatments on total yield and total dry matter production

addition of Zn was reported by Khan *et al.* (2000) in legumes. Also Zn is involved in the metabolism of of plant hormones such as auxin whose activity declined in Zn deficiency condition, confirming that Zn can activate auxin formation which is capable of accelerating the growth parameters (Marschner, 1995)

The increase in number of branches might be due to the higher nutrient use efficiency, photosynthetic rates and N uptake efficiency with the application of Zn humate. This is in accordance with the findings of Naruka *et al.* (2000). The short duration of flowering might be attributed by the activation of Zn- finger transcription factors that were involved in the development and functioning of floral tissues such as anthers, tapetum, pollen and pistil secretory tissues. These results corroborated with the findings of Kobayashi *et al.* (1998).

5.2. EFFECT OF TREATMENTS ON YIELD CHARACTERISTICS OF TOMATO

The total yield and yield attributes were significantly influenced by the different treatments. The treatment T_7 with the application of N, P, K+ Zn Humate (44 kg ha^{-1}) recorded the highest values for total yield and dry matter production per plant. The increase in yield and dry matter production might be due to the enhanced availability of macro and micronutrients in Zn-Humate. The optimum availability of nutrients increased the plant metabolites, which were responsible for photosynthesis, cell division and elongation (Datir *et al.*, 2010). Similar results were reported by Abdel Monaim *et al.*, 2012; Aman and Rab, 2013; Kazemi, 2013, 2014; Asri *et al.*, 2015; Farnia and Moradi, 2015.

The maximum dry matter accumulation might be due to the increased plant height, maximum number of branches, greater nutrient availability and increased photosynthetic rate. Zn-humate derived from humic acid improved the translocation of nutrient cations within the plant system and nutrient use efficiency by providing more balanced supply of nutrients (Rady, 2011). Similar results were reported by Anburani and Manivannan (2002).

Increase in yield might be possibly due to the balanced application of N, P and K which might have improved the N and P efficiency in the presence of K and Zn resulting in better reproductive growth. The increase in activity of carbonic anhydrase might have also led to the betterment in yield and dry matter production as this plays an important role in photosynthesis and biomass production. These observations are similar with the findings of Ohki, 1976; Dell and Wilson, 1985.

5.3. EFFECT OF TREATMENTS ON PHOTOSYNTHETIC ENZYMES

The treatments had significantly influenced the plant enzymes carbonic anhydrase, PEP carboxylase and Carboxy dismutase. In general, an increase in activity of these enzymes were noted in treatments applied with Zn sources. Zn is an essential constituent of carbonic anhydrase, the main function of these enzyme is to catalyses the reversible hydration of carbon dioxide to bicarbonate and hydrogen ions (Graham and Reed, 1971). This might have increased the photosynthetic CO_2 fixation along with this, Zn also have an important role in chlorophyll and carotenoid metabolism (Kumar *et al.*, 1976). Thus an increase in carbonic anhydrase along with photosynthetic pigments might have triggered an increase in activity of PEP carboxylase (Fig. 5) and carboxy dismutase thereby exhibiting a positive correlation between Zn and plant enzymes (Table 11). Similar results were also reported by Ohki (1976) and Shotri *et al.* (1983).

5.4. EFFECT OF TREATMENTS ON OXIDO- REDUCTASES IN PLANT TISSUE

From the study, it was observed that the treatments had imposed a significant effect on oxido- reductases such as dehydrogenase, peroxidase and catalase. These enzymes showed an increase in activity during the peak stage of fruiting. It was noticed that the treatment T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1}) recorded the highest value of dehydrogenase. This might be due to the role of Zn in enhancing the activity of dehydrogenases like alcohol dehydrogenase, glutamic dehydrogenase, D-glyceraldehyde-3-phosphate dehydrogenase and malic dehydrogenase. The increased rate of Zn assimilation, N metabolism,

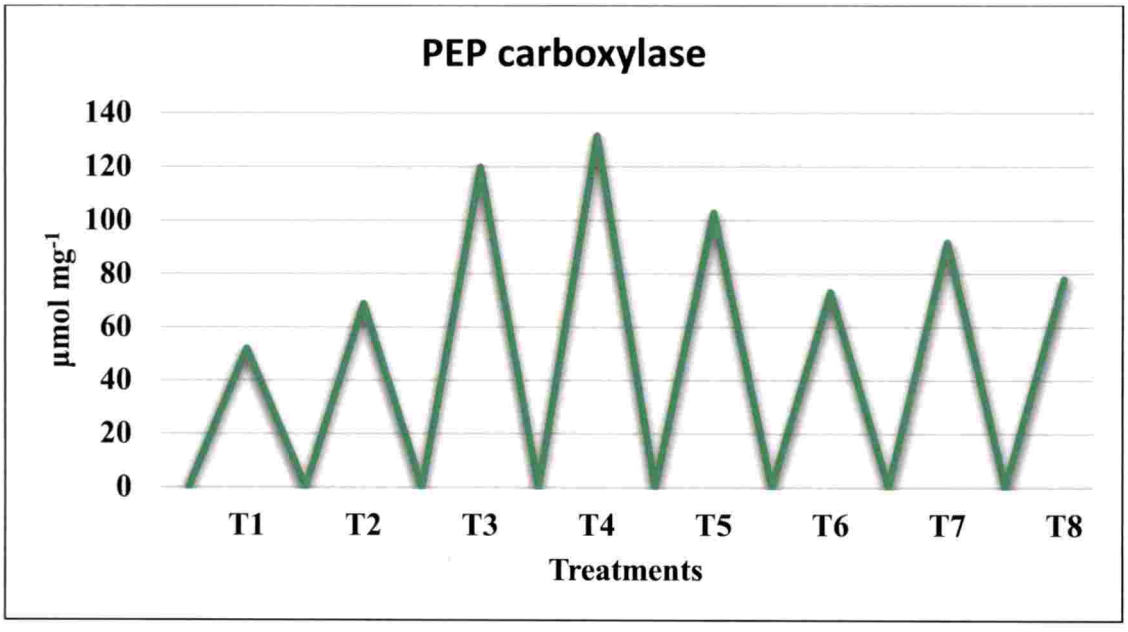

Fig 5. Activity of PEP carboxylase

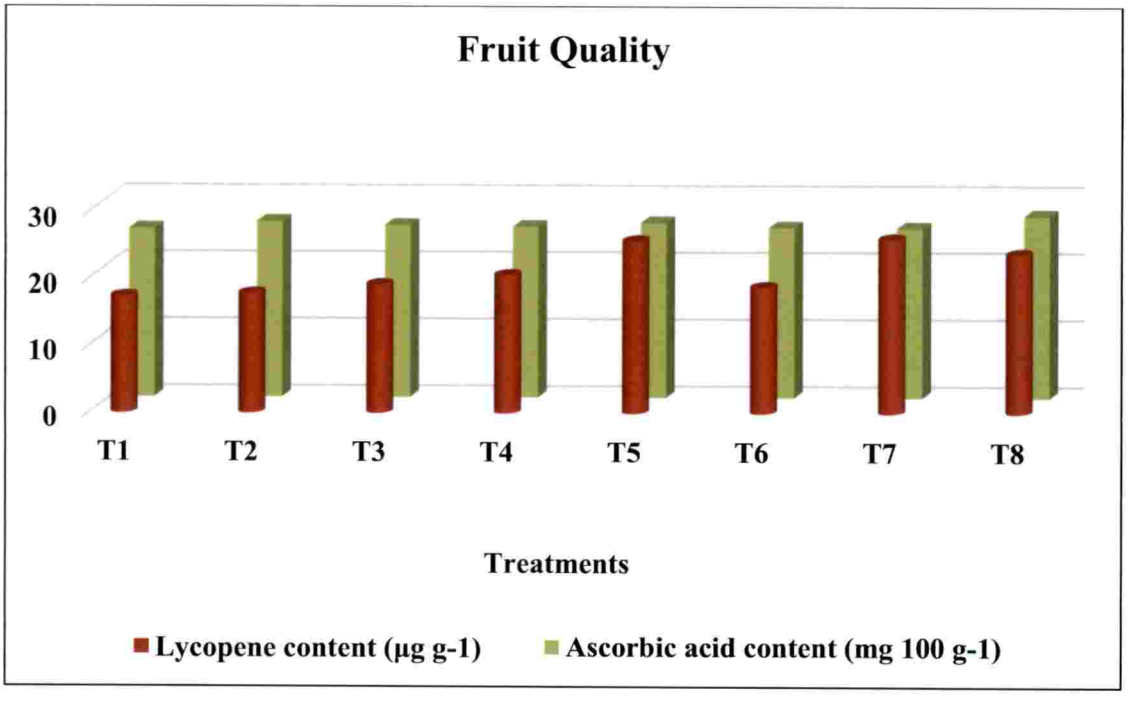

Fig 6. Effect of treatments on fruit quality

photosynthesis, respiration and carbohydrate metabolism might have also triggered an increase in dehydrogenase activity. Similar observations were recorded by Moore and Patrick (1988).

From the study, it was observed that the treatment T_7- N, P, K+ Zn Humate (44 kg ha^{-1}) registered the highest value for peroxidase and catalase. Peroxidase and catalase constitute the H_2O_2 scavenging system in cells. The high peroxidase and catalase activity might be due to the exposure to Zn. This is in accordance with the findings of Jain *et al.* (2010). The excess of Zn might have triggered an intensive oxidative stress along with this, humic acid activated several biochemical processes which resulted in increased enzymatic activity. These metabolic changes led to an increase in the concentration of catalase and peroxidases. This findings corroborated with the results of Bertrand and Poirier (2005).

5.5. EFFECT OF TREATMENT ON PLANT PIGMENT, PLANT GROWTH REGULATORS AND FRUIT QUALITY

From the study it was noticed that the treatments had a significant influence on the plant growth regulator (IAA) and fruit quality. With respect to plant pigment the treatments showed a non significant effect. Treatment T_7- N, P, K+ Zn Humate (44 kg ha^{-1}) recorded the highest value for IAA and lycopene.

The increase in IAA might be due to the combined effect of humic acid and Zn. Humic acid can hinder the oxidative destruction of IAA by IAA oxidase while Zn is required for the synthesis of tryptophan which is a precursor of IAA. This is in accordance with the findings of Brennan (2005). Treatment T_7- N, P, K+ Zn Humate (44 kg ha^{-1}) registered the highest N uptake. The increase in lycopene content might have been due to increase in nitrogen content. Similar observations were reported by Montagu and Goh (1990).

From the investigation carried out, it was observed that T_8- N, P, K+ K solubilizer (5%) registered the highest value for ascorbic acid. Increased K uptake might have led to the increased production of mannose which serves as the primary

substance for the synthesis of ascorbic acid. This is in conformity with the findings of Guha and Ghosh (1935) and Wheeler *et al.* (1998).

5.6. EFFECT OF TREATMENT ON ELECTRO CHEMICAL PROPERTIES OF SOIL

In general, an increase in soil pH and EC were observed with the application of treatments. The increase in pH might be due to the combined effect of agricultural lime along with farm yard manure. Manure could reduce the concentrations of aluminium, manganese and iron ions through complex formation (Narambuye and Haynes, 2006). Increase in pH due to the application of lime might be attributed to the neutralization of hydrogen ions by hydroxyl group resulting in the formation of water whereas the calcium ions resulted in the formation of complex with aluminium, iron and manganese (Anderson *et al.*, 2013). Similar results were reported by Khoi *et al.* (2010) and Kisiniyo *et al.* (2012)

Treatment T_7 with the application of N, P, K+ Zn Humate (44 kg ha^{-1}) recorded the highest value for EC. The increase in EC might be due to the release of protons and exudations from roots while humic acid might have enhanced the formation of complexes with metal ions, hydroxides and oxides by the processes of ion exchange, surface-adsorption, chelation, coagulation, and peptization and thereby promoted the total soluble salt content of the soil. These results corroborated with the findings of Boguta and sokolowska, 2016.

5.7. AVAILABLE MAJOR NUTRIENT STATUS OF THE SOIL

In the case of major nutrients, the treatments had a significant influence on available P and K but the effect was non significant on available N (Fig. 7). Treatment T_3- N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1}) and T_7- N, P, K+ Zn Humate (44 kg ha^{-1}) registered the highest value for available N. Application of inorganic nitrogen source might have promoted N mineralization, leading to the buildup of available N content. Similar results were also reported by Sharma and Gupta (1998). Combination of fertilizers with manures might have also led to the

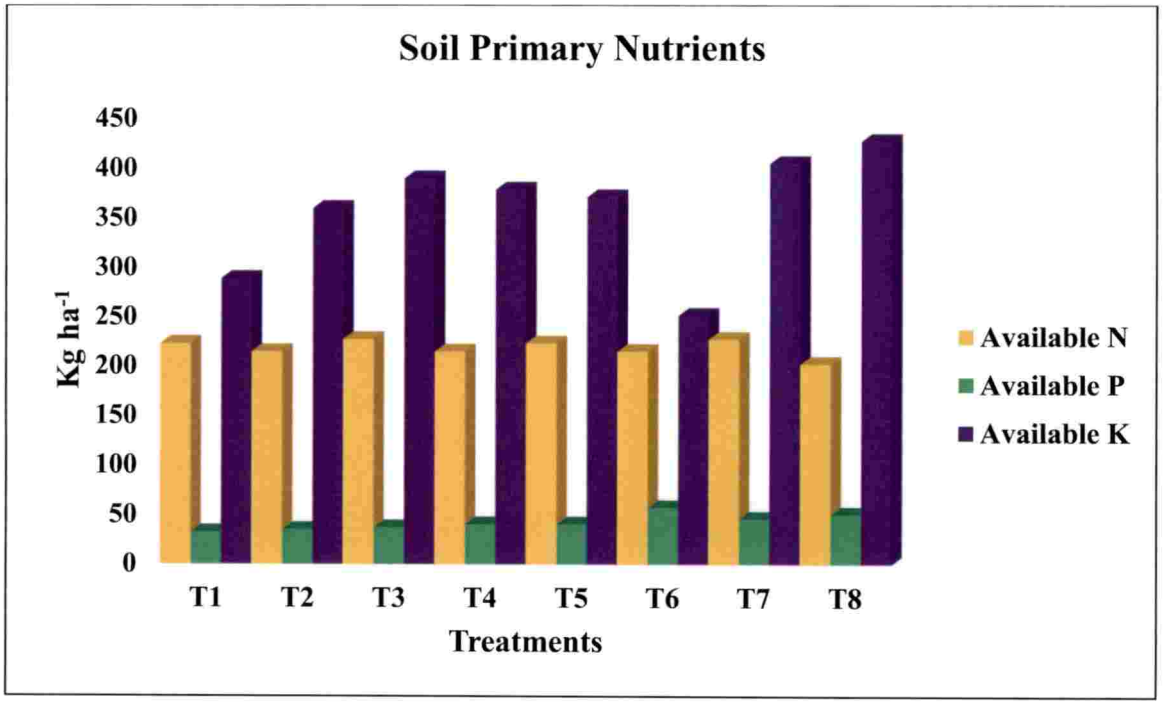

Fig 7. Soil primary nutrient status

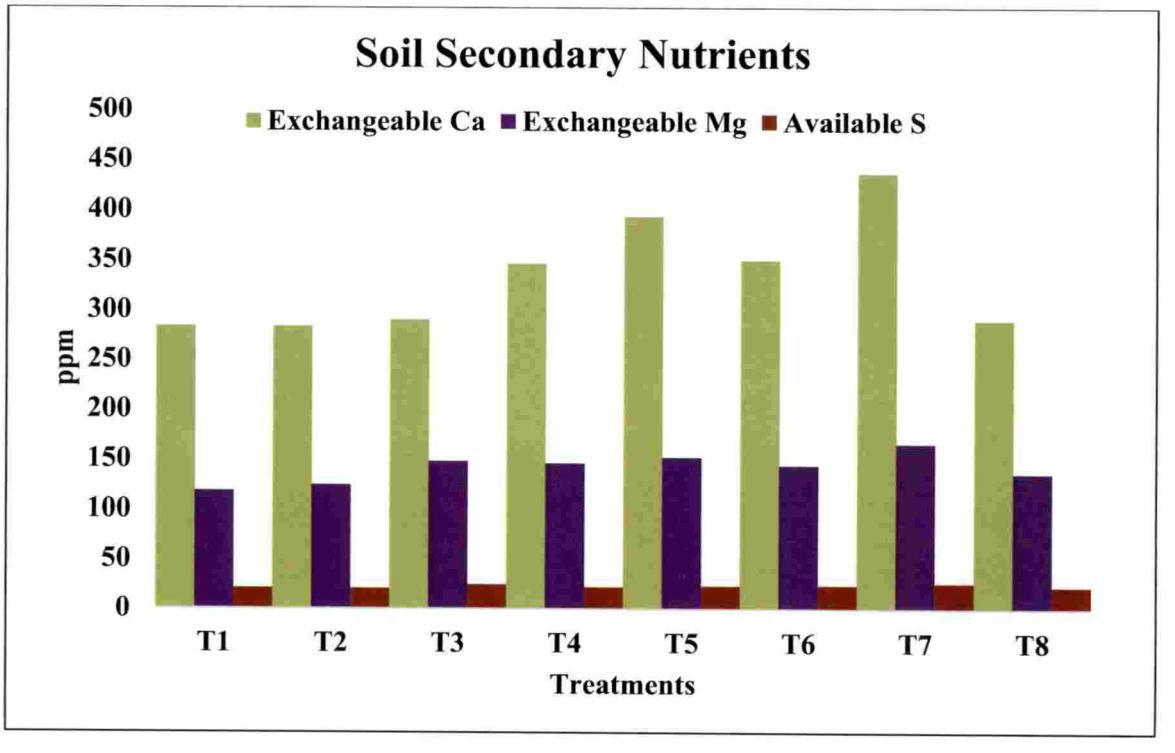

Fig 8. Soil secondary nutrient status

higher synthesis of microbial biomass, which promoted the release of N by mineralization. This were in agreement with the findings of Banerjee *et al.* (2006).

Phosphorus plays an important role in crop growth and yield, particularly in the development of root growth. The higher content of available P in the experimental soil might be due to the application of higher quantity of P fertilizers which would have masked the antagonist effect of Zn. This result is in accordance with the findings of Singh *et al.* (1986).

From Table 16 it was observed that treatment T_6- N, P, K+ Zn solubilizer (5 %) registered the highest value for available P. This might be due to the solubilization of P by the production of organic acids. The production of organic acids like gluconic acid, proton extrusion and production of chelating agents accelerated the solubilisation of P by Zn solubilizer (Di Simine *et al.,* 1998). Similar results were reported by Goteti *et al.* (2013).

Potassium is an essential element for the growth of plants and are required in large quantities during the life cycle. It is needed for the movement of water and carbohydrates in the plant tissue, opening and closing of stomata and helps in imparting resistance to the plant diseases. From the study, an increase in available K was observed with the application of treatments. This might to be due to the addition of K fertilizer like Muriate of Potash. Similar observations were also made by Kumar *et al.* (2007).

From Table 16, it was observed that treatment T_8- N, P, K+ K solubilizer (5%) recorded the highest value for available K and was found to be on par with T_7- N, P, K+ Zn Humate (44 kg ha^{-1}). This might be due to the multiplication of K solubilising microorganisms, *Bacillus* and *Pseudomonas* species which are capable of releasing K from K bearing minerals. K solubilizers can dissolve silicate minerals and convert the insoluble K to soluble forms by the production of organic and inorganic acids, acidolysis, polysaccharides, complexolysis, chelation, and exchange reactions. This result corroborated with the findings of Etesami *et al.* (2017).

5.8. AVAILABLE SECONDARY NUTRIENT STATUS OF THE SOILS

Calcium, it is a component of cell wall and is mainly involved in cell nucleus formation and metabolism. Generally, satisfactory levels of exchangeable Ca was observed in the experimental site. Application of lime in soil might have resulted in better availability of Ca in all the treatments irrespective of the antagonistic effect of Zn. It is obvious from the study that treatment T_7- N, P, K+ Zn Humate (44 kg ha^{-1}) recorded the highest value for exchangeable Ca. This might be due to the production of humic acid. The humic substances released by the dissociation of Zn humate can act as a source of nutrients for microorganisms (Tikhonova *et al.* 2010). These microorganisms might have produced exudates with a special pH which are capable of binding and extracting nutrients like phosphorus, calcium, and potassium. These results corroborated with the findings of Kudrina (1951).

Magnesium is required for the production of sugars, oils and fats. It is also an integral part of chlorophyll which is needed for photosynthesis. With regard to exchangeable Mg, the highest values were given by treatment T_7- N, P, K+ Zn Humate (44 kg ha^{-1}). Increase in exchangeable Mg might be due to the formation of humic substances that were capable of chelating cations such as magnesium (Mg^{2+}) and calcium (Ca^{2+}). Additions of farm yard manure along with humic substances might have also contributed to higher CEC there by higher Mg content. This is in agreement with the findings of Lakshmi *et al.* (2007).

It was noticed that T_7- N, P, K+ Zn Humate (44 kg ha^{-1}) registered the highest value for available S and it was on par with T_3- (N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1}) and T_6 (N, P, K+ Zn solubilizer (5 %) (Fig 8.). The increased S content may be attributed to the proliferation of microorganisms that are capable of decomposition of organic materials leading to the production of ammonia and hydrogen sulphide that can be later oxidized to form sulfuric acid (H_2SO_4) leading to the buildup of available S content in the soil. Similar results were also reported by Huang *et al.* (2013) and Etesami *et al.* (2017).

5.9. AVAILABLE MICRONUTRIENT STATUS OF THE SOIL

Iron is a universal element, which act as an electron carrier in respiration, photosynthesis and oxygen transport. From the present investigation it was observed that there was a general decrease in available Fe in the soils treated with Zn fertilizers (Table 18). This might be due to the antagonist effect of Fe and Zn resulting in low Fe content.Similar reports regarding the antagonist effect of Fe and Zn were reported by Sadana and Takkar (1983); Agib and Jarcass (2008).

The treatment T_1- Absolute control registered the highest value for Fe and was found to be on par with treatment T_8- N, P, K+ K solubilizer (5%). This might be due to the solubilisation of Fe with the production of organic acids which are capable of acidifying the micro environment through the sequesteration of cations. This is in accordance with the findings of Welch (1995).

Zinc is a key component of many enzymes and plays an important role in plant metabolic processes viz., hormone production and internode elongation. With regard to available Zn, it was observed from Fig. 9, that treatment T_7- N, P, K+ Zn Humate (44 kg ha^{-1}) recorded the highest value which was statistically on par with T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1}). This might be due to the low interaction of Zn with soil components, preventing reactions like fixation, adsorption etc. The low interaction was attributed by the bonding of Zn in the chelate rings which might have led to the loss of their cationic characteristics (Giordano and Morvedt, 1973). The results were in agreement with the findings of Chatterjee and Mandal (1985).

From the study, it was noticed that treatments had significantly influenced the available B. Even though the available B content is still deficient in the soil, it was treatment T_7- N, P, K+ Zn Humate (44 kg ha^{-1}) which recorded the highest value. This might be due to the high number of sorption sites in humus. Humic substances were considered to have an important role in B adsorption (Goldberg, 1997). The formation of complex between B and dihydroxy organic compounds present in humus might have resulted in the adsorption of B, thus increasing their

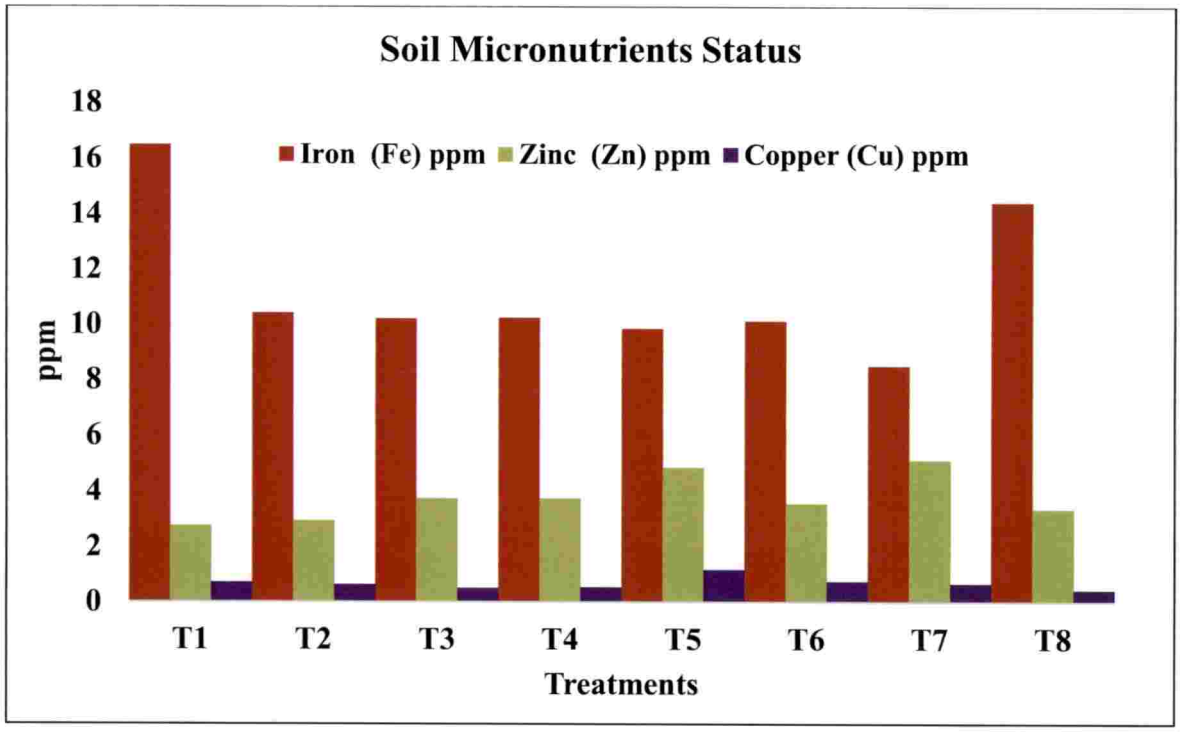

Fig 9. Soil micronutrient status

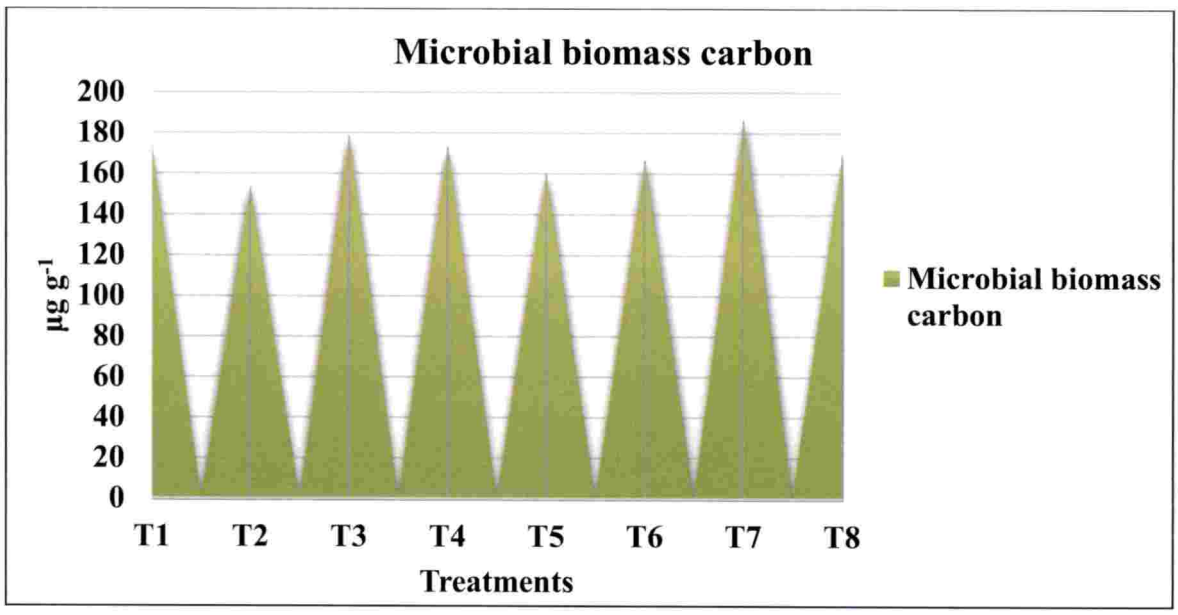

Fig 10. Microbial biomass carbon status

availability in the soil. Similar results were also reported by Raza *et al.*, 2002; Shafiq *et al.*, 2008.

Copper activates many enzymes and is essential for the synthesis of carbohydrates, proteins and lignin. From the study, it was observed that the treatments had significantly influenced the available Cu content. Treatment T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1}) recorded the highest value for available Cu. This might be attributed to the production of organic acids, pH change, release of soluble chelates and enzymes from the decomposing foliage and root secretions.

5.10. MICROBIAL PARAMETERS

5.10.1. Soil Respiration Rate

Soil respiration refers to the CO_2 released as a result of biological activity of soil organisms, including root respiration, microbial decomposition of litter and soil organic matter, mycorrhizal exploration and soil animals. It is a barometer of soil metabolic activity (Phillips and Nickerson, 2015). The treatment T_7- N, P, K+ Zn Humate (44 kg ha^{-1}) recorded the highest value for soil respiration rate which was comparable with T_4-N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$ (Table 19).This might be due to the role of Zn in supporting the growth and functioning of microorganisms, where it serves as the structural proteins and pigment, maintains the ionic balance in redox processes, regulates the osmotic pressure and enzyme component of cells. These results were in conformation with the findings of Kosolapov *et al.*, 2004.

5.10.2. Microbial Biomass Carbon Status (MBC)

Microbial biomass carbon is the quantity of carbon contained within the living component (bacteria and fungi) of soil organic matter. Therefore it is an early indicator of changes in total organic carbon content (Anderson and Domsch, 1989). It is evident from the data present in the Table 19 that the MBC content is significantly influenced by the treatments. Treatment T_7- N, P, K+ Zn Humate (44 kg ha^{-1}) recorded the highest value which was comparable with T_3- N, P, K+ Soil application of Zn as $ZnSO_4$ (10 kg ha^{-1}), T_4-N, P, K+ Foliar application of Zn

as 0.5% $ZnSO_4$ and T_1- Absolute control. The addition of organic material might have improved the relative abundance of C content in macro aggregate fractions, thus acting as a substrate for microbial population. Similar results were reported by Das *et al.* (2014); Bashir *et al.* (2015) and Khan and Parvej (2010).

5.11. SOIL ENZYME STATUS

5.11.1. Dehydrogenase

Dehydrogenase is an enzyme capable of oxidising soil organic matter by transferring protons and electrons from substrate to receptors. Burns (1982) stated that dehydrogenase activity was used as an indicator of biological activity. From the study it was observed that the treatments had a significant influence on dehydrogenase activity. With respect to main effects T_7- N, P, K+ Zn Humate (44 kg ha^{-1}) was observed to be the best treatment as far as soil dehydrogenase activity values are concerned. This might be due to the activity of humic substances which have acted as a suitable substrate for microbial proliferation thereby showed an increased dehydrogenase activity. Similar results were reported by Geethakumari and Shivashankar, 1991. The stabilization of extracellular enzymes through complexation with humic substances might have resulted in greater dehydrogenase activity in soil.

The addition of Zn souces might have resulted in the multiplication of microbial population and thereby an increase in dehydrogenase activity. A positive correlation with Zn and soil dehydrogenase (r= 0.942**) also supports this result. The increase in dehydrogenase activity might be linked to the availability of more substrate (organic matter and Zn) availability. These results corroborated with the findings of Basak *et al.*, 2013.

5.11.2. β- glucosidase

β- glucosidase is a rate limiting enzyme involved in microbial degradation of cellulose to glucose. It is an important source of C energy for microorganisms in the soil (Esen, 1993).

In the study it was noticed that a significant effect in β- glucosidase activity was observed due to the treatments. The highest β-glucosidase activity was noticed in treatment T_7- N, P, K+ Zn Humate (44 kg ha^{-1}).The highest value might be due to the humus substance in Zn humate, which have improved organic carbon content and microbial colonization favouring the release of β- glucosidase. This is in accordance with the findings of Dick, 1994. The availability of Zn and FYM as a potential substrate source might have enhanced the activity of β- glucosidase. A positive correlation of Zn and β- glucosidase (r= 0.803**) also supported this result.

5.12. NUTRIENT UPTAKE STATUS

5.12.1. Uptake of Primary Nutrients

From the Fig. 12, it is observed that, the uptake of nutrients were significantly influenced by the treatments. Treatment T_7 with the application of N, P, K+ Zn Humate (44 kg ha^{-1}) recorded the highest value for N content and uptake. This might be due to the combined effect of humus substances and Zn. The higher nitrogen absorption might be due to the stimulatory effect of zinc on nitrogen uptake, as it is involved in nitrogen fixation and translocation to plant parts. Similar results were also reported by Jeanine *et al.* (2003) and Swami and Shekhawat (2009). Humic substances were able to activate enzymes involved in N assimilation and Krebs cycle. This might have also attributed to the increased N uptake. The results were in agreement with the findings of Vaccaro *et al.*, 2015.

With respect to phosphorus and potassium uptake, treatment T_8- N, P, K+ K solubilizer (5%) recorded the highest value. This might be due to the production of organic and inorganic acids, acidolysis, complexolysis, chelation, production of polysaccharides and exchange reaction by potassium solubilizing bacteria (KSB) in K and P bearing minerals. Similar results were also reported by Lin *et al.* (2002) and Badar *et al.* (2006)

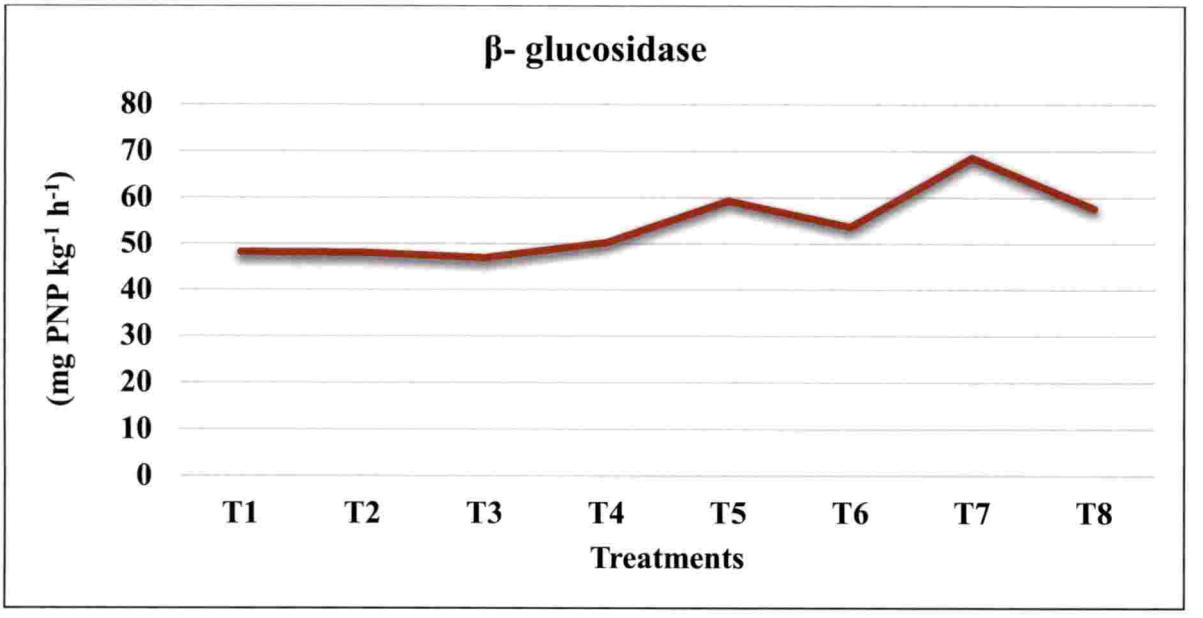

Fig 11. Activity of β- glucosidase

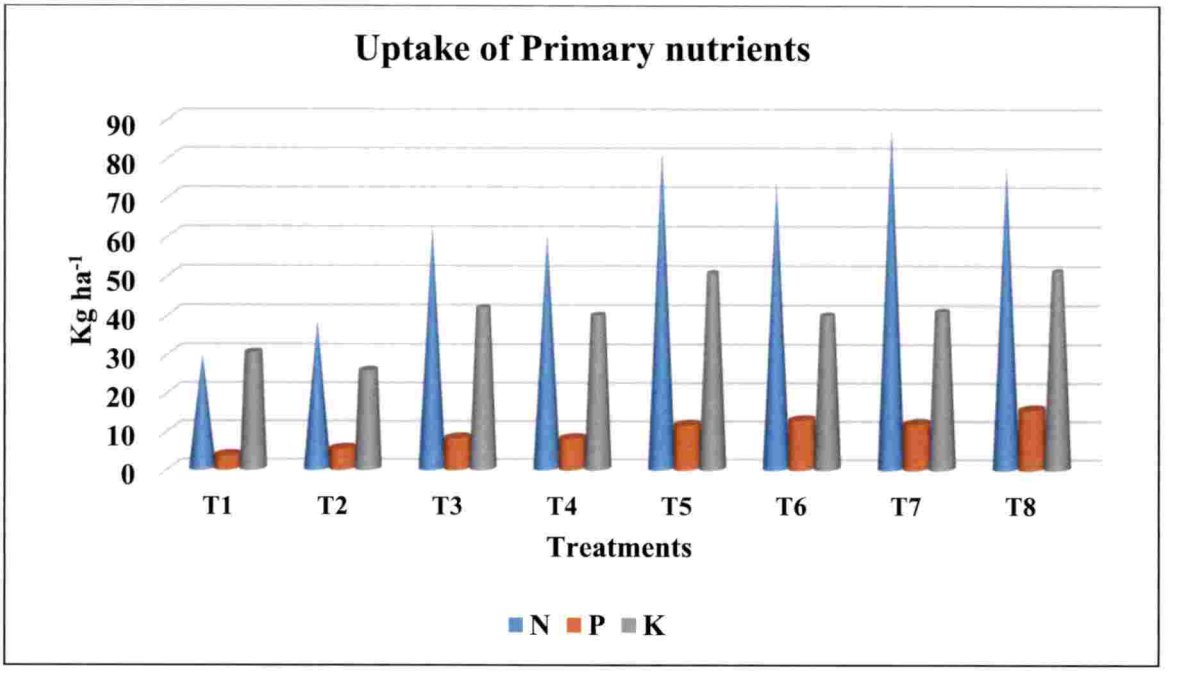

Fig 12. Status of uptake of primary nutrients

5.12.2. Uptake of Secondary Nutrients

From the Fig 13, it was noticed that the treatments had a significant influence on Ca uptake. It was also observed that application of Zn fertilizer decreased the Ca uptake. This might be due to the antagonistic effects of Ca and Zn. Similar results were also reported by Davis- Carter *et al.* (1991).

The highest Ca uptake was reported by T_4-N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$ which was found to be on par with T_7- N, P, K+ Zn Humate (44 kg ha^{-1}). An increase in pH and application of agricultural lime might have promoted the better uptake of Ca by plant roots. In the case of Mg, the treatments had significantly influenced the Mg uptake. This might be due to the positive interaction of Zn with Mg. This result corroborated with the findings of Merrill *et al.* (1953). Along with this the accumulation of sediments and organic matter as the field was near to Vellayani lake might have improved the cation exchange capacity and thereby increased the uptake of Ca and Mg.

The highest value for Mg uptake was reported by T_7- N, P, K+ Zn Humate (44 kg ha^{-1}) followed by T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1}). This might be due to the chelating effect of humate and EDTA since chelates can be easily adsorbed by the plant roots because of their organic nature. These results are in conformation with the findings of Sekhon, 2003.

The treatments show a significant effect on S uptake and the treatment T_7- N, P, K+ Zn Humate (44 kg ha^{-1}) recorded the highest value. This might be due to the positive interaction of S and Zn. Similar reports were given by Cui and Wang (2005). S is mainly taken up by the plant in the form of SO_4^{2-}. The decomposition of organic materials such as humic substances which were capable of producing ammonia and hydrogen sulphide might have under gone oxidation to give SO_4^{2-}, resulting in increased uptake of S. This is in conformation with with the findings of Coolong and Randle, 2003.

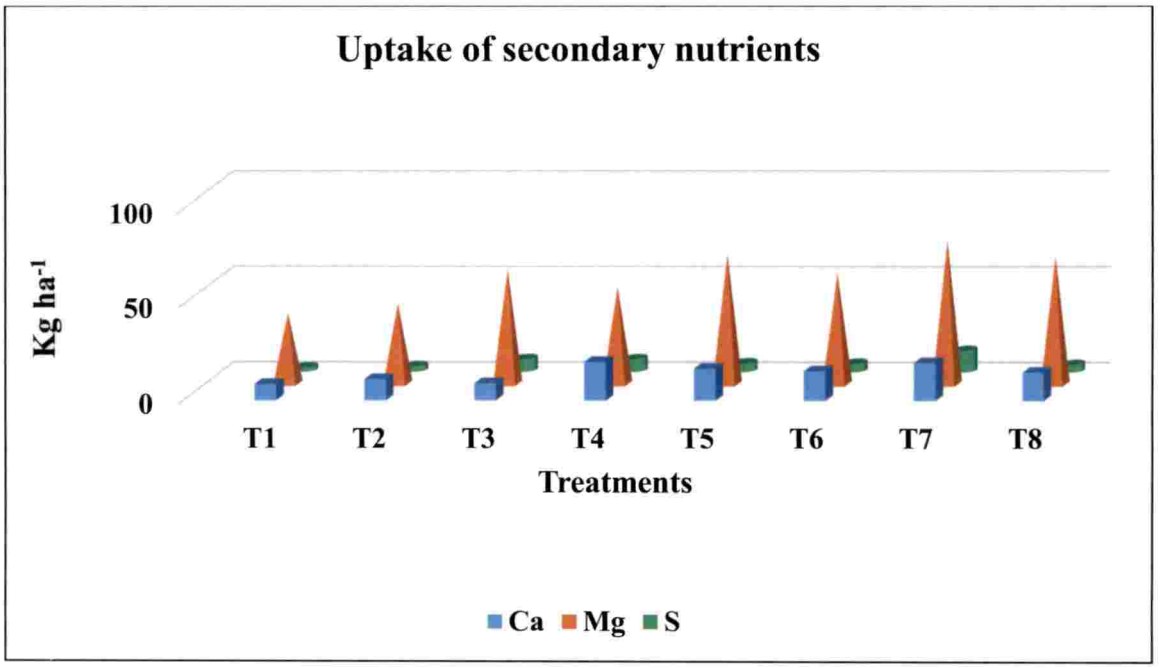

Fig 13. Status of uptake of secondary nutrients

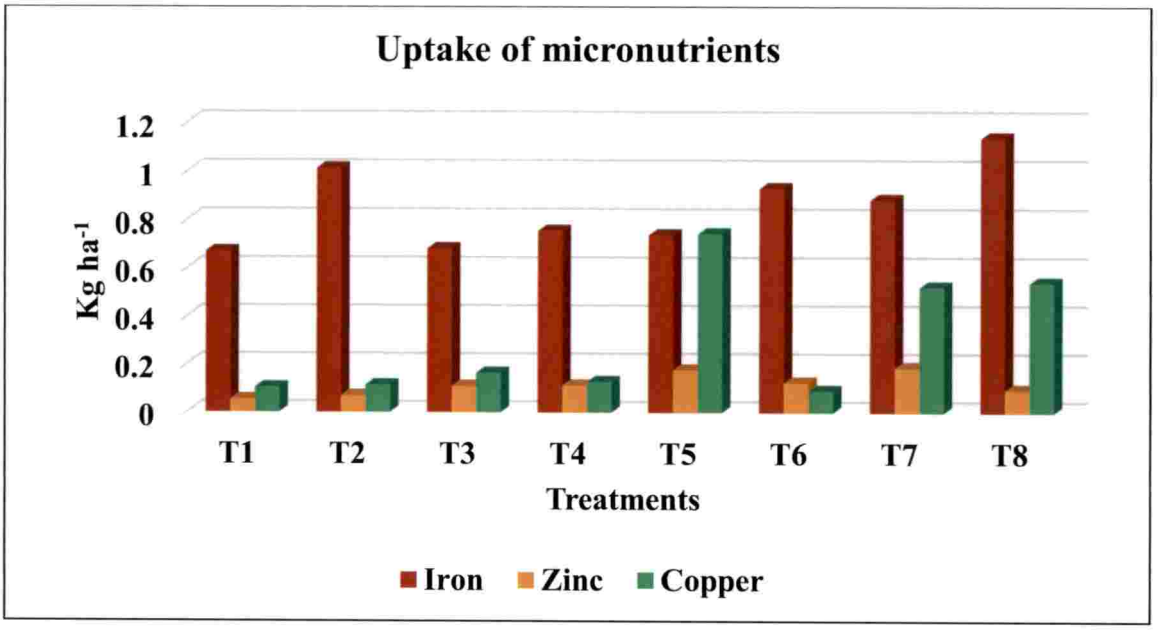

Fig 14. Status of uptake of micronutrients

5.12.3. Uptake of Micronutrients

From the study conducted it was observed that the treatment T_8- N, P, K+ K solubilizer (5%) recorded the highest value for Fe. This might be due to the increased photosynthetic and enzyme activity favouring the higher concentration of Fe in plant. Similar results were reported by Chaturvedi *et al.*, (2010). The production of phytohormones by the bacteria, along with the weathering agents, activated root development that modified the root physiology and root exudation which might have enhanced the nutrient uptake. This is in accordance with the findings of Gahoonia *et al.* (1997).

The treatments significantly influenced the Zn uptake (Fig. 14) and the highest value was recorded by T_7- N, P, K+ Zn Humate (44 kg ha^{-1}) followed by T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1}). This might be due to their chelating nature of Zn humate and Zn EDTA and these chelates can be easily translocated through the pores of leaf and root surfaces, as their mode of action is partly systemic. These results were in accordance with the findings of Sekhon (2003).

Among the micronutrients, uptake of B is significantly influenced by the treatments. The highest value was recorded by treatment T_7- N, P, K+ Zn Humate (44 kg ha^{-1}) followed by T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1}). This might be due to the positive interaction between B and Zn. A positive correlation was obtained for Zn and B ($r= 0.744^{**}$). This might be due to the chelating effect of humus and EDTA that might have enhanced the availability of B. Similar results were reported by Sinha *et al.* (2000).

The treatment T_4-N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$ recorded the highest value for Cu uptake. High microbial biomass carbon might have enhanced the microbial population which were capable of producing siderophores which have high affinity for Cu. These observations were I conformation with the results of Singh *et al.*, 2005.

5.13. EFFECT OF ZN ON ENZYMATIC ACTIVITY

A lab incubation study was carried out to assess the effect of different dosages of Zn on enzymes such as Oxido reductases (dehydrogenase and peroxidase) and Carboxylases (Carbonic anhydrase).

From the study it was observed that dehydrogenase activity was significantly influenced by Zn (Fig.15). A general increase in the activity was observed. This might be due to the addition of FYM. FYM acted as a substrate which provided carbon and energy to the soil microbes resulting in higher dehydrogenase activity. This results corroborated with the findings of Walls-Thumma, (2000).

The highest mean value for dehydrogenase activity was given by T_5-Soil + Zn as $ZnSO_4$ (2 ppm). The addition of Zn souces might have resulted in the multiplication of microbial population and thereby an increase in dehydrogenase activity. A positive correlation with Zn and soil dehydrogenase (r= 0.942**) also supports this result.

It is obvious from the study, that Zn had a significant influence on peroxidase activity (Fig. 16). There was a general decrease in peroxidase activity due to application of Zn. This might be due to the limited generation of O^{2-} and H_2O_2 in Zn sufficient soil.

Treatment T_4: Soil + Zn as $ZnSO_4$ (1.5 ppm) reported the highest value for peroxidase activity. This might be due to the slightly acidic pH provided by 1.5 ppm $ZnSO_4$, as it becomes completely inactive at pH 2.5 and $\geq$ 8.5. Similar results were reported by Mizobutsil *et al.* (2010).

In the case of carbonic anhydrase, a significant influence in activity was observed two month after the application of Zn. There is a general increase in Carbonic anhydrase activity which might be due to the limited production of oxidizing agents. Similar results were reported by Kiese and Hastings (1940).

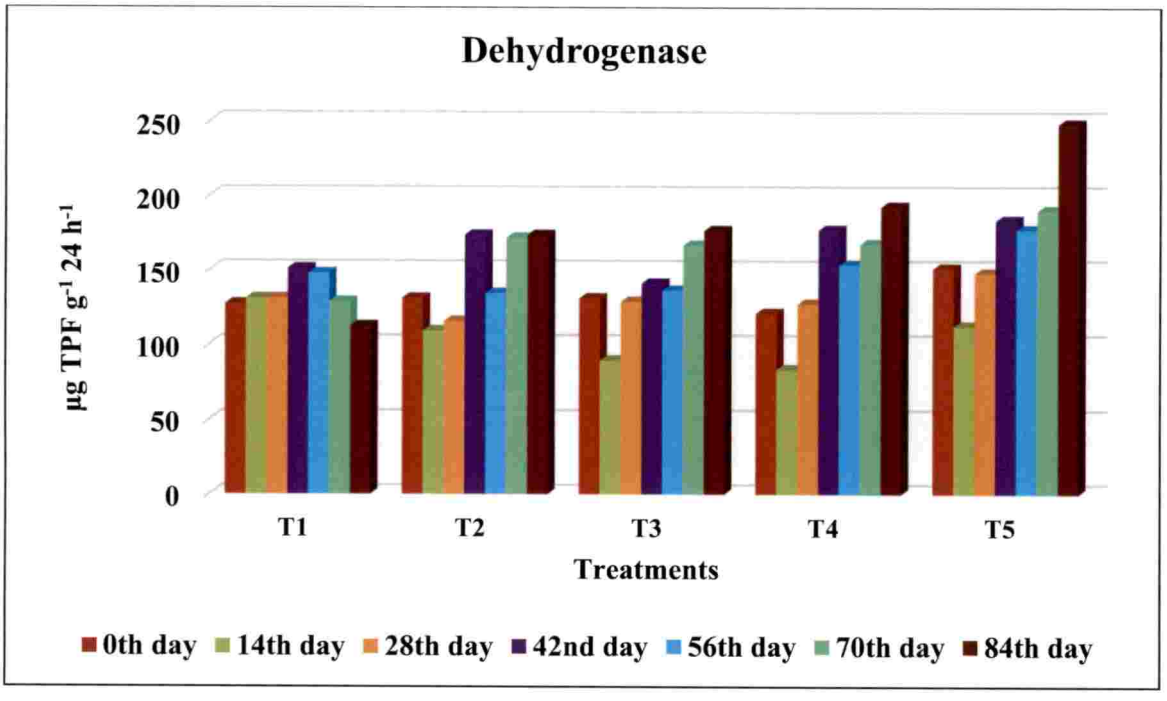

Fig 15. Activity of Dehydrogenase for a period of 3 months

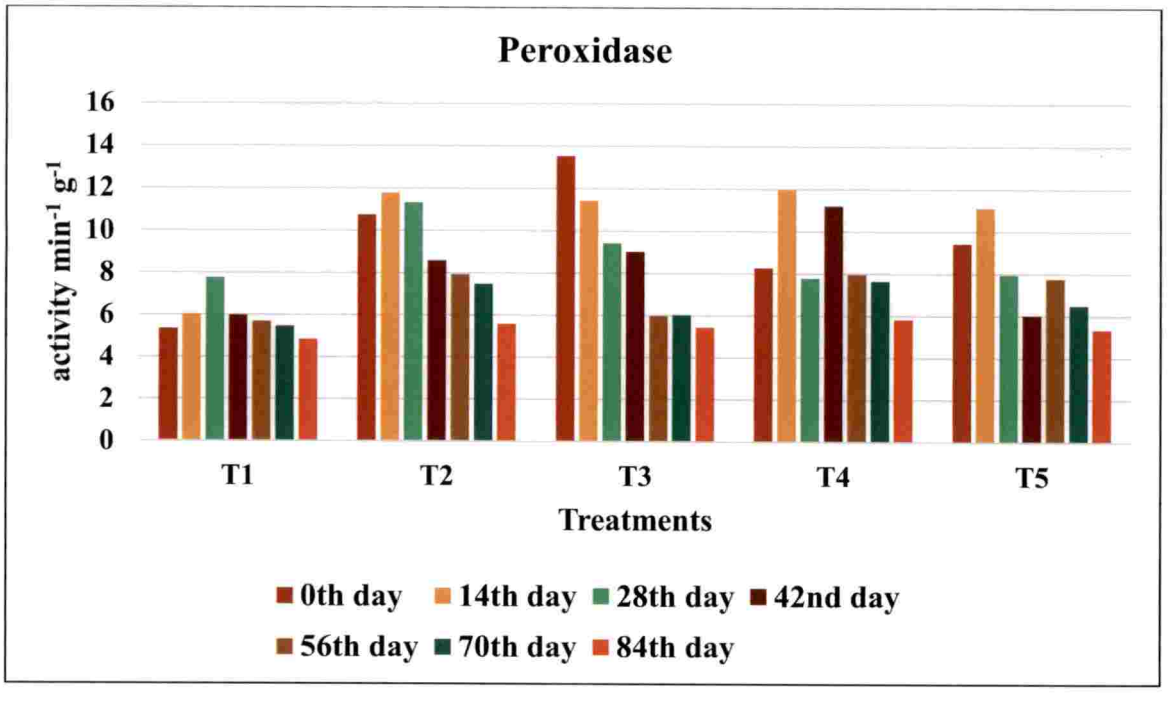

Fig 16. Activity of peroxidase for a period of 3 months

From Fig. 17, it is observed that treatment T_4: Soil + Zn as $ZnSO_4$ (1.5ppm) registered the highest value for carbonic anhydrase activity. This might be attributed by the production of limited oxidising agents and slightly acidic pH. This results corroborated with the findings of Keilin and Mann (1940)

5.14. ENZYME KINETIC PARAMETERS OF MAJOR ENZYMES

Kinetic study of enzymes provide better knowledge about their role in biogeochemical cycles. K_m is the combination of individual reaction constants in the enzyme reaction which is independent of enzyme concentration. V_{max} is the maximum rate of reaction when the enzyme is saturated with substrate. The interaction of enzyme with substrate were determined using K_m value. Line- weaver burk double reciprocal plot is used to plot V_{max} and K_m (Gianfreda *et al.*, 1995).

For the enzymes dehydrogenase, peroxidase and carbonic anhydrase, the enzyme kinetic parameters michaelis-mentan (K_m) and maximum velocity (V_{max}) were worked out.

From the study (Table 27 and 28), it was observed that for dehydrogenase the enzyme kinetic parameter V_{max} was found to be maximum on the 28^{th} day of incubation. While in case of K_m the greater affinity of the enzyme for its substrate was observed on the 84^{th} day by treatment T_5: Soil + Zn as $ZnSO_4$ (2 ppm). The lowest K_m value signifies the maximum adsorption of the enzyme to the soil surface.

In the case of peroxidase, the peak value for V_{max} was observed on the 28^{th} day of incubation by treatment T_5: Soil + Zn as $ZnSO_4$ (2 ppm). This might be due to the increase in the concentration of substrate. Higher the level of substrate more will be the turnover product (Marx *et al.*, 2005).

From the Table 30, it was observed that the substrate affinity was maximum at the 84^{th} day of incubation for peroxidase. The lowest K_m values were observed for treatment T_2: Soil + Zn as $ZnSO_4$ (0.5 ppm). This might be due to the production of isoenzymes with diverse enzyme substrate affinity and those displaying changed active sites. This is in accordance with the findings of Allison and Martiny (2008).

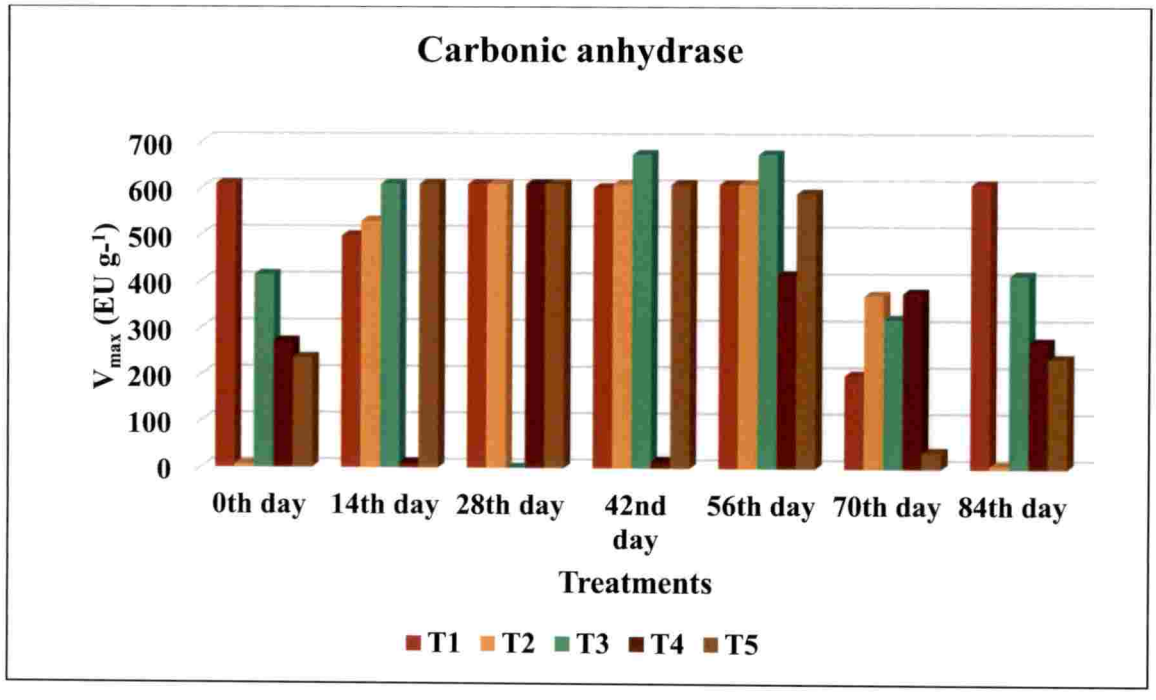

Fig 17. Activity of carbonic anhydrase for a period of 3 months

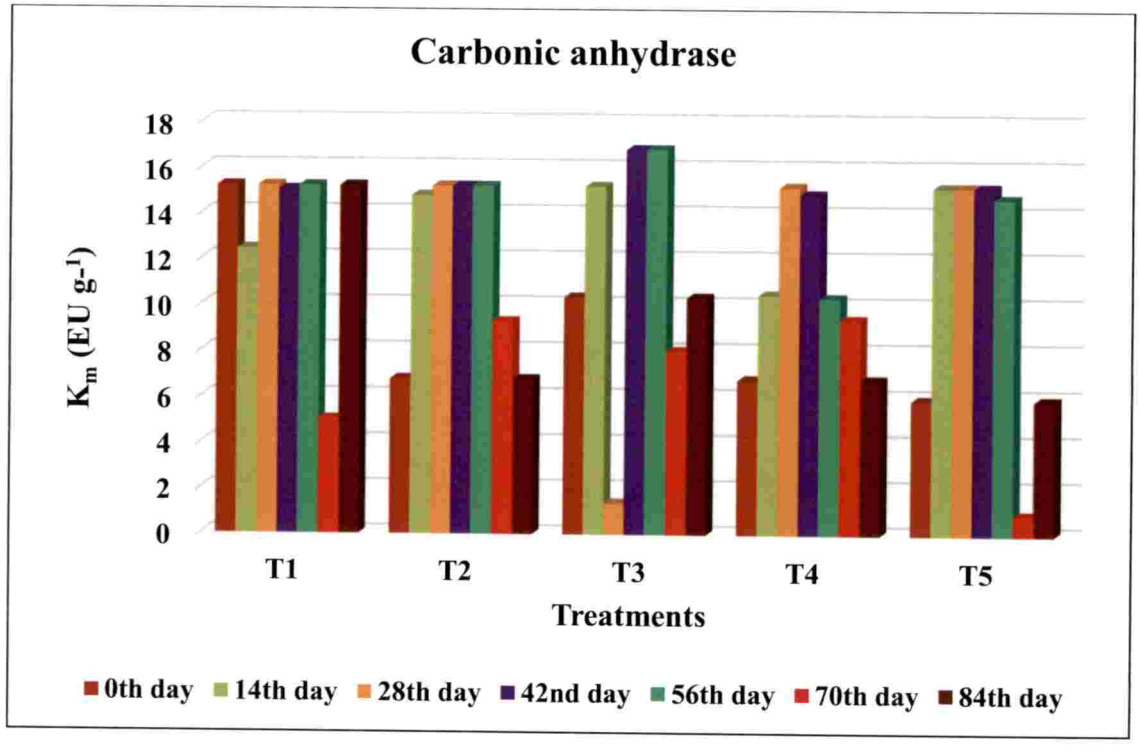

Fig 18. Enzyme kinetics (K_m) of Carbonic anhydrase

With regard to carbonic anhydrase, the enzyme kinetic parameter maximum velocity was registered on the 46th and 52nd day of incubation by treatment T_3 *ie.*, Soil + Zn as $ZnSO_4$ (1 ppm). This might be attributed by the optimum addition of nutrient (Zn) which could have caused more microbial growth and increased the enzyme activities.

The lowest K_m value for carbonic anhydrase (Fig. 18) was registered on the 70th day of incubation. The treatment showing lowest K_m values were T_5: Soil + Zn as $ZnSO_4$ (2 ppm). This might be due to the increased affinity of enzyme for its substrate. The high concentration of Zn promoted the growth of microorganisms which were involved in the production of bicarbonate and hydrogen ions and thereby increased the enzymes affinity for its substrate.

SUMMARY

6. SUMMARY

The study entitled "Effect of zinc fertilization on major plant and soil enzymes in southern laterites" was carried out in the Department of Soil Science and Agricultural Chemistry, College of Agriculture, Vellayani during 2017- 19. The study was envisaged to assess the effect of various sources and methods of application of zinc on the activities of major plant enzymes, soil enzymes, biochemical and microbial parameters in laterite soils using tomato (*Solanum lycopersicum*) as a test crop. The soil samples and plant samples were collected from the experimental plots and were analyzed in the post graduate laboratory for various physical, chemical and biological parameters in addition to biometric, yield and quality attributes. Along with this an incubation study was carried out to monitor the pattern of enzymes activity such as dehydrogenase, peroxidase and carbonic anhydrase and to evaluate their kinetic parameters for a period of three months.

The salient findings emerged from the study are furnished in this chapter.

- Soil samples were collected from the zinc deficient location in Neyattinkara series and were subjected to chemical analysis for confirming the Zn deficiency. Soil with low Zn status (0.506 ppm) was selected for the field experiment.
- The initial soil was deficient in available N, P, exchangeable Ca and Mg and micronutrients such as Zn, B and Cu.
- The treatments had significant influence on plant biometric characters viz., branches per plant, days to first flowering, fruits per plant, fruit length and yield characters like total yield and total dry matter production per plant.
- The treatment T_8- N, P, K+ K solubilizer (5%) registered the highest values for no. of flowers per branch, ascorbic acid content and soil pH. The highest value for EC were recorded by treatment T_7- N, P, K+ Zn Humate (44 kg ha^{-1}).
- The treatment T_4-N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$ registered the highest value for carboxy dismutase and PEP carboxylase.
- Treatment T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1}) registered the highest value for dehydrogenase while T_7- N, P, K+ Zn Humate (44 kg ha^{-1}) recorded the

highest value for peroxidase, catalase, carbonic anhydrase, lycopene and IAA content.

- The favourable soil pH of 5.46 for available Zn was recorded by treatment T_7- N, P, K+ Zn Humate (44 kg ha^{-1}).
- As regards to the effect of treatments on availability of primary nutrients it was observed that status of N, P, K increased from the initial values at the time of harvest. The highest value for available P was registered by T_6- N, P, K+ Zn solubilizer (5 %) while in case of available K it was treatment T_8- N, P, K+ K solubilizer (5%). The available N was found to be non significant.
- Among the secondary nutrients, exchangeable Ca and Mg were significantly influenced by the treatments. The exchangeable Ca and Mg was found to be high from the initial soil status. Treatment T_7- N, P, K+ Zn Humate (44 kg ha^{-1}) was found with the highest value for exchangeable Ca and Mg and available S.
- Among the micronutrients it was observed that available Fe decreased from the initial soil status whereas available B and Cu increased in the post harvest soil. The highest value for Fe was registered by T_1- Absolute control which was on par with treatment T_8- N, P, K+ K solubilizer (5%). In the case of available B, treatment T_7- N, P, K+ Zn Humate (44 kg ha^{-1}) recorded the highest value whereas treatment T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1}) registered the highest value for available Cu.
- Treatment T_7: N, P, K+ Zn Humate (44 kg ha^{-1}) recorded the highest value for available Zn followed by treatment T_5: N, P, K+ Zn as Zn EDTA (18 kg ha^{-1}).
- With regard to the soil biological characters, the highest values for soil respiration, microbial biomass carbon and soil enzymes (dehydrogenase and β- glucosidase) were registered by treatment T_7- N, P, K+ Zn Humate (44 kg ha^{-1}).
- P and K uptake were found to be highest for treatment T_8- N, P, K+ K solubilizer (5%) whereas T_7- N, P, K+ Zn Humate (44 kg ha^{-1}) recorded the highest N uptake.

- Treatment T_7- N, P, K+ Zn Humate (44 kg ha^{-1}) was found to have significant influence on Mg and S uptake whereas Ca uptake was highest for treatment T_4- N, P, K+ Foliar application of Zn as 0.5% $ZnSO_4$.
- With regards to the uptake of micronutrients, treatment T_7- N, P, K+ Zn Humate (44 kg ha^{-1}) recorded the highest value for Zn followed by T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1}).
- Treatment T_7- N, P, K+ Zn Humate (44 kg ha^{-1}) had significant influence on B uptake whereas the highest value for Fe uptake was recorded by treatment T_8- N, P, K+ K solubilizer (5%) while T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1}) registered the highest values for Cu uptake.
- With respect to the incubation study, the treatment T_4: Soil + Zn as $ZnSO_4$ (1.5 ppm) was found to be superior over all treatments for Peroxidase and Carbonic anhydrase whereas T_5: Soil + Zn as $ZnSO_4$ (2 ppm) was the best treatment for dehydrogenase.
- The peak activity of dehydrogenase and carbonic anhydrase were observed on the 84^{th} day of incubation while in the case of peroxidase it was on the 14^{th} day of incubation.
- From the enzyme kinetics study, it was evident that the enzyme's affinity for its substrate (K_m) was the highest in T_5: Soil + Zn as $ZnSO_4$ (2 ppm) for peroxidase and dehydrogenase while incase of carbonic anhydrase, it was treatment T_2: Soil + Zn as $ZnSO_4$ (0.5 ppm) which was found to be the best.
- In dehydrogenase and peroxidase peak values for V_{max} activity were observed on the 28^{th} day of incubation whereas the maximum activity of carbonic anhydrase saturated with substrate was observed on the 84^{th} day of incubation.
- The correlation studies between soil enzymes, plant enzymes and micronutrients showed a significant and positive correlation between dehydrogenase, β- glucosidase, carbonic anhydrase, peroxidase and catalase with Zn and it was observed that the micronutrient Zn reported a negative correlation with B, while Fe showed negative correlation with β- glucosidase, dehydrogenase (plant), carboxy dismutase and catalase.

- The regression studies between soil enzymes and plant enzymes with micronutrients revealed that the soil enzyme (dehydrogenase) and plant enzymes (peroxidase and catalase) were dependent on Zn.

6.1 CONCLUSION

- From the study it was concluded that, treatment T_7- N, P, K+ Zn Humate (44 kg ha^{-1}) was found to be superior over all other treatments.
- Treatment T_4: Soil + Zn as $ZnSO_4$ (1.5 ppm) was found to be best for maximising the activity of soil enzymes in incubation study.
- From the enzyme kinetics study, it was evident that the enzyme's affinity for its substrate (K_m) was highest in T_5: Soil + Zn as $ZnSO_4$ (2 ppm).

6.2 FUTURE LINE OF WORK

- To elucidate mechanisms of action of various commercial humates through field trials using various crops.
- To focus on the physiological effect of humic substances on plants, and their impact on nutrient cycling.
- Through geographical information systems (GIS) – identification of Zn deficient prone areas in Kerala and suggesting better management practices.

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EFFECT OF ZINC FERTILIZATION ON MAJOR PLANT AND SOIL ENZYMES IN SOUTHERN LATERITES

by

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ABSTRACT

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ABSTRACT

The study entitled "Effect of zinc fertilization on major plant and soil enzymes in southern laterites" was carried out in the Department of Soil Science and Agricultural Chemistry, College of Agriculture, Vellayani during the period 2017-19. The study was envisaged to assess the effect of various sources and methods of application of zinc on the activities of major plant and soil enzymes, biochemical and microbial parameters in laterite soils using tomato (*Solanum lycopersicum var.* Anagha) as a test crop.

From the soil test database developed by the Department of Soil Science & Agricultural Chemistry under the project RF-Soil Testing Lab, laterite soils in Neyyatinkkara series with Zn deficiency were identified. Soil samples were collected from these locations and analysed for confirming Zn deficiency. Soil with low Zn status (0.506 ppm) was selected for the study.

The second part of the experiment was a field study aimed at evaluating the efficacy of different sources of Zn using tomato (*var.* Anagha) as the test crop. The study was laid out in Randomized Block Design with eight treatments replicated thrice. The treatments were Absolute control (T_1), N, P, K as per POP- 75:45:25 kg ha^{-1} (T_2), N, P, K+ Soil application of Zn as $ZnSO_4$- 10 kg ha^{-1} (T_3), N, P, K+ Foliar application of Zn as 0.5 per cent $ZnSO_4$ (T_4), N, P, K+ Zn as Zn EDTA-18 kg ha^{-1} (T_5), N, P, K+ Zn solubilizer -5 per cent (T_6), N, P, K+ Zn Humate- 44 kg ha^{-1} (T_7), N, P, K+ K solubilizer 5 per cent (T_8).

From the study, it was observed that the treatment T_7 with the application of N, P, K+ Zn Humate (44 kg ha^{-1}) recorded the highest plant growth and yield attributes such as number of branches per plant (8.3), days to first flowering (31.7), fruits per plant (46.7), fruit length (3.5 cm), total fruit yield (35833.36 kg ha^{-1}) (1.29 kg plant $^{-1}$) and total dry matter production (0.108 kg plant $^{-1}$). The same treatment reported the highest value for enzymes such as carbonic anhydrase, peroxidase and catalase *viz.*, 910 EU g^{-1}, 48.17 activity min^{-1} g^{-1} and $27.06x10^3$ units ml^{-1} respectively. The results of indole 3- acetic acid (IAA) (217.83 µg g^{-1})

and lycopene analysis (26.11 μg g^{-1}) also revealed that the same treatment T_7 recorded the highest values. It was observed that the treatments did not have a significant effect on plant pigments chlorophyll a and b.

The results of post harvest soil analysis revealed a marginal increase in available nutrient content compared to initial status. The treatment T_7 with the application of N, P, K+ Zn Humate (44 kg ha^{-1}) recorded the highest mean value for electrical conductivity (EC) (0.172 dS m^{-1}), exchangeable Ca (436.67 ppm) and Mg (166 ppm), available B (0.221 ppm), soil respiration rate (177.14 mg CO_2 100 g^{-1} d^{-1}), microbial biomass carbon (186.67 μg g^{-1}) and enzymatic activities (dehydrogenase and β- glucosidase). Similarly, T_7 with the application of N, P, K+ Zn Humate (44 kg ha^{-1}) recorded the highest value for available Zn (5.123 ppm) which was on par with treatment T_5 (4.860 ppm).The uptake analysis revealed that the nutrient uptake varied significantly with the treatments. The treatment T_7 registered the highest plant uptake of N, Mg, Zn and B (87.87, 75.29, 0.187 and 0.57 kg ha^{-1} respectively).

An incubation study was conducted to monitor the pattern of activity of enzymes such as dehydrogenase, peroxidase and carbonic anhydrase and to evaluate their kinetic parameters for a period of three months. The study was carried with five treatments each replicated four times in CRD pattern. The treatments included were Soil alone (T_1), Soil + Zn as 0.5 ppm $ZnSO_4$ (T_2), Soil + Zn as 1 ppm $ZnSO_4$ (T_3), Soil + Zn as 1.5 ppm $ZnSO_4$ (T_4) and Soil + Zn as 2 ppm $ZnSO_4$ (T_5). It was observed that the activity of enzymes were significantly influenced by the treatments. The enzymes peroxidase and carbonic anhydrase showed an increasing trend in activity while peroxidase registered a decreasing trend. Treatment T_4 with the application of Soil + Zn as 1.5 ppm $ZnSO_4$ registered the highest value for peroxidase (11.98 activity min^{-1} g^{-1}) and carbonic anhydrase activity (385 EU g^{-1}).

The enzyme kinetics study revealed that the lowest k_m value was noticed for peroxidase (0.17 activity min^{-1} g^{-1}) and dehydrogenase (0.77μg TPF g^{-1} 24 h^{-1}) on the 84th day. The peak value for V_{max} was observed on the 28th day for enzyme

dehydrogenase (75.23 µg TPF g^{-1} 24 h^{-1}) and peroxidase (59.43x10^3activity min^{-1} g^{-1}). The treatment T_5 corresponding with the application of Soil + Zn as 2 ppm $ZnSO_4$ registered the lowest value of K_m for carbonic anhydrase (0.89 EU g^{-1}) and dehydrogenase (0.77µg TPF g^{-1} 24 h^{-1}), and the highest V_{max} values for peroxidase.

A significant positive correlation between soil enzyme (dehydrogenase), plant enzymes (peroxidase and catalase) and Zn with correlation coefficients 0.942^{**}, 0.950^{**} and 0.726^{**} respectively was noticed. Similarly, in the case of regression analysis, the plant enzymes (peroxidase and catalase) and soil enzyme dehydrogenase were dependent on zinc with coefficients (0.001^{*}, 0.001^{*} and 0.041^{*} respectively).

From the study it was observed that treatment T_7 with the application of N, P, K+ Zn Humate (44 kg ha^{-1}) registered the highest values for soil parameters (EC, available nutrients and soil enzymes) and plant parameters viz., growth, yield and uptake. Similarly it was also observed to be the best source for available Zn followed by T_5- N, P, K+ Zn as Zn EDTA (18 kg ha^{-1}). The incubation study revealed that for highest activity of peroxidase and carbonic anhydrase, the rate of Zn application is 1.5 ppm. In enzyme kinetics study, the treatment T_5 with the application of Soil + Zn as 2 ppm $ZnSO_4$ was found to be superior than all other treatments. Hence it can be concluded from the present study that the most efficient source of Zn was Zn Humate with an ideal rate of 44 kg ha^{-1} along with the recommended levels of N, P, K nutrients.

APPENDIX

APPENDIX I

Weather data during the crop season (June - September 2018)

Standard week	Temperature (° C)		Relative humidity (%)	Rainfall (mm)
	Maximum	Minimum		
25	31.00	24.57	88.07	57.00
26	31.46	24.40	85.21	25.20
27	31.56	24.69	81.00	10.20
28	29.63	23.00	89.64	69.30
29	30.41	23.54	85.14	56.30
30	31.41	23.57	81.29	13.10
31	29.49	23.91	85.64	136.20
32	30.29	23.33	88.07	107.30
33	29.09	22.57	92.36	205.20
34	30.96	24.00	83.00	2.80
35	31.97	24.46	80.50	0.00
36	32.17	24.06	79.57	0.00
37	33.00	24.07	78.00	0.00
38	32.00	24.23	80.21	7.20