ZINC BIOFORTIFICATION THROUGH ORGANIC Zn AND BIO-ORGANIC SUPPLEMENTS IN HIGH DENSITY PLANTATION OF APPLE (*Malus × domestica* Borkh.)

Thesis

by

SIMRAN SAINI
(H-2020-45-D)

submitted to

Dr. YASHWANT SINGH PARMAR UNIVERSITY
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Dr. Pramod Kumar
Major Advisor

Department of Fruit Science
Dr. Yashwant Singh Parmar University of Horticulture and Forestry
(Nauni) Solan (HP) – 173 230 India

CERTIFICATE – I

This is to certify that the thesis titled **“Zinc biofortification through organic Zn and bio-organic supplements in high density plantation of apple (*Malus* × *domestica* Borkh.)”** submitted in partial fulfilment of the requirements for the award of the degree of **Doctor of Philosophy Fruit Science** in the discipline of **Horticultural Sciences** to Dr. Yashwant Singh Parmar University of Horticulture & Forestry, (Nauni) Solan (HP) – 173 230 is a bonafide research work carried out by **Ms. Simran Saini (H-2020-45-D)** daughter of Sh. Nem Chand Saini under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been fully acknowledged.

Place: Nauni, Solan
Dated: 28/08/2024

Dr. Pramod Kumar
Major Advisor

CERTIFICATE – II

This is to certify that the thesis titled **"Zinc biofortification through organic Zn and bio-organic supplements in high density plantation of apple (*Malus* × *domestica* Borkh.)"** submitted by **Ms. Simran Saini (H-2020-45-D)** daughter of Sh. Nem Chand Saini to the Dr. Yashwant Singh Parmar University of Horticulture & Forestry, Nauni, Solan (HP) – 173 230, India in partial fulfilment of the requirements for the degree of **Doctor of Philosophy Fruit Science** in the discipline of **Horticultural Sciences** has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner.

Dr. Pramod Kumar
Major Advisor

Dr. A S Rehalia
External Examiner
24/8/24

Advisory Committee:

1. **Dr. D P Sharma**
   Dean, COH&F Neri

2. **Dr. C L Sharma**
   Senior Scientist (Co-opted Member)
   Department of Fruit Science
   24/8/24

3. **Dr. Anjali Chauhan**
   Associate Professor
   Department of Soil Science and Water Management
   24/8/24

Professor and Head
(Department of Fruit Science)

Countersigned

Dean
College of Horticulture
Dr. Yashwant Singh Parmar University of Horticulture & Forestry
(Nauni) Solan (HP) – 173 230 India

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Needless to say errors and omissions are mine.

Place : Nauni, Solan
Dated: 28/08/2024 **(Simran Saini)**

CONTENTS

ABBREVIATIONS USED

@	:	at the rate of
%	:	per cent
/	:	per
ºC	:	degree Celsius
et al.	:	co-workers or ‘and others’
CD	:	critical difference
g	:	gram
µg	:	microgram
mg	:	milligram
kg	:	kilogram
m	:	meter
m^2	:	meter square
m^3	:	cubic meter
mm	:	millimeter
µm	:	micrometer
h	:	hour
ha	:	hectare
i.e.	:	that is
nm	:	nanometer
cm	:	centimeter
cm^2	:	centimeter square
mL	:	milliliter
µL	:	microliter
L	:	liter
ppm	:	parts per million
µM	:	micro molar
mmol	:	millimole
t	:	tones
MT	:	metric tones
s	:	second
π	:	pi
w/w	:	weight/weight
w/v	:	weight/volume
v/v	:	volume/volume
$	:	dollar
viz.	:	videlicet (namely)
h	:	hour
kHz	:	kilohertz
rpm	:	revolutions per minute
cv.	:	cultivar
N	:	normal

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Chapter - 1

INTRODUCTION

Apple (*Malus* × *domestica* Borkh.) is the most important deciduous fruit crop grown worldwide which belongs to family Rosaceae. It is believed to have originated in Central Asia and is well distributed throughout the temperate zones worldwide. India accounts for 80 per cent of the global market supply of the delicious fruits (Amin and Jan, 2020). Its commercial cultivation extends to Jammu and Kashmir, Himachal Pradesh and Uttrakhand with total cultivable area of 3,04,000 ha, annual production of 28,76,000 MT and productivity of 9.4 MT/ha (Anonymous, 2023a). In Himachal Pradesh, apple cultivation extends from Shimla, Kullu, Kinnaur, Chamba, Mandi, Lahaul-Spiti and Sirmaur districts to lower Shiwalik foothills covering total cultivable area of 1,15,680 ha with 6,72,343 MT annual production at productivity of 5.81 MT/ha (Anonymous, 2023b). The fruits are excellent source of organic acid, minerals (0.34-1.23%), antioxidants including phytic acid and ursolic acid, vitamin-C, and dietary fiber (pectin) which helps to maintain insulin level into blood (Lee, 2012). The cultivar, Gala Schniga Schnico is indeed a high yielding Gala sport, and a mutation of ‘Schnitzer Schniga’ found by A. Gruber Genetti and co-workers at Schniga, Bozen, Italy. It has tapered shape, uniform red skin colour and striped blush with light coloured lenticels than the parent, Gala Schniga Schnitzer (Ennammali, 2015; Malachowska and Tomala, 2022).

Zinc (Zn) is an essential micronutrient playing a crucial role in various physiological processes such as photosynthesis, enzyme activation, and protein synthesis (Chiyaneh et al., 2017). Adequate Zn levels ensure optimal photosynthetic activity, which is vital for the production of carbohydrates and overall plant growth (Ponce-Garcia et al., 2019). Zn also helps to maintain the integrity of the tree’s vascular system which transports water and nutrients throughout the plant (Burman et al., 2013). It is involved in the formation of plant hormones and enzymes that regulate flowering, pollination and fruit development. Zn influences the biosynthesis of chlorophylls, tryptophan, the formation of pigments responsible for fruit color, the uptake and transportation of nitrogen, phosphorus and potassium within the plant, the synthesis of sugars, organic acids and other compounds that contribute to fruit flavor (Brennan, 2005; Sadak and Bakry, 2020). Zn deficiency comparatively more prevails in soils with low organic matter content due to its conversion to insoluble form (Gangloff et al., 2006). Besides, its deficiency offers the greatest threat to human health, based on its effect on mortality and quality of life (Bhutta et al., 2013). Human body can store 2-3 g of Zn, and out of which only 0.1 per cent is replenished every day, however, conservative estimates indicate that more than 25 per cent of the population globally faces a potential risk of Zn deficiency (Chien et al., 2006; Maret and Sandstead, 2006; Marukhlenko et al., 2022) or low bio-availability of Zn in food crops (Cakmak et al., 2010). Therefore, to address the Zn deficit, enrichment of food crops with accessible Zn through agronomic biofortification is the quick mean to attain significant health advantage (Wei et al., 2012).

Zinc sulphate ($ZnSO_4$) and other synthetic Zn chelates such as Zn-EDTA and Zn-DTPA are most commonly used Zn fertilizer sources (Bastam et al., 2020). However, the use of inorganic Zn fertilizer is linked with the increased risk of heavy metal toxicity such as cadmium (Tavallali and Karimi, 2017), besides, these are more expensive with lower uptake efficacy and have proven to disrupt micronutrient balance (Rafie et al., 2017; Tavallali and Karimi, 2017). In recent times, organic Zn fertilizer sources have been widely used to improve mineral nutrition in plants under ideal and stress conditions (Sanchez et al., 2005). European Union Regulation of 2019 emphasized the growing significance of bio-stimulants within the framework of EU regulations (EUR-Lex, 2021). These amino acid based complexes are the organic fertilizers which have shown great potential for usage and are environmentally safe along with high efficacy (Souri and Hatamian, 2019). Moreover, three-folds of expansion in the global market for these organic products over the next seven years is estimated a growth increase from $3.2 billion in 2023 to $9.0 billion by 2030 (Cruz and Dias, 2024), and the sale is forecasted to exceed $2 billion by 2025 in horticultural crops (Jones, 2019). These fertilizers are now familiar with farming community including amino chelate fertilizers, which are also quite effective to correct nutrient deficiencies (Souri, 2016). Amino acids are produced from enzymatic and chemical hydrolysis of proteins obtained from agricultural by-products such as animal waste and plant residues (Calvo et al., 2014). These natural chelating agents also have the ability to coordinate metal ions such as Zn *via* carboxyl groups (COOH) and also increase their availability to plants (Ghasemi et al., 2013). Besides, chelation removes the positive charge from micronutrients which allows neutral or slightly negative charge chelates to move more quickly through the pores on the leaf and roots (Sekhon, 2003). Moreover, amino acids are important precursors for the production of hormones and low molecular-weight nitrogenous substances that can form soluble complexes with Zn and thereby increase their bioavailability in humans (Lonnerdal, 2000).

Earlier studies are well documented that amino acids released during protein digestion can bind phytic acid and improve Zn absorption by retaining Zn in solution (Alkhtib et al., 2020). Furthermore, despite the potential effects of amino acid chelates to protect Zn against phytate reactions has a unique biological function compared to inorganic Zn complexes (Schlegel et al., 2013). Zn amino acid complexes are less expensive and more degradable products compared to synthetic chelates (Cieschi et al., 2016). These are smaller and also have low molecular weight than synthetic chelates (Zn-EDTA), and thus absorb more readily. Studies have also contemplated the increased Zn bioavailability through Zn amino acid chelates compared to $ZnSO_4$ (Marukhlenko et al., 2022). Different Zn amino acid fertilizers synthesized by complexation of Zn with varied amino acids such as Zn glycinate, Zn gluconate, Zn amino acid chelate and nano Zn amino acid chelate were used for the study. Glycinate is a smallest hydrophilic and proteinogenic amino acid which is non-polar having molar mass of 75 g/mol. It can be used in amino chelate based plant nutrition that can react acidic or basic in different mediums due to its chemical structure (Souri, 2016; Noroozlo et al., 2019a). Zn-Glu is produced by reaction between one mole of Zn and two moles of gluconic acid. Zn-AAC can be obtained from chelation of Zn with amino acids or partially hydrolyzed peptides/proteins. In addition, Zn-AAC employs the amino acid Aspartic acid to boost the bioavailability and absorption, hence increasing its activity. Besides, nano Zn-AAC is a most effective source of Zn produced from complexation of nano Zn with chelated amino acid (Souri and Hatamian, 2019; Dolev et al., 2020).

Additionally, fertilizing the plants with different organic supplements such as vermi-compost, *Azolla*, AM fungi, plant growth promoting rhizobacteria (PGPR) and humic acid can enrich plant system *via* slow-release mechanism (Chuan-Chuan et al., 2017). Vermi-compost is a rich source of readily available nutrients that contained growth substances and various beneficial microorganisms. Moreover, the earthworm species and microbes present in the vermicompost improve the soil physical properties through increased aeration, porosity and water holding capacity of the soil. *Azolla* is a free floating macrophyte which utilizes solar energy and provide organic matter to the soil (Taha and El-Shahat, 2017) and also helps in regulation of soil pH levels and boosts the accessibility of N and P (Bora et al., 2016) and can substitute 40-50 kg N/ha (Pathak et al., 2017). AM fungi and PGPR can promote plant growth through increased nutrient absorption from the rhizosphere (Ashwini, 2019). Humic substances are known to improve biochemical attributes of fruits through chelation effect and increased nutrient absorption (Chen et al., 2004; Trevisan et al., 2010). Biofortification of apples through the use of organic and bio-organic zinc sources is a multifaceted approach that offers significant benefits for human health, plant productivity and environmental sustainability. Despite the benefits, there are challenges in optimizing zinc biofortification. These include variations in soil properties, environmental conditions, and differences in apple varieties. Moreover, these methods often utilize natural materials and biological processes, reducing the reliance on synthetic fertilizers and minimizing the risk of soil and water pollution. Further research is needed to develop more efficient and cost-effective biofortification techniques, and to understand its impact on soil health and crop performance. Considering the above-mentioned aspects, the current study emphasized the potential impact of organic Zn fertilizers *vis-a-vis* bio-organic Zn sources on sustainable and quality production of apple cv. Gala Schniga Schnico in high density plantation.

Objectives

1) To standardize foliar application of Zn and bio-organic formulations on growth, yield and fruit quality under high density apple plantation
2) To examine the effect of organic Zn and bio-organics fertilization on physiological characteristics and nutrient dynamics of apple
3) To assess chemical and biological properties of soil as a function of organic Zn and bio-organic nutrients in high density apple

Chapter - 2

REVIEW OF LITERATURE

Chemical fertlizers are typically composed of elements in simple or ionic forms, which udergo various detrimental reactions in the soil leading to reduced uptake efficiency (Fageria and Baligar, 2005; Souri, 2016). Besides, processes such as fixation, leaching, precipitation and volatilization significantly contribute to lowering the efficiency of fertilizer utilization (Marschner, 2011). As an alternaive, organic fertilizers such as amino acid based chelates are low-cost and highly efficient fertilizers which are required in lower quantities and aids in higher crop production through biofortification (Jacob et al., 2024). Chelation of amino acids with nutrient elements such as Zn results in significant increase in absorption efficiency and mineral translocation throughout the plant (Sanchez et al., 2005). The crucial aspect contributing to the increased efficacy of aminochelate fertilizers is the long-term stability of chelated bond formed between amino acid with Zn in either the soil or nutrient solution (Ghasemi et al., 2013). Organic Zn increases IAA levels which lead to improvement in plant growth characteristics (Pandey et al., 2010) and maintain soil health and productivity. Earlier workers have established the importance of amino acid-complexed fertilizers on fruit crops (Machado et al., 2008; Naseri et al., 2013; Fahimi et al., 2016 and Souri et al., 2017). Moreover, numerous amino acid transporters have been identified in plant tissues (Haydon and Cobbett, 2007; Svennerstam et al., 2007; Tegeder, 2014), providing new perspecties on the future potential of amino chelates in plant nutrition. Additionally, organic based nutrient sources such as vermicompost and humic acid boost plant growth and productivity through improved soil fertility and soil microbial activity compared to chemical fertilizers which suppress microbial growth due to higher salt effects (Salwa, 2011). Plants form symbiotic relationships with a wide array of beneficial microrganisms, including AMF, *Azolla* and PGPR which have the potential to improve both plant health and productivity through increased nutrient availability (Meena et al., 2019). Keeping in view, a brief review of literature prevailing to the impact of organic Zn and bio-organics amendements have been presented under the following heads and sub-heads:

2.1 VEGETATIVE GROWTH PARAMETERS

Koksal et al. (1999) reported maximum shoot length with the application of zinc amino acid chelate (0.2, 0.4 and 0.6%) in pear cv. Williams. Kalaiselvan et al. (2021) noted highest plant height through application of zinc amino acid chelate at 0.5 per cent in agronomic crops. Datir et al. (2010) found significant improvement in plant height and leaf area through application of zinc amino acid chelate (2%) in agronomic crops. Tabesh et al. (2020) recorded significant increase in leaf area through foliar application of zinc amino acid chelate (6%) in agronomic crops. Jalali et al. (2019) recorded increased leaf area with the application of zinc amino acid chelate (0.2%) in agronomic crops. Rizwan et al. (2017) observed increased plant height and shoot length through application of zinc amino acid chelate (30 mg/L) in agronomic crops. Maximum increase in plant height and canopy area was observed when zinc amino acid chelate was applied at 8 per cent in agronomic crops (Yeboah et al., 2021). Krishnaraj et al. (2020) recorded highest plant height, stem girth and leaf area index through application of zinc amino acid chelate (0.2%) in agronomic crops. Alzreejawi and Al-Juthery (2020) observed increased plant height through application of nano zinc amino acid chelate (2 mL/L) in agronomic crops. Plant height was recorded maximum with the conjoint application of nano zinc and amino acid (400 L/ha) in agronomic crops (Al-Juthery et al., 2019). Jaafar and Abdullah (2020) reported increase in plant height with the application of nano zinc (2 g/L) and amino acid (8 mL/L) in agronomic crops. Souri et al. (2017) noted improved plant height with the application of zinc (2.5%) + amino acid (2%) in agronomic crops. Plant height was noted maximum with the application of zinc (28 kg/ha) and amino acid (3000 mL/ha) in agronomic crops (Zakirullah et al., 2018). Kheir et al. (2021) recorded increased shoot length and leaf area through application of amino acid (2 mg/L) and zinc (2 g/L) in mango cv. Fagri Kalan.

Kasem and Ibrahiem (2021) reported significant increase in shoot length and leaf area through application of amino acid + zinc (0.8%) during first week of March, after fruit set and one month later in fig cv. Sultani. El-Badawy (2019) noted maximum shoot length and leaf area with the application of amino acid (3 mL/L) and zinc (150 ppm) in apricot cv. Canino. Zawi and Mejbel (2022) noted increase in plant height and leaf area through application of nano zinc (2 mg/L) and amino acid (2 mg/L) in agronomic crops. Rahimi et al. (2022) stated that plant height was noted maximum through application of nano zinc (2 g/L) + amino acid (2 mL/L) + humic acid (2 g/L) in agronomic crops. Grzyb et al. (2012) recorded increased tree height, trunk girth and shoot growth through aplication of amino acid (0.5%) in apple cv. Topaz and Ariwa. Tavallali et al. (2018) reported increase in plant height through foliar application of zinc glycinate (0.2%) applied after thinning and before flower initiation in agronomic crops. Soltani et al. (2022) revealed that maximum plant height was observed when zinc glycinate at 1 kg/ha was applied in agronomic crops. Raza et al. (2023) recorded increase in plant height through application of zinc glycinate at 12 mg/kg in agronomic crops.

Soliman et al. (2023) reported highest plant height through application of zinc (200 ppm) + glycine (400 ppm) in agronomic crops. Aslani and Souri (2018) noted that application of zinc (2.5%) + glycine (2%) exhibited maximum plant height and leaf area in agronomic crops. Maximum shoot length was recorded with zinc glycinate at 10 µm in agronomic crops (Mirbolook et al., 2020a). Mosa et al. (2020) noted improved shoot length through application of glycine at 50 ppm in apple cv. Anna. Almutairi et al. (2022) noted maximum shoot length and leaf area through application of glycine at 1000 ppm in guava cv. Maamoura. Mohammadipour and Souri (2019) observed maximum plant height through foliar application of glycine at 500 mg/L in agronomic crops. Noroozlo et al. (2019a) reported increase in plant height and leaf area through application of glycine (500 mg/L) in agronomic crops.

Razzaq et al. (2013) reported maximum plant height through application of zinc sulphate (0.6%) in mandarin cv. Kinnow. Khan et al. (2012) stated that application of zinc sulphate (0.5%) recorded increase in plant height and leaf area of mandarin cv. Feutrell’s Early. Hasani et al. (2012) observed significant increase in leaf area through foliar spray of zinc sulphate (0.3%) in pomegranate. Baiea et al. (2015) recorded increased shoot length and leaf area through application of zinc sulphate at 200 ppm in mango cv. Keitt. Leaf area recorded significant increase through foliar application of zinc sulphate (0.75%) in guava cv. Dharidar (Waskela et al., 2013). Hadi and Saleh (2021) revealed that maximum leaf area was recorded through application of zinc sulphate (3 g/L) at petal fall stage in apple cv. Ibrahimi. Increased leaf area was observed with the application of zinc sulphate (100 mg/L) in strawberry cv. Camarosa (Lolaei et al., 2012). Keshavarz et al. (2011) reported improved vegetative growth through foliar application of zinc sulphate (1050 mg/L) in walnut. Leaf area was significantly improved through application of zinc sulphate (0.5%) in sweet orange cv. Mosambi (Singh et al., 2018). Asaad (2014) recorded maximum shoot length and leaf area with the application of zinc (10.5 g/20L) in apple cv. Anna.

Zuo et al. (2018) reported increased plant height and leaf area through application of 50 per cent of vermicompost in strawberry cv. Yanli. Singh et al. (2008) recorded maximum leaf area through application of vermicompost (10 t/ha) in strawberry cv. Chandler. Kumar et al. (2018) observed maximum TCSA through application of 50 per cent of N through vermicompost in apple cv. Oregon Spur. Singh et al. (2015) inferred maximum plant height and leaf area through conjoint application of vermicompost at 10 t/ha and arbuscular mychorhhizal fungi (AM fungi) at 5 kg/ha in strawberry. Raj and Sharma (2009) reported that application of AM fungi (100 g/m) increased shoot length of apple. Khade and Rodrigues

(2009) recorded increased plant height, trunk girth and number of leaves through application of AM fungi (5 g/plant) in papaya. Kumar et al. (2014) noted that application of AM fungi exhibited maximum shoot length in aonla. Suchen (2000) observed that triple inoculation of AM fungi increased vegetative growth of citrus seedlings. Increased plant growth was noted with application of AM Fungi (250 g) in banana cv. Ardhapuri (Patil and Shinde, 2013). Shamshiri et al. (2012) noted increase in plant height, shoot growth and canopy volume through application of AM fungi (100 g/tree) in mandarin cv. Kinnow. Taha and El-Shahat (2017) stated that application of *Azolla* extract (1 L/20L) and humic acid (200 mL/20L) exhibited maximum leaf area in apricot cv. Canino. Taha et al. (2018) noted maximum leaf area through application of *Azolla* (5 kg/20L) in apple cv. Anna.

Arikan and Pirlak (2016) stated that application of plant growth promoting rhizobacteria (PGPR) significantly improved the shoot length and leaf area in sour cherry cv. Kutahya. Kumari et al. (2018) inferred that inoculation of PGPR (*Bacillus subtilis*) exhibited increase in plant height and leaf area in strawberry cv. Chandler. Pathak et al. (2013) recorded maximum plant height through application of PGPR (*Pseudomonas maltophila* PM4) in guava. Thomas et al. (2022) observed increase in leaf area through application of amino acid (5%) + AM fungi (20 g/plant) + PGPR (50 mL/plant) + humic acid (39%) in apple seedling rootstock. Mosa et al. (2015) reported increase in leaf area and shoot length through application of humic acid at 5 per cent in apple cv. Anna. Shehata et al. (2011) recorded maximum plant height through application of amino acid (2 mL/L) + humic acid (1 g/L) in strawberry cv. Festival. Al-Saif et al. (2023) observed maximum leaf area through application of humic acid (2000 mg/L) applied four times in apricot cv. Canino. Maximum shoot growth was observed in strawberry with humic acid application (Singh et al., 2010). Haggag et al. (2016) found significant improvement in plant height through humic acid (0, 0.25, 0.5%) application in olive cv. Agazze. Shall et al. (2010) noted that foliar application of humic acid (250 mL/100L) improved tree height, trunk girth and length of shoots in plum. Ennab (2016) observed maximum leaf area and canopy volume through application of humic acid (20 mL/tree) in Egyptian lime. Morsey et al. (2015) reported maximum shoot length and leaf area through application of humic acid at 30 g/tree in apple cv. Anna. Khattab et al. (2012) noted significant improvement in shoot length and leaf area through application of amino acid at 16 g/plant and humic acid at 48 g/plant in pomegranate cv. Manfalouty. Ashwini et al. (2022) reported increase in plant height, trunk girth, canopy diameter, shoot growth, leaf area, trunk cross-sectional area (TCSA) and canopy volume (CV) through application of AM fungi (25 g/plant) + PGPR (25 mL/plant) + humic acid (60 mL/L) in guava cv. Lalit.

2.2 FLOWERING TRAITS

Mohammadipour and Souri (2019) revealed that maximum number of flowers was exhibited through foliar application of glycine at 500 mg/L in agronomic crops. Al-Baidhani and Al-Zurfi (2021) recorded increased length of flowering shoot through application of zinc (30 mg/L) and amino acid (6 mL/L) in horticultural crops. Number of flowers was recorded maximum through application of zinc sulphate at 0.4 per cent in strawberry cv. Chandler (Chaturvedi et al., 2005). Kazemi (2014) stated that application of zinc sulphate (150 mg/L) significantly improved number of flowers in strawberry cv. Pajaro. Hada et al. (2014) reported least days for initiation of flowering with the application of zinc sulphate at 0.8 per cent in guava cv. Lucknow-49. Masroor et al. (2015) noted improved number of flowers through zinc sulphate (0.5-1%) application in mango. Minimum days of flowering were exhibited through zinc (0.5-1%) application in sweet orange cv. Blood Orange (Sajid et al., 2010). Ashoori et al. (2013) also found reduced number of days for flowering through application of zinc sulphate (1.5 mg/L) in grapes.

Singh et al. (2008) noted that application of vermicompost at 10 t/ha resulted in least days for initiation of flowering in strawberry cv. Chandler. Singh et al. (2015) revealed that combined application of vermicompost (10 t/ha) and AM fungi (5 kg/ha) exhibited minimum days for initiation of flowering and number of flowers in strawberry. Moctezuma et al. (2005) reported increased flowering through conjoint application of vermicompost, AM fungi and PGPR (*B. pumilus*) in papaya. Mia et al. (2010) found improved days for flower initiation through application of PGPR in banana cv. Berangan. Arunima et al. (2022) found that application of *Azolla* extract (1 kg/L) resulted in increased number of flowers in agronomic crops. Abbas et al. (2013) observed significantly improved flowering through application of humic acid (80 mL/plant) in mandarin cv. Kinnow. Maximum number of flowers was recorded through humic acid (0.5 g/L) application in mango cv. Ewais and Keitt (Kosary et al., 2011). Khattab et al. (2012) stated that application of amino acid at 16 g/plant and humic acid at 48 g/plant showed significant increase in number of flowers per shoot in pomegranate cv. Manfalouty. Ashwini et al. (2022) revealed that maximum length of flowering shoot and number of flowers was exhibited through application of AM fungi (25 g/plant) + PGPR (25 mL/plant) + humic acid (60 mL/L) in guava cv. Lalit

2.3 YIELD CONTRIBUTING PARAMETERS

Kamiab and Zamanibahramabadi (2016) stated that application of zinc amino acid chelate (2 g/L) significantly improved fruit set and yield in almond. Alzreejawi and Al-

Juthery (2020) recorded increased yield through foliar application of nano zinc amino acid chelate (2 mL/L) in agronomic crops. Hassan et al. (2010) stated that application of zinc amino acid chelate (0.25%) increased number of fruits, fruit set and fruit yield in plum cv. Hollywood. Fruit yield and yield efficiency (yield/trunk cross-sectional area) were improved through application of zinc amino acid chelate (0.2, 0.4, 0.6%) in pear cv. Williams (Koksal et al., 1999). Najizadeh and Khoshgoftarmanesh (2019) observed increase in fruit yield through application of zinc amino acid chelate at 3 L/tree in pistachionut cv. Akbari. Panahi et al. (2023) revealed that foliar application of zinc amino acid chelate (4%) exhibited increase in fruit yield when applied during Hababook and Khalal stage in datepalm cv. Mazafati. Datir et al. (2010) reported maximum yield through application of zinc amino acid chelate (2%) in agronomic crops. Mirsardoo et al. (2023) stated that highest yield was noted through application of zinc amino acid chelate at 2 mL/L in agronomic crops. Sun et al. (2018) noted highest yield through foliar application of zinc amino acid chelate (70 mL/m^2) applied thrice at seven days' interval in agronomic crops. Increased yield was noted through application of zinc amino acid chelate at 0.5 per cent (Rafie et al., 2017). Mirbolook et al. (2020a) recorded maximum yield through application of zinc glycinate at 10 µm compared to control. Maximum fruit yield was reported through application of zinc glycinate (10 µM) in agronomic crops (Mirbolook et al., 2020b). Seddigh et al. (2016) noted increased yield through application of zinc glycinate at 40 mg/kg in agronomic crops. Aslani and Souri (2018) observed increased number of fruits and yield through application of zinc (2.5%) + glycine (2%) in agronomic crops. Soliman et al. (2023) revealed that maximum number of fruits and yield was exhibited through application of zinc (200 ppm) + glycine (400 ppm) in agronomic crops. Almutairi et al. (2022) recorded increase in fruit set and fruit yield through application of glycine at 1000 ppm in guava cv. Maamoura. Ghasemi et al. (2013) found that application of zinc glycinate (0.2%) significantly improved the yield in agronomic crops. Yeboah et al. (2021) reported maximum yield with the application of zinc amino acid chelate at 8 per cent in agronomic crops. Kalaiselvan et al. (2021) exhibited maximum increase in yield through application of zinc amino acid chelate (0.5%) in agronomic crops. Jaafar and Abdullah (2020) observed maximum increase in fruit weight with the application of nano zinc (2 g/L) + amino acid (8 mL/L) in agronomic crops.

Shalan (2020) revealed that maximum fruit set and yield was noted when zinc (0.5%) + amino acid (150 mL/tree) was applied in datepalm cv. Zaghloul. Szczepaniak et al. (2018) recorded increased yield through application of zinc (500 g/L) + amino acid (93 g/L) in agronomic crops. Fruit yield was observed maximum through application of zinc amino acid chelate at 6 per cent in agronomic crops (Tabesh et al., 2020). Zakirullah et al. (2018) noted maximum yield through foliar application of zinc (28 kg/ha) and amino acid (3000 mL/ha) in agronomic crops. Krishnaraj et al. (2020) recorded highest number of fruits and yield with the application of zinc amino acid chelate (0.2%) in agronomic crops. Fruit yield was observed maximum through application of zinc amino acid chelate (2%) in agronomic crops (Datir et al., 2012). Wei et al. (2012) noted maximum yield through application of zinc amino acid chelate at 0.2 per cent in agronomic crops. Souri et al. (2017) recorded significant increase in yield through application of zinc (2.5%) + amino acid (2%) in agronomic crops. El-Badawy (2019) observed maximum fruit set and yield through application of amino acid (3 mL/L) and zinc (150 ppm) in apricot cv. Canino. Kheir et al. (2021) reported maximum yield with the application of amino acid (2 mg/L) and zinc (2 g/L) in mango cv. Fagri Kalan. Elshamly et al. (2024) noted maximum yield through application of zinc (1.25 kg/ha) + amino acid (200 mL/L) in agronomic crops. Bashir et al. (2021) recorded maximum yield with the application of zinc amino acid chelate (1.5%) in agronomic crops. Gourkhede et al. (2020) recorded highest number of fruits and yield through application of zinc gluconate in agronomic crops.

Hadi and Saleh (2021) found that application of zinc sulphate (3 g/L) at petal fall stage exhibited maximum fruit set in apple cv. Ibrahimi. Amiri et al. (2008) observed maximum fruit yield through foliar application of zinc sulphate (110 g/tree) in apple cv. Golden Delicious. Mohammad et al. (2017) recorded maximum fruit set and yield through zinc sulphate (0.1%) application in apple cv. Red Delicious. Jat and Kacha (2014) reported maximum number of fruits and fruit yield through zinc sulphate (0.6%) application in guava cv. Bhavnagar. Maximum fruit set was found through application of zinc sulphate (2.5 kg/m^3) in olive (Saadati et al., 2016). Modi et al. (2012) noted highest number of fruits through foliar application of zinc sulphate at 0.5 per cent in papaya. Baranwal et al. (2017) reported increased fruit set through application of zinc sulphate (0.75%) in winter season guava. Hada et al. (2014) revealed that number of fruits and fruit set was improved through zinc sulphate (0.8%) application in guava cv. Lucknow-49. Abdel-Karim et al. (2015) noted significant improvement in fruit set and yield through application of zinc sulphate (1, 2 g/L) in avocado cv. Fuerte. Obaid and Al-Hadethi (2013) recorded increased fruit set and yield through foliar application of zinc sulphate at 3 per cent in pomegranate cv. Salemy. Singh et al. (2018) noted increased fruit set and yield with the application of zinc sulphate (0.5%) in sweet orange cv. Mosambi.

Asaad (2014) observed highest fruit set and yield through application of zinc (10.5 g/20L) in apple cv. Anna. Mosa et al. (2020) recorded maximum fruit set and yield through application of glycine at 50 ppm in apple cv. Anna. Singh et al. (2008) noted maximum number of fruits and fruit yield through application of vermicompost at 10 t/ha in strawberry cv. Chandler. Zuo et al. (2018) recorded maximum fruit yield through application of 50 per cent of vermicompost in strawberry cv. Yanli. Singh et al. (2015) inferred that maximum fruit set and fruit yield were exhibited through conjoint application of vermicompost (10 t/ha) and AM fungi (5 kg/ha) in strawberry. Das et al. (2017) recorded highest fruit yield through inoculation of AM fungi in guava. Hazarika et al. (2011) reported increased fruit yield through AM fungi application in banana cv. Grand Naine. Kundu et al. (2011) recorded maximum fruit yield through application of AM fungi in mango cv. Amrapalli. Patil and Shinde (2013) revealed increase in fruit yield exhibited by application of AM fungi (250 g) in banana cv. Ardhapuri. Taha et al. (2018) noted increase in fruit set and fruit yield through application of *Azolla* at 5 kg/20L in apple cv. Anna. Taha and El-Shahat (2017) reported improved fruit set and fruit yield through combined application of *Azolla* extract (1 L/20L) + humic acid (200 mL/20L) in apricot cv. Canino.

Mia et al. (2010) observed that fruit yield was significantly improved with the application of PGPR in banana cv. Berganan. Kumari et al. (2018) reported increased number of fruits and fruit yield through inoculation of PGPR (*B. subtilis*) in strawberry cv. Chandler. Ashwini et al. (2022) noted increase in fruit set and fruit yield through application of AM fungi (25 g/plant) + PGPR (25 mL/plant) + humic acid (60 mL/L) in guava cv. Lalit. Mosa et al. (2015) observed increased fruit set and fruit yield through application of humic acid (5%) in apple cv. Anna. Morsey et al. (2015) noted increase in fruit set and fruit yield through application of humic acid (30 g/tree) in apple cv. Anna. Khattab et al. (2012) observed increased fruit set and number of fruits through humic acid (32-48 g/tree) application in pomegranate. Abbas et al. (2013) found that application of humic acid (80 mL/plant) reported increased number of fruits in mandarin cv. Kinnow. Al-Saif et al. (2023) noted that fruit set and fruit yield were significantly increased through four times application of humic acid (2000 mg/L) in apricot cv. Canino. Kosary et al. (2011) noted improved fruit set through application of humic acid (0.5 g/L) in mango cv. Keitt and Ewais. Hagagg et al. (2013) recorded improved fruit set and yield with the application of humic acid (0.5%) applied at beginning of fruit set in olive cv. Aggizi. Sotiropoulos et al. (2024) noted increase in fruit yield through application of humic acid at 0.5 per cent applied at petal fall stage and after twenty days in olive cv. Koroneiki. Khattab et al. (2012) revealed that application of amino acid at 16 g/plant and humic acid at 48 g/plant showed significant increase in number of fruits and fruit yield in pomegranate cv. Manfalouty.

2.4 FRUIT QUALITY CHARACTERISTICS

2.4.1 Physical

Kamiab and Zamanibahramabadi (2016) observed maximum fruit length, breadth and weight with the application of zinc amino acid chelate (2 g/L) in almond. Hassan et al. (2010) recorded highest fruit length, breadth, weight and lowest fruit firmness through aplication of zinc amino acid chelate (0.25%) in plum cv. Hollywood. Panahi et al. (2023) observed that foliar application of zinc amino acid chelate (2%) applied at Hababook and Khalal stage showed increase in fruit length, breadth and weight in datepalm cv. Mazafati. Mirsardoo et al. (2023) noted increased fruit weight through application of zinc amino acid chelate at 2 mL/L in agronomic crops. Wei et al. (2012) revealed that fruit weight was recorded maximum through application of zinc amino acid chelate (0.2%) in agronomic crops. Kasem and Ibrahiem (2021) reported maximum fruit length, breadth and weight through application of amino acid + zinc (0.8%) during first week of March, after fruit set and one month later in fig cv. Sultani. Fruit breadth was recorded maximum with the application of zinc amino acid chelate (0.5%) in agronomic crops (Rafie et al., 2017). Datir et al. (2010) noted highest fruit weight through application of zinc amino acid chelate at 2 per cent in agronomic crops. Jaafar and Abdullah (2020) observed increased fruit weight through application of nano zinc (2 g/L) and amino acid (8 mL/L) in agronomic crops. Datir et al. (2012) recorded maximum fruit length and weight with the application of zinc amino acid chelate (2%) in agronomic crops. Bashir et al. (2021) found significantly increased fruit length, breadth and weight through application of zinc amino acid chelate (1.5%) in agronomic crops. Krishnaraj et al. (2020) observed highest fruit length, breadth and weight through application of zinc amino acid chelate (0.2%) in agronomic crops. Maximum fruit firmness and weight were recorded through application of amino acid (3 mL/L) and zinc (150 ppm) in apricot cv. Canino (El-Badawy, 2019). Shalan (2020) recorded maximum fruit length, breadth and weight through application of zinc (0.5%) + amino acid (150 mL/tree) in datepalm cv. Zaghloul. Soltani et al. (2022) noted highest fruit weight through application of zinc glycinate at 1 kg/ha in agronomic crops. Aslani and Souri (2018) observed that fruit length was improved through application of zinc (2.5%) + glycine (2%) in agronomic crops. Soliman et al. (2023) stated that maximum fruit weight was found through application of zinc (200 ppm) + glycine (400 ppm) in agronomic crops. Highest fruit length, diameter and weight were exhibited through application of glycine (1000 ppm) in guava cv. Maamoura (Almutairi et al., 2022). Gourkhede et al. (2020) recorded significant increase in fruit weight through application of zinc gluconate in agronomic crops.

Mohammad et al. (2017) observed maximum fruit length and breadth through application of zinc sulphate at 0.1 per cent in apple cv. Red Delicious. Amiri et al. (2008) noted maximum fruit weight and minimum fruit firmness through foliar application of zinc sulphate (110 g/tree) in apple cv. Golden Delicious. Goswami et al. (2012) recorded that foliar application of zinc sulphate at 0.4 per cent revealed maximum fruit length and fruit breadth in guava cv. Sardar. Jat and Kacha (2014) observed maximum fruit weight through application of zinc sulphate at 0.6 per cent in guava cv. Bhavnagar Red. Jagtap et al. (2013) observed increase in fruit breadth and weight through zinc sulphate (0.5%) application in acid lime cv. Kagzi Lime. Kar et al. (2002) noted enhancement in fruit weight through zinc sulphate (0.2, 0.4 and 0.6%) application in pineapple. Modi et al. (2012) observed heaviest fruits through application of zinc sulphate at 0.5 per cent in papaya. Parmar et al. (2014) revealed that application of zinc sulphate (0.6%) significantly improved the fruit breadth and weight in guava cv. Bhavnagar Red. Fruit dimension and weight were reported maximum through application of zinc (10.5 g/20L) in apple cv. Anna (Asaad, 2014). Singh and Banik (2011) reported that application of zinc (0.5%) increased the fruit size in mango cv. Himsagar. Arabloo et al. (2017) recorded maximum fruit weight and firmness through application of amino acid (4 mg/L) in apple cv. Granny Smith. Mosa et al. (2020) noted improved fruit length, breadth, weight and firmness with the application of glycine (50 ppm) in apple cv. Anna. Singh et al. (2008) reported highest fruit weight through application of vermicompost (10 t/ha) in strawberry cv. Chandler. Zuo et al. (2018) recorded maximum fruit weight through application of 50 per cent of vermicompost in strawberry cv. Yanli. Application of AM fungi (200 g/tree) reported increased fruit length, diameter and weight in guava cv. L-49 (Dey et al., 2005). Chandra et al. (2012) revealed that inoculation of AM fungi at 400 g significantly improved the fruit weight in guava.

Taha and El-Shahat (2017) revealed that application of *Azolla* extract (1 L/20L) + humic acid (200 mL/20L) showed significantly higher fruit length, breadth and diameter in apricot cv. Canino. Taha et al. (2018) recorded maximum fruit length, breadth and weight through application of *Azolla* (5 kg/20L) in apple cv. Anna. Kumari et al. (2018) observed improved fruit length, breadth and weight through inoculation of PGPR (*B. subtilis*) in strawberry cv. Chandler. Mosa et al. (2015) noted increase in fruit length, breadth and weight through application of humic acid at 5 per cent in apple cv. Anna. Morsey et al. (2015) revealed that application of humic acid at 30 g/tree exhibited maximum fruit length and breadth in apple cv. Anna. Al-Saif et al. (2023) observed that fruit length, breadth and weight were increased through application of humic acid (2000 mg/L) applied four times in apricot cv. Canino. Increased fruit weight was exhibited through application of amino acid (2 mL/L) and humic acid (1 g/L) in strawberry cv. Festival (Shehata et al., 2011). Maximum fruit weight and volume were exhibited through application of humic acid (30 mL/tree) in Egyptian lime (Ennab, 2016). Ferrara and Brunetti (2010) observed significantly improved fruit breadth and weight through application of humic acid at 100 mg/L at full bloom in grapes cv. Italia. Hagagg et al. (2013) recorded maximum fruit weight and shape index through application of humic acid (0.5%) at beginning of fruit set in olive cv. Aggizi. Ashwini et al. (2022) reported highest fruit length, breadth and weight through application of AM fungi (25 g/plant) + PGPR (25 mL/plant) + humic acid (60 mL/L) in guava cv. Lalit.

2.4.2 Biochemical

Bastam et al. (2020) noted maximum increase in phenolic content through application of zinc amino acid chelate (0.2%) in olive cv. Beledy. Hassan et al. (2010) observed significant increase in TSS and reduction in titratable acidity through application of zinc amino acid chelate (0.25%) in plum cv. Hollywood. Maximum TSS content was noted through application of zinc amino acid chelate (0.5%) in agronomic crops (Rafie et al., 2017). Zawi and Mejbel (2022) recorded maximum ascorbic acid content through application of nano zinc (2 mg/L) and amino acid (2 mg/L) in agronomic crops. Rahimi et al. (2022) noted highest TSS content and total phenols through application of nano zinc (2 g/L) + amino acid (2 mL/L) + humic acid (2 g/L) in agronomic crops. Al-Khaqani and Aboohanah (2021) recorded maximum ascorbic acid content with the application of nano zinc amino acid chelate at 2 mL/L in agronomic crops. Kasem and Ibrahiem (2021) found that application of amino acid + zinc (0.8%) significantly increased TSS, reducing and total sugars and decreased titratable acidity in fig cv. Sultani. Souri et al. (2017) reported increase in TSS and ascorbic acid content through conjoint application of zinc (2.5%) + amino acid (2%) in agronomic crops. Kheir et al. (2021) recorded maximum TSS and total sugars through application of amino acid (2 mg/L) + zinc (2 g/L) in mango cv. Fagri Kalan. El-Badawy (2019) stated that maximum TSS, total sugars and ascorbic acid content were noted through application of amino acid (3 mL/L) and zinc (150 ppm) in apricot cv. Canino. Shalan (2020) noted highest TSS, total sugars, anthocyanin content and lowest titratable acidity through application of zinc (0.5%) + amino acid (150 mL/tree) in datepalm cv. Zaghloul.

Tavallali and Karimi (2017) recorded that application of zinc glycinate (20 mg/kg) exhibited increased ascorbic acid and phenolic content in pistachionut cv. Badami. Aslani and Souri (2018) observed maximum TSS content and ascorbic acid content through application of zinc (2.5%) + glycine (2%) in agronomic crops. Almutairi et al. (2022) observed significant increase in TSS, reducing sugars, non-reducing sugars, total sugars, ascorbic acid content and decrease in titratable acidity through foliar application of glycine (1000 ppm) in guava cv. Maamoura. Noroozlo et al. (2019a) revealed that ascorbic acid content was improved through application of glycine (500 mg/L) in agronomic crops. Maximum ascorbic acid content was exhibited through application of glycine at 500 mg/L in agronomic crops (Noroozlo et al., 2019b). Mohammad et al. (2017) found that foliar application of zinc sulphate (0.1%) exhibited maximum TSS, total sugars and ascorbic acid in apple cv. Red Delicious. Amiri et al. (2008) observed maximum TSS through foliar application of zinc sulphate (110 g/tree) in apple cv. Golden Delicious. Arabloo et al. (2017) noted increased TSS through application of amino acid (2 mg/L) in apple cv. Granny Smith. Asaad (2014) observed highest TSS and total sugars with the application of zinc (10.5 g/20L) in apple cv. Anna. Increased TSS, reducing, non-reducing and total sugars was observed with the application of glycine (50 ppm) in apple cv. Anna (Mosa et al. 2020). Shehata et al. (2011) found that application of amino acid (2 mL/L) and humic acid (1 g/L) increased the TSS and anthocyanin content in strawberry cv. Festival.

Zuo et al. (2018) recorded maximum TSS and ascorbic acid content through application of 50 per cent of vermicompost in strawberry cv. Yanli. Singh et al. (2008) noted highest TSS and ascorbic acid content and lowest titratable acidity through application of vermicompost (10 t/ha) in strawberry cv. Chandler. Taha et al. (2018) revealed that application of *Azolla* at 5 kg/20L exhibited maximum reducing sugars, total sugars and ascorbic acid content in apple cv. Anna. Taha and El-Shahat (2017) revealed that application of *Azolla* extract (1 L/20L) + humic acid (200 mL/20L) showed significantly higher TSS, total sugars, ascorbic acid content and lower tiratable acidity in apricot cv. Canino. Huang et al. (2020) reported maximum TSS content when inoculated with AM fungi (*Rhizophagus irregularis*) in apple seedlings. Application of AM fungi significantly improved the fruit quality in fruit cuttings of passion fruit (Womocho et al., 2001). Chandra et al. (2012) noted maximum TSS through inoculation of AM fungi at 400 g in guava. Kumari et al. (2018) stated that inoculation of PGPR (*B. subtilis*) inferred increase in TSS, reducing sugars, non-reducing sugars, total sugars, ascorbic acid, anthocyanin content and decrease in titratable acidity in strawberry cv. Chandler. Ashwini et al. (2022) recorded maximum TSS, total sugars, ascorbic acid content and minimum titratable acidity through application of AM fungi (25 g/plant) + PGPR (25 mL/plant) + humic acid (60 mL/L) in guava cv. Lalit. Mosa et al. (2015) observed highest TSS, reducing sugars, non-reducing sugars, total sugars, anthocyanin content and lowest titratable acidity through application of humic acid (5%) in apple cv.

Anna. Highest fruit TSS and lowest titratable acidity was exhibited through application of humic acid (30 g/tree) in apple cv. Anna (Morsey et al., 2015). Al-Saif et al. (2023) observed highest TSS, reducing sugars, non-reducing sugars, total sugars, ascorbic acid content and lowest titratable acidity through application of humic acid (2000 mg/L) applied four times in apricot cv. Canino. Ennab (2016) revealed that maximum TSS and mnimum titratable acidity were noted through application of humic acid (30 mL/tree) in Egyptian lime. Ferrara and Brunetti (2010) recorded maximum TSS and minimum titratable acidity through application of humic acid (100 mg/L) at pre bloom and full bloom stages in grapes cv. Italia. Hagagg et al. (2013) recorded decreased titratable acidity through application of humic acid (0.5%) at beginning of fruit set in olive cv. Aggizi.

2.5 SOIL CHEMICAL PROPERTIES

Raza et al. (2023) observed decrease in soil pH and electrical conductivity (EC) through application of zinc glycinate at 12 mg/kg as compared to control soil. They also reported significant increase in available K content compared to zinc sulphate. Moreira et al. (2015) reported increased available P, DTPA extractable Cu, Fe, Zn content and decreased soil pH through foliar application of zinc (12 mg/kg) and amino acid chelate (0.005%) compared to control soil. They also observed maximum available K and DTPA extractable Mn content with the application of zinc (8 mg/kg) and amino acid chelate (0.005%) compared to control. Khardia et al. (2022) recorded highest available N, K, Zn and Cu content through foliar application of nano zinc (1.25 mL/L) in agricultural field. Amiri et al. (2008) noted that available N, P, K and DTPA extractable Cu, Fe, Zn, and Mn were exhibited maximum through foliar application of zinc sulphate (110 g/tree) in apple cv. Golden Delicious. Rousk et al. (2012) recorded significant increase in soil pH through application of zinc sulphate (0.5-2.5 mmol/g) in organic and mineral soil. Boaretto et al. (2002) observed significant influence on DTPA extractable Zn content through foliar application of zinc sulphate at 0.7 mg/L in orange.

Singh et al. (2020) noted maximum organic carbon content (OC), available N, P and K content through application of vermicompost (10 kg/plant) in custard apple cv. Raydurg. Adak et al. (2014) stated that application of vermicompost at (10 kg/tree) exhibited maximum OC content in soils of mango orchard. Mahmud et al. (2020) found increase in soil N, P, K, Ca, Mg and Zn content through application of vermicompost (10 t/ha) in pineapple. Taha et al. (2018) found that application of *Azolla* at 5 kg/20L inferred minimum pH, EC and maximum OC content in apple cv. Anna. Taha and El-Shahat (2017) observed increase in

OC, available N, P and K content through application of *Azolla* extract (1 L/20L) and humic acid (200 mL/20L) in apricot cv. Canino. El-Kamar (2020) reported decrease in soil pH and electrical conductivity through application of humic acid (8 g/L) compared to untreated soil. Chauhan (2008) noted significant improvement in OC, available N, P, K and exchangeable Ca content through application of vermicompost (20 kg/tree) and AM fungi (60 g/tree) in plum. Baba et al. (2010) observed maximum OC content through application of PGPR (*Bacillus* sp.) in strawberry. Gogoi et al. (2010) reported enhanced soil chemical properties through AM fungi + PGPR application in guava. Kholer et al. (2007) noted that application of AM fungi significantly improved the OC content compared to control soil. Ashwini et al. (2022) reported maximum OC, available N, P and K content through application of AM fungi (25 g/plant) + PGPR (25 mL/plant) + humic acid (60 mL/L) in guava cv. Lalit. Ennab (2016) observed maximum available N, P, K and DTPA extractable Fe, Zn, Mn content through application of humic acid (30 mL/tree) in Egyptian lime. Thomas et al. (2022) noted increase in available N, P and K content through application of amino acid (5%) + AM fungi (20 g/plant) + PGPR (50 mL/plant) + humic acid (39%) in apple seedling rootstock.

2.6 SOIL MICROBIOLOGICAL PROPERTIES

2.6.1 Total viable microbial count

Khardia et al. (2022) observed significant improvement in bacteria, fungi and antinobacterial count through foliar application of nano zinc (1.25 mL/L) in agronomic crops. Singh et al. (2020) recorded maximum soil bacterial, fungal and actinobacterial count through application of vermicompost (10 kg/plant) in custard apple cv. Raydurg. Adak et al. (2014) stated that application of vermicompost at 10 kg/tree exhibited maximum bacterial, fungal and actinobacterial count in soils of mango orchard. Chauhan (2008) recorded significantly improved soil bacterial, actinobacterial, *Azotobacter* and AM fungal count through application of vermicompost (20 kg/tree) and AM fungi (60 g/tree) in plum rhizosphere. Choudhary et al. (2022) reported maximum soil bacterial, fungal and actinobacterial count through application of vermicompost at 24.79 kg/plant in pomegranate cv. Bhagwa. Das et al. (2017) found that inoculation with AM fungi significantly increased the soil microbial population in guava cv. L-49. Aseri et al. (2008) recorded maximum AM fungi spores through inoculation of AM fungi in pomegranate. Maximum root colonization was observed through AM fungi application in pecan seedlings (Joolka et al., 2004). Wu et al. (2011) noted improved AM fungi population through application of AM fungi (*Glomus mosseae, G. versiformae* and *Paraglomus occultum*) in the rhizosphere of peach.

Taha et al. (2018) observed maximum bacterial population through application of *Azolla* at 5 kg/20L in rhizosphere of apple cv. Anna. Taha and El-Shahat (2017) inferred increase in total bacterial count through application of *Azolla* extract (1 L/20L) + humic acid (200 mL/20L) in apricot cv. Canino. Thomas et al. (2022) observed maximum bacteria, fungi and actinomycetes count through application of amino acid (5%) + AM fungi (20 g/plant) + PGPR (50 mL/plant) + humic acid (39%) in apple seedling rootstock. Kumar et al. (2020) noted maximum bacteria, fungi, actinomycetes, potassium solubilizing bacteria (KSB), *Azotobacter* and AM fungi count through inoculation of PGPR consortium (250 g) in strawberry cv. Chandler. Ashwini et al. (2022) stated that maximum actinomycetes, phosphorus solubilizing bacteria (PSB), *Azotobacter* and AM fungi count was reported through application of AM fungi (25 g/plant) + PGPR (25 mL/plant) + humic acid (60 mL/L) in guava cv. Lalit. Ram and Pathak (2007) reported increased bacterial count through humic acid application in rhizosphere of guava soils. Ennab (2016) found that application of humic acid exhibited maximum soil microbial activity in Egyptian lime.

2.6.2 Soil enzymes

Khardia et al. (2022) observed maximum acid phosphatase, alkaline phosphatase and dehydrogenase activity through foliar application of nano zinc (1.25 mL/L) in agronomic crops. Yang et al. (2006) reported increased activity of alkaline phosphatase through application of zinc (400 mg/kg) compared to control soil. Gocmez et al. (2019) noted that application of vermicompost at 40 per cent exhibited maximum alkaline phosphatase and dehydrogenase activity in olive cv. Gemlik. Maximum dehydrogenase activity was recorded through application of vermicompost (10 kg) in soils of mango orchard (Adak et al., 2014). Kumar et al. (2018) observed maximum dehydrogenase activity through application of 50 per cent of N through vermicompost in apple cv. Oregon Spur. Zuo et al. (2018) recorded improved soil enzymatic activity through application of 50 per cent of vermicompost in rhizosphere of strawberry cv. Yanli. Guo et al. (2023) noted that application of vermicompost at 60 per cent inferred maximum enzymatic activity in agronomic crops. Kumar et al. (2020) found that inoculation of PGPR consortium at 250 g recorded maximum acid phosphatase, alkaline phosphatase and dehydrogenase enzyme in strawberry cv. Chandler. Aseri et al. (2008) observed increase in alkaline phosphatase and dehydrogenase enzyme through inoculation of AM fungi (*G. mosseae*) in pomegranate. Taha and El-Shahat (2017) stated that application of *Azolla* extract (1 L/20L) and humic acid (200 mL/20L) exhibited increase in dehydrogenase activity in apricot cv. Canino.

2.7 PHYSIOLOGICAL PARAMETERS

Panahi et al. (2023) reported significant increase in total chlorohyll content through foliar application of zinc amino acid chelate at 4 per cent applied during Hababook and Khalal stage in datepalm cv. Mazafati. Al-Juthery et al. (2019) noted maximum total chlorophyll content through combined application of nano zinc and amino acid (400 L/ha) in agronomic crops. Jalali et al. (2019) recorded significantly improved chlorophyll content through application of zinc amino acid chelate (0.2%) in agronomic crops. Kalaiselvan et al. (2021) recorded maximum chlorophyll content through application of zinc amino acid chelate at 0.5 per cent in agronomic crops. Highest chorophyll content was noted through application of zinc amino acid chelate (2 mL/L) in agronomic crops (Mirsardoo et al., 2023). Rizwan et al. (2017) observed maximum chlorophyll content through application of zinc amino acid chelate at 30 mg/L in agronomic crops. Moreira et al. (2015) noted highest chlorophyll content and maximum photosynthetic efficiency through application of zinc (16 mg/kg) and amino acid chelate (0.005%) in agronomic crops. Hussain et al. (2018) recorded maximum chlorophyll content and stomatal conductance through foliar application of zinc amino acid chelate (30 mg/L) in agronomic crops. Yeboah et al. (2021) reported maximum chlorophyll content and stomatal conductance through application of zinc amino acid chelate at 8 per cent in agronomic crops. Al-Khaqani and Aboohanah (2021) observed maximum chlorophyll and leaf carbohydrate content through application of nano zinc amino acid chelate (2 mL/L) in agronomic crops.

Krishnaraj et al. (2020) observed highest chlorophyll content and leaf area index with the application of zinc amino acid chelate (0.2%) in agronomic crops. Leaf chlorophyll and fruit carbohydrate content was reported maximum with the application of zinc amino acid chelate (6%) in agronomic crops (Tabesh et al., 2020). Raza et al. (2023) noted that application of zinc glycinate at 12 mg/kg exhibited significant increase in chlorophyll content, photosynthetic efficiency, transpiration rate and stomatal conductance in agronomic crops. Kasem and Ibrahiem (2021) reported maximum chlorophyll content through application of amino acid + zinc at 0.8 per cent during first week of March, after fruit set and one month later in fig cv. Sultani. Souri et al. (2017) recorded maximum chlorophyll content through conjoint application of zinc (2.5%) and amino acid (2%) in agronomic crops. Elshamly et al. (2024) stated that maximum chlorphyll content and leaf carbohydrate was noted through application of zinc (1.25 kg/ha) + amino acid (200 mL/L) in agronomic crops. Kheir et al. (2021) observed that application of amino acid (2 mg/L) and zinc (2 g/L) exhibited maximum carbohydrate and chlorophyll content in mango cv. Fagri Kalan. Shalan

(2020) noted maximum chlorophyll content through application of zinc (0.5%) + amino acid (150 mL/tree) in datepalm cv. Zaghloul. Bashir et al. (2021) recorded highest chlorophyll content through application of zinc amino acid chelate (1.5%) in agronomic crops. Rahimi et al. (2022) reported maximum chlorophyll content through application of nano zinc (2 g/L) + amino acid (2 mL/L) + humic acid (2 g/L) in agronomic crops.

Li et al. (2023) noted maximum chlorophyll content through application of zinc glycinate (150 mg/L) in agronomic crops. Mirbolook et al. (2020b) stated that maximum chlorophyll content was observed through application of zinc glycinate at 10 µM in agronomic crops. Increased total chlorophyll content was exhibited by the application of zinc gluconate in agronomic crops (Gourkhede et al., 2018). Mosa et al. (2020) reported maximum chlorophyll content through application of glycine at 50 ppm in apple cv. Anna. Mohammadipour and Souri (2019) revealed that maximum chlorophyll content was noted through foliar application of glycine at 500 mg/L in agronomic crops. Noroozlo et al. (2019a) observed maximum chlorophyll content through application of glycine (500 mg/L) in agronomic crops. Highest chlorophyll content was recorded through applicaton of glycine at 500 mg/L in agronomic crops (Noroozlo et al., 2019b). Zuo et al. (2018) recorded improved chlorophyll content through application of 50 per cent of vermicompost in strawberry cv. Yanli. Mahmud et al. (2019) observed increase in total chlorophyll content through two times application of vermicompost (10 t/ha) in pineapple. Huang et al. (2020) noted increase in total chlorophyll content, photosynthetic efficiency, transpiration rate and stomatal conductance through inoculation with AM fungi (*Rhizophagus irregularis*) in apple seedlings. Taha and El-Shahat (2017) recorded maximum chlorophyll content through conjoint application of *Azolla* extract (1 L/20L) + humic acid (200 mL/20L) in apricot cv. Canino. Kumari et al. (2018) revealed that inoculation of PGPR (*B. subtilis*) inferred increase in chlorophyll content, photosynthetic efficiency, transpiration rate and stomatal conductance in strawberry cv. Chandler. Junior et al. (2019) found that maximum chlorophyll content was recorded through application of humic acid (150 mg/L) in mangosteen seedlings. Al-Saif et al. (2023) recorded highest chlorophyll content through four times application of humic acid (2000 mg/L) in apricot cv. Canino.

2.8 NUTRIENT DYNAMICS

2.8.1 Leaf nutrients

Hassan et al. (2010) observed highest leaf N, P, K and Zn content through application of zinc amino acid chelate (0.25%) in plum cv. Hollwood. Kamiab and Zamanibahramabadi (2016) found that application of zinc amino acid chelate at 2 g/L significantly increased leaf

Zn content in almond. Koksal et al. (1999) observed increased leaf Zn content with the application of zinc amino acid chelate at 0.2, 0.4 and 0.6 per cent in pear cv. Williams. Najizadeh and Khoshgoftarmanesh (2019) recorded improved leaf Zn content through zinc amino acid chelate (3 L/tree) in pistachionut cv. Akbari. Panahi et al. (2023) reported increase in leaf K and Mg content through foliar spray of zinc amino acid chelate (4%) at Hababook and Khalal stage in datepalm cv. Mazafati. Wang et al. (2020) observed maximum leaf Zn content through foliar application of zinc (0.4%) + amino acid (0.1%) in agronomic crops. Baghaie and Keshavarzi (2021) recorded maximum leaf Zn content through zinc amino acid chelate (0.2%) in agronomic crops. Hadi et al. (2020) reported that zinc amino acid chelate (5 µM) influenced leaf Zn content in agronomic crops. Hussain et al. (2018) noted maximum leaf Zn content with the application of zinc amino acid chelate (30 mg/L) in agronomic crops. Highest leaf Zn content was recorded through application of zinc amino acid chelate (1.5%) in agronomic crops (Bashir et al., 2021). Al-Khaqani and Aboohanah (2021) reported that nano zinc amino acid chelate (2 mL/L) exhibited significant improvement in leaf N, K and Zn content of agronomic crops. Rahimi et al. (2022) revealed that highest leaf N, P and K content was exhibited through application of nano zinc (2 g/L) + amino acid (2 mL/L) + humic acid (2 g/L) in agronomic crops.

Leaf N, P, K and Zn were recorded highest through application of amino acid (3 mL/L) and zinc (150 ppm) in apricot cv. Canino (El-Badawy, 2019). Shalan (2020) noted maximum leaf N, P, K, Ca, Mg and Zn content through application of zinc (0.5%) + amino acid (150 mL/tree) in datepalm cv. Zaghloul. Kasem and Ibrahiem (2021) observed maximum N, P, K, Mg and Zn content with the application of amino acid + zinc (0.8%) during first week of March, after fruit set and one month later in fig cv. Sultani. Elshamly et al. (2024) reported maximum leaf N, P, K, Ca, Mg and Zn content through application of zinc (1.25 kg/ha) and amino acid (200 mL/L) in agronomic crops. Souri et al. (2017) exhibited significant improvement in leaf N content through application of zinc (2.5%) and amino acid (2%) in agronomic crops. Mirzapour et al. (2019) noted increased leaf Zn content through application of zinc + amino acid (10 µm) in agronomic crops. Maximum leaf N and Zn content was exhibited through application of zinc glycinate (10 µM) in agronomic crops (Mirbolook et al., 2020b). Maximum leaf Zn content was observed with zinc glycinate (0.2%) in olive cv. Beledy (Bastam et al., 2020). Mohammadi and Khoshgoftarmanesh (2014) recorded highest increase in leaf Ca and Zn content through application of zinc glycinate (2 mmol) in agronomic crops. Ghasemi et al. (2013) found significant improvement in leaf Zn content through application of zinc glycinate (10 µM) in agronomic crops. Li et al. (2023) observed significant increase in leaf

Zn content through application of zinc glycinate (150 mg/L) in agronomic crops. Tavallali et al. (2018) reported maximum leaf Zn content when zinc glycinate at 0.2 per cent was sprayed after thinning and before flowering in agronomic crops. Soltani et al. (2022) found maximum leaf Zn content through application of zinc glycinate at 1.5 kg/ha in agronomic crops. Almutairi et al. (2022) observed that foliar application of glycine (1000 ppm) exhibited significant increase in leaf N, P and K content in guava cv. Maamoura.

Asaad (2014) recorded maximum leaf Zn content through application of zinc (10.5 g/20L) in apple cv. Anna. Increased leaf N, P, K, Ca and Zn content was noted through application of glycine (50 ppm) in apple cv. Anna (Mosa et al., 2020). Mohammadipour and Souri (2019) stated that foliar application of glycine at 500 mg/L exhibited maximum leaf N, K and Ca content in agronomic crops. Noroozlo et al. (2019a) noted maximum leaf N, K, Ca, Mg and Zn content through application of glycine (500 mg/L) in agronomic crops. Amiri et al. (2008) revealed increase in leaf N, P, K Ca, Mg and Zn content through foliar application of zinc sulphate (110 g/tree) in apple cv. Golden Delicious. Mahmud et al. (2020) reported increase in leaf P, K and Mg content through application of vermicompost at 10 t/ha in pineapple. Taha and El-Shahat (2017) recorded maximum leaf N, P, K and Mg content through application of *Azolla* extract (1 L/20L) and humic acid (200 mL/20L) in apricot cv. Canino. Taha et al. (2018) noted increase in leaf N, P, K and Mg content through application of *Azolla* (5 kg/20L) in rhizosphere of apple cv. Anna. Shamshiri et al. (2012) stated that maximum leaf N, P, K, Ca, Mg and Zn content was exhibited through application of AM fungi (100 g/tree) in mandarin cv. Kinnow. Chandra et al. (2012) found that application of AM fungi at 400 g increased the leaf N and P content in guava. Ibraheim et al. (2018) noticed maximum leaf N, P, K content through inoculation of AM fungi and PGPR in guava. Wang et al. (2016) found that leaf N and P content were significantly improved through application of AM fungi and PGPR in trifoliate oranges.

Erdogan et al. (2018) stated that PGPR application increased leaf N, P and K content in grapes. Mosa et al. (2015) recorded maximum leaf N, P, K, Ca, Mg and Zn content through foliar application of humic acid (5%) in apple cv. Anna. Morsey et al. (2015) noted increase in leaf N, P, K and Zn content through application of humic acid (30 g/tree) in apple cv. Anna. Al-Saif et al. (2023) revealed increase in leaf N, P, K, Ca, Mg and Zn content through application of humic acid (2000 mg/L) applied four times in apricot cv. Canino. Sotiropoulos et al. (2024) observed highest leaf N, P, K, Ca and Mg content through application of humic acid (0.5%) applied at petal fall stage and after twenty days in olive cv. Koroneiki. Asgharzade and Babaeian (2012) found that application of humic acid (300 mg/kg) significantly improved leaf

P and K content in grapes. Baldotto et al. (2009) found maximum leaf N, P and K content through humic acid application in pineapple. Mahmoudi et al. (2013) reported that humic acid (100 mg/L) application exhibited highest leaf N, P and K content in kiwifruit. Ashwini et al. (2022) recorded maximum leaf N, P and K content through application of AM fungi (25 g/plant) + PGPR (25 mL/plant) + humic acid (60 mL/L) in guava cv. Lalit.

2.8.2 Fruit nutrients

Al-Juthery et al. (2019) reported highest fruit N, P, K and Zn content through conjoint application of nano zinc and amino acid (400 L/ha) in agronomic crops. Najizadeh and Khoshgoftarmanesh (2019) recorded improved nut Zn content with the application of zinc amino acid chelate (3 L/tree) in pistachionut cv. Akbari. Bastam et al. (2020) reported significant increase in fruit Zn content with the application of zinc amino acid chelate (0.2%) in olive cv. Beledy. Highest fruit K content was observed through application of zinc amino acid chelate (2 mL/L) in agronomic crops (Mirsardoo et al., 2023). Panahi et al. (2023) reported increase in fruit Ca and Mg content through foliar application of zinc amino acid chelate (2%) applied during Hababook and Khalal stage in datepalm cv. Mazafati. Sun et al. (2018) stated that fruit Zn content was increased through foliar application of zinc amino acid chelate (70 mL/m^2) applied 3 times at weekly intervals in agronomic crops. Maximum leaf Zn content was recorded through application of zinc amino acid chelate at 0.2 per cent in agronomic crops (Baghaie and Keshavarzi, 2021). Rizwan et al. (2017) reported increased fruit Zn content with the application of zinc amino acid chelate (30 mg/L) in agronomic crops. Tabesh et al. (2020) observed that application of zinc amino acid chelate at 6 per cent increased fruit N content in agronomic crops. Wei et al. (2012) recorded maximum leaf Zn content through application of zinc amino acid chelate (0.2%) in agronomic crops.

Maximum fruit Zn content was observed when zinc + amino acid (10 µm) was applied in agronomic crops (Mirzapour et al., 2019). Bashir et al. (2021) noted maximum fruit Zn content through application of zinc amino acid chelate (1.5%) in agronomic crops. Wang et al. (2020) reported significant increase in fruit N, P, K and Zn content through application of zinc and amino acid (0.01%) in agronomic crops. Maximum fruit Ca content was exhibited through application of amino acid (4 mg/L) in apple cv. Granny Smith (Arabloo et al., 2017). Shalan (2020) observed maximum fruit Ca content through application of zinc (0.5%) + amino acid (150 mL/tree) in datepalm cv. Zaghloul. Ghasemi et al. (2013) also reported increase in fruit Zn and Fe content when zinc glycinate was applied at 0.2 per cent compared to zinc sulphate (0.2%) in agronomic crops. Xu et al. (2022) observed increase in fruit Zn content when zinc glycinate was applied at 0.2 per cent in agronomic crops. Amiri et al. (2008) recorded increase in fruit P, K Ca, Mg content and K/Ca, (K+Mg)/Ca fruit nutrient ratio through foliar application of zinc sulphate (110 g/tree) in apple cv. Golden Delicious. Taha and El-Shahat (2017) reported highest fruit P, K, Ca, Mg and Zn content through application of *Azolla* extract (1 L/20L) + humic acid (200 mL/20L) in apricot cv. Canino.

2.9 ANTIOXIDANTS

Wei et al. (2012) noted minimum phytic acid content through application of zinc amino acid chelate (0.2%) in agronomic crops. Wang et al. (2020) observed significant decrease in phytic acid content and PA: Zn through application of Zn + amino acid at 0.01 per cent in agronomic crops. Seddigh et al. (2016) observed lower phytic acid content through application of zinc glycinate (40 mg/kg) compared to zinc sulphate in agronomic crops. Raza et al. (2023) revealed that application of zinc glycinate at 12 mg/kg showed significant decrease in phytic acid (PA) content and PA: Zn in agronomic crops. Decrease in phytic acid content and PA: Zn was exhibited through application of zinc glycinate (0.25%) in agronomic crops (Mirbolook et al., 2020b). Ghasemi et al. (2013) revealed that application of zinc glycinate (0.2%) showed decrease in phytic acid compared to zinc sulphate in agronomic crops. Baligah et al. (2020) noted reduction in phytic acid content and PA: Zn through application of zinc gluconate (2.5 kg/ha) as compared to zinc sulphate in agronomic crops.

3.0 ZINC BIOFORTIFICATION

More than half of the global population in developing nations predominantly suffers from micronutrient malnutrition (Gupta et al., 2015) such as Zn, posing significant challenges to their overall health and well-being (Zaman et al., 2018). While insufficient dietary Zn intake might contribute to suboptimal Zn levels in some cases, another common factor is low bioavailability of Zn (Ghasemi et al., 2013). In certain scenarios, for directly meeting the nutritional requirements of humans by facilitating the production of healthy edible parts, crop biofortification emerges as a wholesome approach (Bhatt et al., 2020). Zn absorption is further affected by some dietary elements such as, phytic acid (Gargari et al., 2007). Phytic acid plays a crucial role as a chelator of Zn in food products, potentially hindering the bioavailability, absorption and digestion of Zn in the human body (Welch and Graham, 2004). As phytic acid is recognized as anti-nutritional factor (Hotz and Gibson, 2007), lowering its levels in food will enhance Zn bioavailaility (Praharaj et al., 2021). Unlike phytic acid, higher concentration of amino acids and protein can enhance the availability of microelements (Ghasemi et al., 2014). Zn functionality in biological system heavily relies on protein, as protein plays significant role in facilitating catalytical activity of Zn (Hoell et al., 2010). The application of chelated amino acid Zn fertilizers has been known to increase Zn bioavailability in plants compared to conventional form (Ghasemi et al., 2013). Moreover, the increase in Zn biofortification is reported through foliar application over soil application (Ram et al., 2011). Graham et al. (2001) elucidated that the breakdown of proteins during digestion liberate amino acids, which could subsequently bind with phytic acid, thereby augmenting the absorption of Zn.

Recently, ursolic acid, a pentacyclic terpenoid have emerged as a potential group of polyphenols for mitigating global health issues (Odun-Ayoa et al., 2022). Earlier documented research indicates that ursolic acid exhibits numerous pharmacological activities encompassing, antitumorous, anti-inflammatory, neuroprotective, cardioprotective and antidiabetic properties (Yamaguchi et al., 2008). Polyphenol compounds recognized as biologically active metabolites with antioxidant potential are highly recognized as health benefiting substances in fruits (Slavin and Lloyd, 2012). Apple peel is the major constituent of polyphenols, and thereby is gaining attention (Wolfe et al., 2003). Besides, the application of organic Zn is also known to stimulate the accumulation of phenolic compounds and augment the antioxidant capacity in apples (Soppelsa et al., 2018).

Chapter - 3

MATERIALS AND METHODS

The current study, **"Zinc biofortification through organic Zn and bio-organic supplements in high density plantation of apple (*Malus* × *domestica* Borkh.)"** was carried out during two consecutive years between 2022 and 2023. The detail of the material and methodologies employed during the course of study has been elucidated under the following heads and sub-heads:

3.1 GEOGRAPHICAL SITE

The experiment was undertaken at the Experimental block of Department of Fruit Science, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh. The study area falls within the sub-temperate zone with geographical coordinates of 30°51′49″ North latitude and 77°9′23″ East longitude at an elevation of 1279 m above mean sea level. The maximum mean air temperature during the experiment was 25.83 ºC and minimum mean air temperature was 11.20 ºC during the cropping period. The annual rainfall ranged between 140 to 145 cm in the months of July and September accounting for around 80 per cent of the total.

3.2 EXPERIMENTAL DETAIL

3.2.1 Layout and Planting Material

The experimental orchard of healthy budded feathered plants of apple was established in 2019. Four-year-old uniform apple trees of the cultivar, Gala Schniga Schnico budded on EMLA 111 (scion-stock combination) were selected. Trees were spaced at 2.5 × 2.0 m with planting density of 2000 plants per hectare. Pollinizer trees within the experimental orchard were maintained in the ratio of 3:1 (variety: pollinizer). Selected plants were kept under standard and uniform cultural practices including protection measures, irrigation scheduling and weeding operations etc. during the course of investigation. Observations on vegetative growth characteristics, flowering, yield contributing traits and quality attributes of fruit samples were recorded.

3.2.2 The edaphic conditions

During the course of investigation, two sets of experiments were laid out to understand Zn nutritional aspects of fruit production. The experimental soil was collected at 0-30 cm depth to determine the initial chemical and biological characteristics of soil. In the first set of experiment, the soil exhibited as sandy clay loam in texture (pH 6.82) in a 1:2 w/v soil-water suspension (Jackson, 1973), electrical conductivity of 0.20 dS/m, organic carbon content of 1.73 per cent (Walkey and Black, 1934), alkaline $KMnO_4$ extractable-N of 283.41 kg/ha (Subbiah and Asija, 1956), available $NaHCO_3$-extractable P of 97.68 kg/ha (Olsen *et al.*, 1954), and available NH_4OAC-K of 194.35 kg/ha (Merwin and Peach, 1951). DTPA-extractable micronutrient cations *viz.*, Zn (8.24 mg/kg), Fe (20.31 mg/kg), Cu (10.75 mg/kg) and Mn (4.36 mg/kg) were determined according to Lindsay and Norvell (1978). The initial microbial biomass in the rhizosphere soil as colony forming units per g of soil (cfu/g) of bacteria (11.52×10^6), soil fungi (2.43×10^3), actinomycetes (5.34×10^4), *Azotobacter* (1.12×10^5), P-solubilizing bacteria (PSB, 2.30×10^4), K-solubilizing bacteria (KSB, 3.43×10^5), and Zn-solubilizing bacteria (ZSB, 5.67×10^5) were recorded. Furthermore, the initial viable count of AM fungi spores corresponds to 70.84 spores per 50 g of the moist soil.

In the second set of experiment, the soil was sandy clay loam in texture, with a pH of 6.60, electrical conductivity of 0.17 dS/m, organic carbon content of 1.77 per cent, alkaline $KMnO_4$ extractable-N of 295.67 kg/ha, available $NaHCO_3$-extractable P of 98.11 kg/ha and available NH_4OAC-K of 198.75 kg/ha. The concentration of DTPA-extractable micronutrient cations (7.3 ± 0.05 pH) namely, Zn, Fe, Mn and Cu were 8.85, 21.73, 4.93 and 12.02 mg/kg, respectively. Initial assessment of microbial population revealed total bacteria, soil fungi, actinomycetes, *Azotobacter*, PSB, KSB and ZSB with corresponding values of 12.81×10^6, 3.22×10^3, 5.75×10^4, 2.03×10^5, 2.45×10^4, 3.87×10^5 and 6.34×10^5 cfu/g, respectively. Besides, the initial enumeration of viable spore count of AM fungi indicated 77.51 spore per 50 g of the moist soil.

3.2.3 Fertilizer inputs

The relative effectiveness of various organic Zn nutrient sources and bio-organics on growth patterns, fruit quality and soil properties of apple was studied.

3.2.3.1 Organic Zn nutrient sources

Five Zn fertilizer sources namely, zinc glycinate (Zn-Gly), zinc gluconate (Zn-Glu), zinc amino acid chelate (Zn-AAC), nano zinc amino acid chelate (NZn-AAC) along with zinc sulphate ($ZnSO_4$) were used in the experiment. Zn nutrient analogue including, i) Zn-Gly contained 15 per cent Zn (15% Zn) with average particle size of 0.55 nm, ii) 15 per cent Zn chelated by Zn-Glu with 99.5 per cent purity, iii) Zn-AAC (15% Zn), iv) NZn-AAC (12% Zn) with 99.9 per cent purity, 10 per cent solubility, and 4-6 per cent of total N content (w/w) and v) $ZnSO_4$ (33% Zn). The required amount of each Zn fertilizer source was weighed and the final volume was made to one liter. The stock solution of different Zn nutrient sources including, Zn-Gly (6.6 g/L), Zn-Glu (6.6 g/L), Zn-AAC (6.6 g/L), NZn-AAC (8.3 g/L) and $ZnSO_4$ (3.0 g/L) was prepared using distilled water.

3.2.3.2 Foliar spray

Foliar application of Zn was carried out at four stages i.e. 15 days before bud break, petal fall, fifteen days after petal fall/pea nut stage and after harvest. Stock and further diluted spray solutions of Zn fertilizers were prepared using distilled water. The required quantity of each fertilizer was precisely weighed before adjusting the final volume to one liter. From the stock solution, further dilutions of desired concentrations were prepared before use. The final dilutions were further foliar applied uniformly. Clear day and morning hours were selected for the foliar application. Spray application was administered uniformly on both sides of the tree via motorized backpack sprayer at a concentration of 1 L per tree up to drift level.

3.2.3.3 Organic fertilizers

Nutritive organic fertilizers *viz.*, vermicompost (VC) and humic acid (HA) were applied in the apple tree basin during the month of November. The vermicompost was prepared by following the standard protocol, employing the earthworm species *Eisenia fetida*. The nutrient profile of vermicompost was composed of N (3%), P (1%) and K (1.5%). The humic acid was comprised of 100 per cent water soluble granules with a humic acid content of 3 per cent (w/w) along with traces of fulvic acid and calcium.

3.2.3.4 Bio-inoculants

Azolla pinnata (AZ) was acquired in the month of October and was subsequently incorporated into the apple tree basins on a fresh weight basis (containing 4.5% N) by mixing it with the soil. Consortia of arbuscular mycorrhizal fungi (AMF) and plant growth promoting rhizobacteria (PGPR) were applied to the respective treatments in the rhizosphere during the month of November. The AMF comprised of roasted bentonite clay contained moisture content of 15-20 per cent along with viable 100 propagules per gram of the product. PGPR procured for application was *Bacillus* species.

3.2.3.5 Inorganic fertilizers

The fertilizers used for NPK application included urea, single super phosphate and muriate of potash containing 46 per cent N, 16 per cent P_2O_5 and 60 per cent K_2O, respectively. The application of inorganic fertilizers in tree basin was done via broadcasting method at 30 cm distance from tree trunk. Full dose of P and K were applied in the month of

December. Two split doses of N were applied *viz.*, first half was applied two weeks before flowering (March) and the remaining half after one month of full bloom.

3.2.4 Treatment details

Experiment 1: Effect of foliar feeding of organic Zn fertilizers in apple

Four different foliar organic Zn fertilizers *viz.*, zinc glycinate (200 and 400 mg/L), zinc gluconate (200 and 400 mg/L), zinc amino acid chelate (200 and 400 mg/L), and nano zinc amino acid chelate (200 and 400 mg/L) in comparison to that of zinc sulphate were used. The trial included nine treatments *viz.*, T_1 : zinc glycinate @200 mg/L (Zn-Gly_{200}); T_2 : zinc glycinate @400 mg/L (Zn-Gly_{400}); T_3 : zinc gluconate @200 mg/L (Zn-Glu_{200}); T_4 : zinc gluconate @400 mg/L (Zn-Glu_{400}); T_5 : zinc amino acid chelate @200 mg/L (Zn-AAC_{200}); T_6 : zinc amino acid chelate @400 mg/L (Zn-AAC_{400}); T_7 : nano zinc amino acid chelate @200 mg/L (NZn-AAC_{200}); T_8 : nano zinc amino acid chelate @400 mg/L (NZn-AAC_{400}); T_9 : $ZnSO_4$ @0.5%. All the treatments were supplemented organically through FYM on 100% N equivalence basis.

Experiment 2: Conjoint effect of organic Zn fertilizers and bio-organics fertilization

The experiment was laid out with four different foliar organic Zn fertilizers *viz.*, zinc glycinate (200 and 400 mg/L), zinc gluconate (200 and 400 mg/L), zinc amino acid chelate (200 and 400 mg/L) and nano zinc amino acid chelate (200 and 400 mg/L), and bioorganic nutrient sources, namely, vermicompost (VC), *Azolla* (AZ), arbuscular mycorrhizal fungi (AMF), consortia of plant growth promoting rhizobacteria (PGPR) and humic acid (HA) were evaluated to determine the optimum and best combination in comparison with the recommended application of zinc sulphate and NPK fertilizers. Different inputs of bioorganic sources namely, Vermicompost @80 per cent of RDN (VC_{80}); Vermicompost @75 per cent of RDN (VC_{75}); *Azolla* @300 g/plant (AZ_{300}); *Azolla* @600 g/plant (AZ_{600}); AM fungi @20 g/plant (AMF_{20}); AM fungi @25 g/plant (AMF_{25}); PGPR @200 mL/plant ($PGPR_{200}$); PGPR @250 mL/plant ($PGPR_{250}$); Humic acid @100 g/plant (HA_{100}); Humic acid @120 g/plant (HA_{120}) were applied in 9 different combinations (T_1–T_8) along with a control as Inorganic N:P:K (105:53:105 g/plant) + $ZnSO_4$ @0.5% (T_9). Different treatments were comprised of the following combinations, T_1 : Zn-Gly_{200} + VC_{80} + AZ_{300} + AMF_{25} + $PGPR_{200}$ + HA_{100}; T_2 : Zn-Gly_{400} + VC_{75} + AZ_{600} + AMF_{20} + $PGPR_{250}$ + HA_{120}; T_3 : Zn-Glu_{200} + VC_{80} + AZ_{300} + AMF_{25} + $PGPR_{200}$ + HA_{100}; T_4 : Zn-Glu_{400} + VC_{75} + AZ_{600} + AMF_{20} + $PGPR_{250}$ + HA_{120}; T_5 : Zn-AAC_{200} + VC_{80} + AZ_{300} + AMF_{25} + $PGPR_{200}$ + HA_{100}; T_6 : Zn-AAC_{400} + VC_{75} + AZ_{600} + AMF_{20} + $PGPR_{250}$ +

HA_{120}; T_7 : NZn-AAC_{200} + VC_{80} + AZ_{300} + AMF_{25} + $PGPR_{200}$; T_8 : NZn-AAC_{400} + VC_{75} + AZ_{600} + AMF_{20} + $PGPR_{250}$; T_9 : Inorganic N:P:K (105:53:105 g/plant) + $ZnSO_4$ @0.5%.

3.3 OBSERVATIONS RECORDED

3.3.1 Vegetative growth parameters

3.3.1.1 Plant height

Plant height was measured twice, once before the experiment began in February and further at the end of the growing season in December, using a measuring tape from the soil surface to the top of a tree. The increase in tree height throughout the growing season was computed and expressed in centimeters (cm).

3.3.1.2 Trunk girth

Trunk girth was measured at the end of the growing season using measuring tape, positioned 15 cm above the graft union. The average trunk girth was calculated and expressed in centimeters (cm).

3.3.1.3 Canopy diameter

The horizontal distance across the canopy was measured in two directions i.e., north-south and east-west, from one end to the other using a measuring tape. The average values were then computed and expressed in centimeters (cm).

3.3.1.4 Shoot growth

A representative sample of five uniform shoots from the current season's growth was randomly selected in each of the four directions of selected tree. The shoot growth was measured at the end of the growing season using a measuring tape, and the average values were expressed in centimeters (cm).

3.3.1.5 Leaf area

Twenty fully expanded leaves were randomly collected from each selected tree in the month of August. Leaf area was measured using LI-3000 CAP Leaf Area Meter, and the average values were expressed in square centimeters (cm^2).

3.3.1.6 Leaf area index

Leaf area index was estimated by using Flour PEN-PSI Canopy analyser.

3.3.1.7 Trunk cross-sectional area

Trunk cross-sectional area (TCSA) was calculated using the formula TCSA = πr^2, where 'r' represents the radius of the trunk, following the method suggested by Westwood (1978).

3.3.1.8 Canopy volume

Canopy volume (CV) was determined according to Westwood (1978) by calculating the total above-ground volume of each tree using the following different formulae:

i) For the trees that were taller than their width

$TCV = 4/3\ \pi\ ab^2$

ii) For the trees that were wider than their height

$TCV = 4/3\ \pi\ a^2b$

Where, $\pi = 3.14$, 'a' represents half of the length of the major axis (height), and 'b' represents half of the length of the minor axis (width). The resulting canopy volume values were then expressed in cubic meters (m^3).

3.3.1.9 Canopy area

Canopy area (CA) was determined by taking average of canopy diameter as suggested by Westwood (1978) using formula, $CA = \pi r^2$ [radius (r) = canopy diameter/2] and was expressed as square meter (m^2).

3.3.2 Flowering characteristics

3.3.2.1 Date of pink bud emergence

The selected trees were visually observed for the emergence of pink buds, and the date of their emergence was recorded accordingly.

3.3.2.2 Date of initiation of flowering

The date of initiation of flowering was determined as the day when 4 to 5 floral buds had opened.

3.3.2.3 Date of full bloom

Date of full bloom was interpreted as the date with 75 and 80 per cent of the flowers opened.

3.3.2.4 Duration of flowering

From the beginning to the end of flowering, the observations on flowering period were consistently recorded. The duration of flowering was then calculated and expressed in terms of days.

3.3.2.5 Days from full bloom to harvest

The number of days from full bloom to harvest was described as the period from the date of full bloom to the date of harvesting, and a mean value was computed for this duration.

3.3.2.6 Length of flowering shoot

To measure the length of the flowering shoot, four healthy shoots from the current season's growth in each of the four directions were selected at random. Using a measuring tape, the length was measured and the values were expressed in centimeter (cm).

3.3.2.7 Spur density

Four shoots of uniform length (30 cm) at random were selected around the tree periphery. Before flowering, the spurs on the selected shoots were counted, and the spur density was computed as the number of spurs per centimeter of shoot length.

3.3.3 Yield contributing parameters

3.3.3.1 Number of flowers/shoot

To count the number of flowers on flowering shoot, four uniform shoots in each of the four directions were chosen. After that, the average value was calculated, and then expressed as the number of flowers per shoot.

3.3.3.2 Number of fruits/shoot

To count the number of fruits on each shoot, four equal-length shoots facing four directions were selected. Number of fruits per shoot was determined to calculate the average count.

3.3.3.3 Fruit set

Total number of flowers on the selected trees was initially counted, followed by a count of the total number of fruits three weeks after petal fall. Subsequently, the final fruit set was determined according to Westwood (1978).

3.3.3.4 Fruit yield

Fruit yield under various treatments was measured at the time of harvest by weighing all the fruits retained on individual tree and was expressed as kilograms per tree (kg/tree).

3.3.3.5 Yield efficiency

Yield efficiency of the trees assess the potential yield per unit area which was determined by dividing the fruit yield by leaf area, TCSA, TCV, and CA (Westwood, 1978). The results for yield efficiency were then expressed in terms of kg/cm^2 of leaf area and TCSA, kg/m^3 of TCV, and kg/m^2 of CA.

3.3.4 Fruit quality characteristics

3.3.4.1 Physical

3.3.4.1.1 Fruit dimension

Ten fruits were chosen at random, and their polar lengths were measured between the calyx and stylar ends, while, the diameters of the same fruits were recorded by measuring the distance between their cheeks using a digital vernier calipers. All the measurements were then expressed in millimeters (mm).

3.3.4.1.2 Shape index

Shape index was computed by dividing the values of fruit’s length by its diameter.

3.3.4.1.3 Fruit weight

Ten randomly selected fruits were weighed using an electronic top pan balance, and the average fruit weight was expressed in grams (g).

3.3.4.1.4 Fruit volume

Fruit volume was determined using water displacement method. Ten fruits randomly selected for recording fruit weight were subsequently immersed in a graduated beaker filled with water. The fruit volume was obtained from the difference between the initial and final readings and was expressed in cubic centimeters.

3.3.4.1.5 Specific gravity

Specific gravity of fruits was recorded by measuring weight in air and in water using the formula as:

$$\text{Specific gravity} = \frac{\text{Weight of fruit}}{\text{Volume of fruit}}$$

3.3.4.1.6 Fruit firmness

Fruit firmness at the time of harvest was assessed using an Acucal Digital Fruit Pressure Tester (Model ACSY4). A thin layer of fruit skin was carefully removed with a stainless steel knife at three locations on each fruit, and the penetrometer was inserted into the fruit. The resulting pressure readings were recorded and expressed in kilograms per square centimeter (kg/cm^2).

3.3.4.2 Biochemical

3.3.4.2.1 Total soluble solids

Total soluble solids (TSS) content was determined in the fruit samples using a Milwaukee Digital Refractometer (0-32 °Brix). Prior to each measurement, the refractometer was calibrated using distilled water. Following this, a drop of fruit juice was placed on the prism plate of the refractometer, and the readings were recorded. The resulting values were expressed in °Brix (°B).

3.3.4.2.2 Titratable acidity

Twenty five grams of fruit sample (pulp along with peel) was homogenized using pestle and mortar, and the volume was made to 250 mL using distilled water. The contents were then filtered through Whatman No. 1 filter paper and 50 mL of the extract was used for titration against a 0.1 N NaOH solution, with the endpoint being marked by the appearance of a light pink color. The titratable acidity was computed based on malic acid content and was expressed in percentage.

$$\text{Titratable acidity (\%)} = \frac{T \times N \times V1 \times E}{V2 \times W \times 1000} \times 100$$

Where,

T = Titre value

N = Normality of NaOH

V1 = Volume made

E = Equivalent weight of malic acid

V2 = Volume of aliquot taken

W = Weight of sample (g)

3.3.4.2.3 Total sugars

Total sugars content of fruits samples was assessed using the Lane and Eynon’s volumetric method (A.O.A.C., 1980). 200 mL of the extract remaining after titratable acidity estimation was taken and 10 mL of a standard lead acetate solution (45%) was added into it. Following this, after 10 minutes, 10 mL of a potassium oxalate solution (22%) was added to precipitate excess lead acetate. The volume was subsequently made to 250 mL with distilled water, which was then filtered through the Whatman No. 1 filter paper. After this, 50 mL of the filtrate was taken and 5 mL of concentrated HCl was added to it. The solution was then left at room temperature overnight to undergo hydrolysis. On the next day, excess of HCl was neutralized by using a saturated 1 per cent NaOH solution, and the final volume of 250 mL was made with distilled water. Total sugars were determined by titrating a boiling mixture consisting of 5 mL each of Fehling A and Fehling B along with 50 mL of distilled water against the hydrolyzed solution, using methylene blue as an indicator. The presence of a brick-red color at the end of the titration signified the endpoint. The total sugars content in the fruit samples was expressed as a percentage of the fresh weight of the fruit.

$$\text{Total sugars (\%)} = \frac{\text{Factor } (0.05) \times \text{Dilution}}{\text{Titre value } \times \text{Weight of sample taken} \times \text{Volume of aliquot taken}} \times 100$$

3.3.4.2.4 Reducing sugars

The unhydrolyzed solution that remained after determining the total sugar content was titrated against a boiling mixture consisting of 5 mL each of Fehling A and Fehling B along with 50 mL of distilled water using methylene blue as an indicator (A.O.A.C., 1980). The appearance of a brick-red color marked the endpoint. The content of reducing sugars was expressed as a percentage of the fresh weight of the fruit.

$$\text{Reducing sugars (\%)} = \frac{\text{Factor } (0.05) \times \text{Dilution}}{\text{Titre value } \times \text{Weight of sample taken}} \times 100$$

3.3.4.2.5 Non-reducing sugars

Non-reducing sugars content was computed by subtracting the reducing sugars from total sugars and multiplying the difference by a standard factor of 0.95. The final values were expressed in per cent.

3.3.4.2.6 Ascorbic acid

Extraction solution

Metaphosphoric acid pellets weighing 15 g were dissolved in a solution containing 40 mL of glacial acetic acid and 200 mL of distilled water. The volume was brought to 500 mL by the addition of distilled water. The solution was then promptly filtered using Whatman No. 1 filter paper and placed in a refrigerator in a coloured container.

Preparation of solution

Precisely 100 grams of analytical grade ascorbic acid was weighed and dissolved in 10 mL of a meta-phosphoric acid extraction solution. The resulting mixture was subsequently transferred into a 100 mL volumetric flask, with the final volume made to 100 mL using meta-phosphoric acid solution. Before use, the solution was further diluted to a total volume of 1 liter with meta-phosphoric acid solution to minimize dye consumption.

Standard solution (Indophenol)

150 mL of distilled water was used to dissolve 50 mg of 2,6-dichlorophenol indophenol sodium salt, and 42 mg of sodium bicarbonate was then added to this. The mixture was vigorously shaken, and once the 2,6-dichlorophenol indophenol had completely

dissolved, it was diluted to a final volume of 200 mL with distilled water. Following dilution, the solution was filtered and stored in a dark-coloured bottle within a refrigerator.

Estimation

Total of 25 grams of fruit pulp was homogenized in meta-phosphoric acid, which served as the extraction solution. The volume was subsequently made to 100 mL using a volumetric flask. 10 mL of the solution was then titrated against 2, 6-dichlorophenol indophenol dye with phenolphthalein indicator, and the endpoint was signified by the appearance of a light pink color. The ascorbic acid content in the fruit juice was determined using the following formula:

$$\text{Ascorbic acid (mg/100g)} = \frac{\text{Dye factor} \times \text{Titre value} \times \text{Volume madeup}}{\text{Weight of fruit taken} \times \text{Volume used for estimation}} \times 100$$

3.3.4.2.7 Anthocyanins content

The total anthocyanins content of fruit samples was assessed using the method proposed by Harborne (1973). The procedure consisted of extracting anthocyanins from 1-gram apple peel using a known quantity of methanol containing 1 per cent HCl. The solution was kept overnight in a deep freezer (0 °C) for the extraction of anthocyanin pigment. The following day, red colour of the resulting solution was measured at a wavelength of 535 nm against a methanolic-HCl blank, using a Nukes UV-VIS spectrophotometer. Anthocyanins content was quantified and expressed as mg per 100 g using the following formula:

$$\text{Total OD/100 mg} = \frac{\text{OD} \times \text{Volume made up for colour measurement} \times \text{Total volume}}{\text{Volume of extract used} \times \text{Weight of sample taken}} \times 100$$

$$\text{Total anthocyanins content (mg/100 g)} = \frac{\text{Total OD} \div 100\text{ mg}}{\text{E}}$$

where, E represents the Extinction coefficient, taking a value of 98.2, and OD represents optical density.

3.3.4.2.8 Total phenols

The method suggested by Ranganna (1997) was employed to determine the total phenols in fruit samples. One gram of the fresh sample was pulverized using 10 mL of 80 per cent ethanol with a pestle and mortar. The resulting homogenate was then subjected to centrifugation at 10,000 rpm for 20 minutes, and the supernatant was collected carefully. Any remaining residues were subjected to a second extraction using 5 mL of 80 per cent ethanol, followed by centrifugation, and the supernatants from both extractions were combined. These supernatants were subsequently evaporated until dry. The resulting residues were dissolved in a known volume of 5 mL of distilled water, from which a 1 mL aliquot was taken into a test tube. To this, 2 mL of water was added, bringing the final volume to 3 mL. To each test tube, 0.5 mL of Folin-Ciocalteu reagent was added, and after a 3-minute interval, 2 mL of 20 per cent Na_2CO_3 was added. The contents were mixed thoroughly and test tubes were immersed in boiling water for one minute, and then allowed to cool. The absorbance was measured at 760 nm using a Nukes UV-VIS spectrophotometer. The total phenol content in the sample was determined by referring to a standard curve created using various concentrations of catechol and the final values were expressed in mg per 100 g of fresh weight.

$$\text{Total phenolics content (mg/100g)} = \frac{\text{OD at 760 nm} \times \text{Volume made up} \times 100}{\text{Aliquot taken} \times \text{Weight of sample} \times 1000}$$

3.3.5 Soil chemical properties

Soil samples were collected at depth of 0-30 cm within the tree basin, and for each treatment, a composite sample weighing up to 1 kg was prepared. After collection, these samples were allowed to air dry, followed by grinding and passing them through a 2 mm mesh sieve. The prepared samples were placed in plastic bags with appropriate labels, ready for the subsequent analysis of chemical properties.

Soil pH and EC was measured in a soil-to-water suspension (1:2), following the protocol outlined by Jackson (1973). Organic carbon content was determined using the wet digestion method described by Walkley and Black (1934). Available N content was assessed through the alkaline potassium permanganate method (Subbiah and Asija, 1956), available P content was determined using Olsen’s method (Olsen et al., 1954) using Nukes UV-VIS spectrophotometer (660 nm wavelength), and available K content was measured with the 1N neutral ammonium acetate method using flame photometer Model TMF-45 (Merwin and Peach, 1951). Exchangeable Ca and Mg were extracted using 1N neutral ammonium acetate method and quantified using an Analyst 400 Atomic Absorption Spectrophotometer, and the results were expressed in mg/kg. DTPA-extractable Fe, Cu, Zn and Mn were analyzed with the use of an atomic absorption spectrophotometer, maintained at a pH level of 7.3 ± 0.05, as per the method outlined by Lindsay and Norvell (1978).

3.3.6 Microbiological properties

3.3.6.1 Total viable microbial count

3.3.6.1.1 Serial dilution

After collection from the field, moist soil samples from each treatment were passed through a 2 mm sieve. Microbes were isolated using the serial dilution technique by placing 1 g of soil into a 9 mL sterilized distilled water blank, and agitated on a shaker for 5-10 minutes. This initial step resulted in 10^{-1} dilution, followed by subsequent preparations of 10^{-2} to 10^{-9} dilutions.

3.3.6.1.2 Quantification of rhizosphere microflora

For the quantification of total microbial count, the ‘Standard Plate Count’ technique was employed (Wollum, 1982). Media specific to different microorganisms were prepared and sterilized through autoclaving. After the media had cooled to room temperature, it was poured into sterile petri plates under aseptic conditions. A 0.1 mL aliquot from the dilution blank was evenly spread over the solidified media. Specifically, Nutrient agar media was used for bacteria (Rangaswamy, 1966), Potato dextrose agar media for fungi, Kenknight and Munaires agar media for actinomycetes (Rangaswamy, 1966), Pikovaskayas’s agar media for phosphorus solubilizing bacteria (Pikovskaya, 1948), Aleksandrow agar media for potassium solubilizing bacteria (Hu et al., 2006), Liquid salt media for zinc solubilizing bacteria (Subba Rao, 1977), and Jensen’s agar media for *Azotobacter* (Jensen, 1987). Following inoculation, the plates were incubated in an inverted position at 28 ± 2 °C for 48 hours in the incubator. Upon the completion of the incubation period, the microbial count was quantified and expressed as colony forming units per gram (cfu/g) of soil. The composition of the respective media for the enumeration of microbial count in the rhizosphere samples is described as follows:

3.3.6.2 Arbuscular Mycorrhizal Fungi (AMF)

3.3.6.2.1 Collection of samples

Soil samples representing each treatment were collected from 0-30 cm depth from the apple rhizosphere in the months of May and June. The collected samples were kept in polythene bags and were stored in a refrigerator (4°C) for isolation and analysis in the future.

3.3.6.2.2 Enumeration of AM Fungal spore population

The population of arbuscular mycorrhizal (AM) fungi was assessed through spore isolation by employing wet sieving and decanting method (Gerdemann and Nicolson, 1963). 50 g of soil sample was mixed in 200 mL of water in a 500 mL flask. The mixture was stirred vigorously, allowing heavier particles to settle down for few minutes. Afterward, the suspension was poured through a sequence of sieves arranged in descending order of size (500, 350, 250, 105, 45 µm), enabling the separation of AM fungal spores from the soil. The remaining material on each sieve was meticulously rinsed under running tap water. After washing, any remaining residue left on the sieve was collected in a beaker, and the final volume was adjusted to 50 mL. From this suspension, a known quantity (2 mL) was transferred to petri plate and observed under a stereoscopic binocular microscope. The spore count per 50 g of moist soil was then calculated based on this examination. The final AMF spore count was composed of both live and dead spores.

3.3.6.2.3 Identification of AM fungal spores

AMF isolates were collected in the form of dispersed clusters of germinating spores, or alternatively, sporocarps or chlamydospores were carefully picked using an auto pipette and then prepared for examination by mounting them in either lactophenol or polyvinyl lactic acid (Omar et al., 1979). The individual spores isolated were identified, at a minimum, to the genus level by referring a collection of scanned and photographic slides, which were compiled with the assistance of the synoptic key provided by Trappe (1982), Hall (1984) and Benny et al. (2001).

3.3.6.3 Soil enzymes

3.3.6.3.1 Phosphatase

Phosphatase enzyme activity was estimated using the method described by Tabatabai and Bremner (1969). 1 g of soil sample was mixed with toluene (0.2 mL), modified universal buffer (MUB) (4 mL) and p-nitrophenyl phosphate solution (1 mL) and was incubated at 37 °C for 1 hour. Following this, calorimetric analysis of the extract obtained by treating the incubated soil with 0.5 M $CaCl_2$ (1 mL) and 4 mL 0.5 M NaOH (1 mL) was carried out. The resulting suspension was filtered through Whatman No. 42 filter paper. The yellow colour of the released p-nitrophenol was assessed in spectrophotometer at a wavelength of 420 nm. A standard curve was prepared by utilizing various concentrations of p-nitrophenol ranging between 10 to 100 ppm and the findings were expressed as µmoles of p-nitrophenol released per hour per gram of soil.

3.3.6.3.2 Dehydrogenase

The dehydrogenase enzyme was assessed by reducing 2,3,5-triphenyl tetrazolium chloride (TTC) to triphenyl formazan (TPF) by following calorimetric method as suggested by Casida et al. (1964). 1 g of moist soil sample was placed in a screw-cap tube along with calcium carbonate (0.01 g), 3 per cent TTC solution (0.5 mL), and 1 per cent glucose solution (0.5 mL). To eliminate the trapped oxygen, bottom of the tube was gently tapped before the incubation at 37°C for 24 hours. Following it, 10 mL of methanol was added to the solution and was shaken for 1 minute. Subsequently, the mixture was kept in the dark for an additional

6-hour period. The red colour of the sample was measured using a Nukes UV-VIS spectrophotometer at an absorbance of 485 nm. The standard curve was generated from various concentrations of TPF (10 to 60 ppm) and the dehydrogenase activity was expressed as amount of TPF produced per hour per gram of the soil (µg TPF/g/h soil)

3.3.7 Physiological Parameters

3.3.7.1 Leaf chlorophylls

From each experimental tree, ten fully expanded leaves were collected from the current season’s growth during the morning hours in the month of July. Immediately after collection, the leaf samples were placed in an icebox and transported to the laboratory (Halfacre et al., 1968). Further the leaf samples were taken out of the icebox only before analysis so as to prevent degradation of chlorophyll pigments.

Extraction

The leaf samples for each treatment were thoroughly washed and chopped finely under subdued light. 100 mg of finely chopped leaf material was placed in a test tube and mixed with 7 mL of dimethyl sulphoxide (DMSO). The tubes were cotton plugged and subjected to incubation at a temperature of 65 °C for thirty minutes. Following this, the final volume was adjusted to 10 mL using DMSO, in accordance with the methodology described by Hiscox and Israelstam (1979).

Estimation

The optical density of the extract was estimated using a Nukes UV-VIS Spectrophotometer against a DMSO blank solution at 645 and 663 nm. Total chlorophyll content was determined using a following formula:

$$\text{Total chlorophylls (mg/g)} = \frac{20.2\ A645 + 8.02\ A663}{a \times 1000 \times W} \times V$$

Where,

V = Volume of extract used

a = Length of light path in cell (usually 1 cm)

W = Weight of the sample (g)

A_{645} = Absorbance at 645 nm

A_{663} = Absorbance at 663 nm

The results obtained were reported as milligrams of total chlorophyll per gram of fresh weight.

3.3.7.2 Photosynthetic efficiency, transpiration rate and stomatal conductance

The photosynthetic efficiency (µmol $CO_2/m^2/s$), transpiration rate ($mmol/m^2/s$), and stomatal conductance (mmol $H_2O/m^2/s$) were assessed between 10:00 AM and 12:00 noon using the LI-6400XT (LI-COR) portable photosynthesis system. Firstly, reference air (CO_2) at 400 µmol/s and a light intensity of 1000 µmol m^2/s were set. Afterwards, measurements on five fully matured leaves present at tree's periphery were carried out (Erf and Proctor, 1987).

3.3.7.3 Total carbohydrate content

3.3.7.3.1 Collection and preparation of sample

Fully expanded leaves of each experimental tree were plucked from the current season's growth. The collected leaf samples were promptly transported to the laboratory, washed properly and air dried. Subsequently, they were placed in paper bags and subjected to final drying in a hot air oven at 65 ± 5 ºC. The dried samples were then ground using a pestle and mortar, and stored in butter paper bags for final estimation of total carbohydrates. In another set of experiment, both fruiting and non-fruiting shoots were sampled 10 days prior to fruit harvesting and taken to the laboratory for total carbohydrate estimation following standardized procedure. For fruit carbohydrate determination, fully matured and healthy fruits were collected from each experimental tree.

3.3.7.3.2 Determination of total carbohydrates

The estimation of total carbohydrate content was carried out using the Anthrone reagent method, as suggested by Hodge and Horfreiter (1962). 100 mg of dried sample was weighed, put in a screw-cap tube and hydrolyzed using 5 mL of 2.5 N HCl for three hours in a boiling water bath. After cooling it down, sodium carbonate (Na_2CO_3) was added gradually to neutralize the acid in the solution until effervescence ceases. The final volume was made to 100 mL using distilled water, and then subjected to centrifugation. 1 mL of the aliquot taken from the supernatant was transferred into a test tube, followed by addition of 4 mL of anthrone reagent. The test tubes were immersed in boiling water bath for 8 minutes and then rapidly cooled. The green to dark green colour developed was recorded at 630 nm absorbance using Nukes UV-VIS spectrophotometer. A standard curve was prepared using glucose at various concentrations and subsequently the carbohydrate content in the sample was determined using the following formula:

$$\text{Total carbohydrates} = \frac{\text{Calculated value from standard curve} \times \text{Total volume of extract} \times 100}{\text{Aliquot of sample used} \times \text{Weight of sample taken} \times 1000}$$

Total carbohydrate content was expressed as milligram per gram on dry weight basis.

3.3.8 Nutrient analysis

3.3.8.1 Collection and preparation of samples

Leaves along with petioles were collected from the middle portion of terminal shoots of the current season growth for each experimental treatment (Kenwothy, 1964). The collected leaf samples were brought to the laboratory, washed under tap water and 0.1 N HCl and then rinsed with distilled water to ensure thorough cleaning, following the procedure suggested by Chapman (1964). After this, leaf samples were air dried, and subsequently oven dried (65 ± 5 °C) for 48 hours after keeping them in paper bags. Additionally, mature and healthy fruits were also collected from the experimental trees for further nutrient analysis. The collected fruit samples were brought to laboratory, washed under running tap water and then by distilled water. For determination of nutrients in the flesh, finely cut fruit slices were kept in petri dishes, whereas, for fruit peel nutrient analysis, only fruit peel was kept in petridishes for final drying in hot air oven. After the drying process, samples were ground using a pestle and mortar, and stored in butter paper bags for further nutrient elements estimation.

3.3.8.2 Digestion of samples

The digestion process for estimating total nitrogen involved subjecting 1 g of samples to concentrated H_2SO_4 along with a digestion mixture constituting of Potassium sulphate, K_2SO_4 (400 parts); Copper sulphate, $CuSO_4$ (20 parts); Mercuric oxide, HgO (3 parts); and Selenium powder, Se (1 part). To determine the P, K, Ca, Mg and Zn content in the leaves and fruits, 0.5 g of the sample underwent digestion in a di-acid mixture comprising HNO_3 and $HClO_4$ with a ratio of 4:1 as recommended by Piper (1996).

3.3.8.3 Determination of nutrient element

3.3.8.3.1 Nitrogen

Leaf nitrogen content was determined using the micro-Kjeldhal method. 5 mL aliquot of the digested sample was taken in a micro-Kjeldhal apparatus, to which 5 mL of NaOH (40%) was slowly added. The liberated ammonia was collected in a 25 mL solution of boric acid (4%) that contained a mixture of methyl red and bromocresol green as an indicator. Boric acid solution turned bluish-green in colour on coming in contact with ammonia. This solution was then titrated against N/100 H_2SO_4 until wine-red colour was achieved, and the results were expresses as percentage on a dry weight basis.

3.3.8.3.2 Phosphorus

Phosphorus content was determined using vanado-molybdate phosphoric yellow colour method. 5 mL of the digested sample was pipetted into a 25 mL volumetric flask, followed by the addition of 5 mL of vanado-molybdate reagent. After that, the solution was diluted with distilled water to 25 mL and allowed to develop colour for thirty minutes. After colour development, Nukes UV-VIS Spectrophotometer operating at 470 nm wavelength was used to determine the amount of phosphorus present in the solution. A blank was run before the samples, to set the absorbance to zero. The phosphorus content in both leaf and fruit (peel and flesh) was expressed as percentage on a dry weight basis.

3.3.8.3.3 Potassium

Potassium content in leaves was determined using Flame Photometer MODEL TMF-45 and the results obtained were expressed as percentage on a dry weight basis. Fruit potassium content of both the peel and flesh of the fruit were determined using Agilent 5110 ICP-OES, with the results expressed as percentage based on dry weight basis.

3.3.8.3.4 Calcium, Magnesium and Zinc

Calcium, Magnesium and Zinc content in leaves were analyzed and quantified using Analyst 400 Atomic Absorption Spectrophotometer and the fruit calcium, magnesium and zinc content in fruits were assessed using Agilent 5110 ICPOES. The results were expressed as percentage on a dry weight basis.

3.3.9 Fruit nutrient ratios

3.3.9.1 Potassium to calcium (K/Ca)

The K/Ca ratio for apple peel was determined by dividing peel K value by the peel Ca value. Similarly, the fruit flesh K/Ca ratio was calculated.

3.3.9.2 (K+Mg)/Ca

The (K+Mg)/Ca ratio for apple peel was determined by dividing peel K+Mg value by the peel Ca value. Similarly, the fruit flesh (K+Mg)/Ca ratio was calculated.

3.3.10 Antioxidants

3.3.10.1 Phytic acid (PA)

0.2 g of fruit sample was weighed into a 250 mL conical flask and soaked in 100 mL of concentrated HCl (20%) for 3 hours. Subsequently, the sample was filtered, and 50 mL of filtrate was transferred to a 250 mL beaker. To this, 100 mL of distilled water was added. Then, 10 mL of 0.3 per cent ammonium thiocyanate (NH_4SCN) solution was added as an indicator, followed by titration with standard iron chloride ($FeCl_3$) solution that contained 0.00195 g of iron (Fe) per mL (Lucas and Markakes, 1975).

$$\text{Phytic acid (\%)} = \frac{\text{Titre value} \times 0.00195 \times 1.19 \times 100}{2}$$

3.3.10.2 Phytic acid/ Fruit nutrient

The PA: Ca, PA: Mg and PA: Mg ratios were determined by dividing the corresponding value of fruit PA to fruit Ca, fruit Mg and fruit Zn value, respectively.

3.3.10.3 Ursolic acid

3.3.10.3.1 Chemicals

Ursolic acid (UA) analytical standard was procured from Sigma-Aldrich Co. (St. Louis, Missouri, USA) having molecular weight 456.7 and purity (HPLC area %) ≥ 98.5%. Methanol (HPLC grade) was purchased from M/s Merck Life Science Private Limited (Mumbai, India). All the other chemicals used in the process were of analytical grade. The Aquaplus ultra water purification equipment was used to prepare the ultra-pure distilled water needed for all HPLC operations.

3.3.10.3.2 Preparation of standard solution

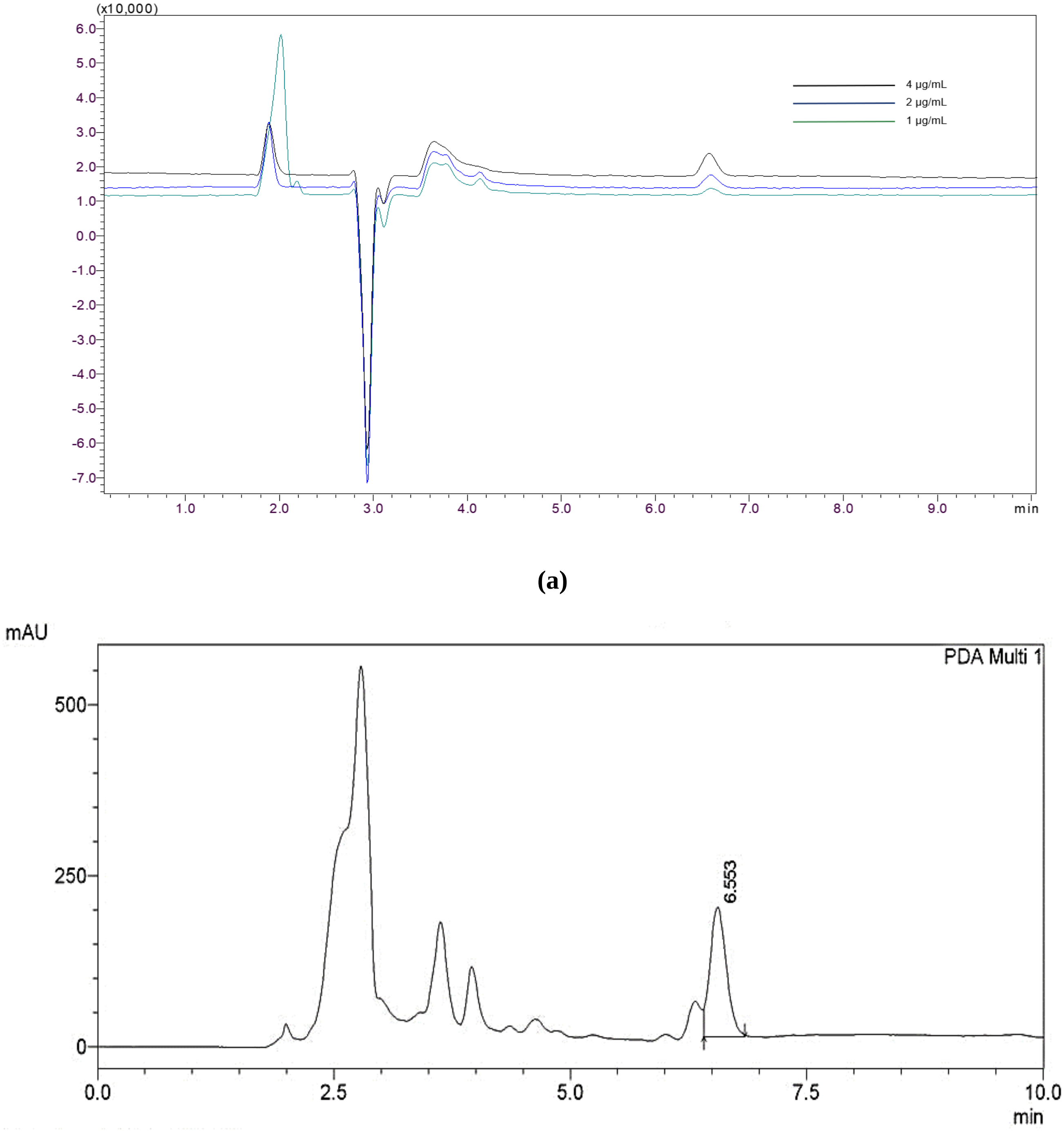
Stock solution of UA (100 µg/mL) was prepared in HPLC grade methanol. The stock solution was subsequently diluted to working standards of 4, 2 and 1 µg/mL in HPLC grade methanol. 20 µL of each of these solutions was used to build the standard curve for UA.

3.3.10.3.3 High performance liquid chromatography

High performance liquid chromatography (HPLC) was performed using SHIMADZU LC-20AT system equipped with SPD-M20A Photodiode Array Detector (PDA), DGU-20A5 degasser and SIL-20 AHT auto injector. The system was coupled with a Merck Li Chrosorb @ RP C18 column (2.1 mm × 30 mm, 5µm). The detector was configured to measure at wavelengths of 204, 220, 254 and 260 nm. The mobile phase was comprised of methanol and ultra-pure water (95:5, v/v), isocratic run, flow rate: 0.8 mL/min, sample volume: 20 µL, analytical wavelength (λ): 204 nm. The mobile phase was filtered through filter assembly with PVDF filter paper (0.2 µm) before use. The column temperature was held steady at 20 °C. The identification relied on the analysis of standard's DAD absorbance spectra and retention time.

3.3.10.3.4 Sample preparation

For each experimental treatment, five to seven healthy fruits were harvested at full maturity, washed and dried in hot air oven (WISWO Instruments, Delhi) at 35 °C for 48 hours. The dried peels were further subsequently peeled. The freshly peeled skins were ground using pestle and mortar in the laboratory and stored in deep freezer (-20 °C) until further analysis.



(a)

(b)

Fig. 1: (a) HPLC overlay plot of different concentrations of UA (1, 2 and 4 µg/mL), (b) HPLC chromatogram of apple peel samples with chromatographic conditions: Merck Li Chrosorb @ RP C18 (2.1 mm × 30 mm, 5µm) column, methanol: ultra-pure water (95:5), λ: 204 nm, flow rate: 0.8 mL/min, retention time: 6.55 min

An accurately weighed 1 g of sample was dissolved in 25 mL of methanol for 30 minutes, followed by 30 minutes of ultrasonic extraction using Ultrasonic water bath UCS-3000H (40 kHz ultrasound frequency). The samples were extracted twice and the resulting extracts were combined and diluted to a final volume of 50 mL with methanol. Before conducting HPLC analysis, the solution underwent filtration using a 0.2 µm syringe filter.

3.3.11 Statistical analysis

Statistical analysis was performed with MS-Excel and OP-STAT, employing one-way ANOVA for mean of each parameter which replicated thrice. The experiment was laid out in Randomized Block Design (RBD) within the main plots. The treatment differences were assessed employing ‘F’ test using the critical difference (CD) at 5 per cent level of confidence, wherever significant (Panse and Sukhatme, 1989). The computed F-values were compared against tabulated F-values. Upon significance of the F-test, the $CD_{0.05}$ was subsequently calculated to assess the relative effectiveness among various organic Zn and bio-organic sources applied. Principal component analysis (PCA) was carried out to classify a large set of data into major components, and to examine the effect of various organic Zn and bio-organic sources on generative traits, fruit quality parameters and soil properties. The PCA analysis was performed using XLSTAT (version 2022.1.2).

Chapter - 4

RESULTS AND DISCUSSION

The present study entitled **“Zinc biofortification through organic Zn and bio-organic supplements in high density plantation of apple (*Malus* × *domestica* Borkh.)”** was carried out at Department of Fruit Science, Dr. YS Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh during two consecutive years between 2022 and 2023. The results obtained on zinc biofortification through bio-organic Zn have been presented and discussed under the following heads and sub-heads:

EXPERIMENT 1:

EFFECT OF FOLIAR FEEDING OF ORGANIC Zn FERTILIZERS IN APPLE

4.1.1 Cropping behaviour

4.1.2 Yield contributing parameters

4.1.3 Fruit quality

4.1.4 Soil properties

4.1.5 Physiological parameters

4.1.6 Leaf nutrients

4.1.7 Fruit nutrients

4.1.8 Fruit nutrients ratio

4.1.9 Antioxidants activity

4.1.1 CROPPING BEHAVIOUR

4.1.1.1 VEGETATIVE GROWTH

The data depicting the influence of bio-organic Zn supplements on the vegetative growth characteristics such as plant height, trunk girth, canopy diameter, shoot growth, leaf area and leaf area index of apple in high density plantation has been presented in Table 1.

4.1.1.1.1 Plant height

Among different Zn sources applied, the annual increase in plant height ranged from 28.42 cm to 39.37 cm (Table 1). Maximum (39.37 cm) increase in plant height was recorded in nano zinc amino acid chelate at 400 mg/L (NZn-AAC$_{400}$) followed by nano zinc amino acid chelate at 200 mg/L (NZn-AAC$_{200}$), zinc amino acid chelate at 400 mg/L (Zn-AAC$_{400}$), zinc amino acid chelate at 200 mg/L (Zn-AAC$_{200}$), zinc glycinate at 400 mg/L (Zn-Gly$_{400}$) and zinc gluconate at 400 mg/L (Zn-Glu$_{400}$) with the corresponding values of 38.49, 37.67, 37.26, 35.50 and 34.52 cm, whereas, it was least (28.42 cm) in $ZnSO_4$ (control). Compared to all other Zn sources applied, NZn-AAC$_{400}$ had statistically superior results, whereas NZn-AAC$_{200}$ exhibited similar effects with Zn-AAC$_{400}$. The progression of plant height under varied Zn sources revealed as NZn-AAC$_{400}$ > NZn-AAC$_{200}$ > Zn-AAC$_{400}$ > Zn-AAC$_{200}$ > Zn-Gly$_{400}$ > Zn-Glu$_{400}$. In addition, the plant height was 1.4 times higher in treatment application of NZn-AAC$_{400}$ than that of the control.

4.1.1.1.2 Trunk girth

The trunk girth of apple trees was significantly affected by the application of different Zn bio-organic sources. The trunk girth ranged between 8.59 cm and 12.23 cm. The treatment application of NZn-AAC$_{400}$ exhibited maximum trunk girth of 12.23 cm which was statistically similar to NZn-AAC$_{200}$ and Zn-AAC$_{400}$. Application of $ZnSO_4$ showed the lowest value of trunk girth (8.59 cm) and however, was statistically similar to Zn-Glu$_{200}$ (8.96 cm). The hierarchical arrangement of various Zn sources for trunk girth was obtained as NZn-AAC$_{400}$ > NZn-AAC$_{200}$ > Zn-AAC$_{400}$ > Zn-AAC$_{200}$ > Zn-Gly$_{400}$ > Zn-Glu$_{400}$. Furthermore, NZn-AAC$_{400}$ showed a 42.4 per cent increase over $ZnSO_4$ application, followed by NZn-AAC$_{200}$ (37.4%), Zn-AAC$_{400}$ (34.9%) and Zn-AAC$_{200}$ (24.3%).

Table 1. Vegetative growth indices of apple affected by foliar bio-organic Zn sources

Zinc nutrition	Increase in plant height (cm)	Trunk girth (cm)	Canopy diameter (cm)	Shoot growth (cm)	Leaf area (cm^2)	Leaf area index	TCSA (cm^2)	CV (m^3)	CA (m^2)
Zn-Gly$_{200}$	32.72	9.32	109.06	33.69	31.08	1.61	6.95	1.34	0.94
Zn-Gly$_{400}$	35.50	10.28	119.74	35.45	31.32	1.69	8.54	1.67	1.13
Zn-Glu$_{200}$	31.05	8.96	106.50	33.39	27.19	1.58	6.44	1.25	0.89
Zn-Glu$_{400}$	34.52	9.82	113.93	34.61	31.26	1.66	7.73	1.49	1.02
Zn-AAC$_{200}$	37.26	10.68	126.60	37.04	32.20	1.72	9.17	1.90	1.26
Zn-AAC$_{400}$	37.67	11.59	129.30	39.27	34.85	1.77	10.73	2.01	1.32
NZn-AAC$_{200}$	38.49	11.80	133.00	40.39	37.71	1.80	11.16	2.18	1.40
NZn-AAC$_{400}$	39.37	12.23	139.40	44.07	39.70	1.83	12.06	2.50	1.53
$ZnSO_4$	28.42	8.59	101.03	31.23	26.25	1.54	5.91	1.08	0.80
CD$_{0.05}$	0.83	0.81	2.96	1.34	1.07	0.04	1.53	0.15	0.07

TCSA, Trunk cross-sectional area; CV, canopy volume; CA, canopy area

4.1.1.1.3 Canopy diameter

The perusal of the data depicted in Table 1 illustrated a pronounced effect on canopy diameter when supplemented with different organic Zn and bio-organic sources in apple trees. Canopy diameter varied from 101.03 cm to 139.40 cm. The treatment of NZn-AAC$_{400}$ demonstrated the maximum canopy diameter of 139.40 cm which was statistically superior to all other Zn sources treatments applied. The lowest value (101.03 cm) however, was recorded in control. Application of Zn-AAC$_{400}$ and Zn-AAC$_{200}$ also elicited similar effects on canopy diameter with corresponding values of 129.30 and 126.60 cm. Different Zn organic sources exhibited canopy diameter of apple trees in the order of NZn-AAC$_{400}$ > NZn-AAC$_{200}$ > Zn-AAC$_{400}$ > Zn-AAC$_{200}$ > Zn-Gly$_{400}$ > Zn-Glu$_{400}$.

4.1.1.1.4 Shoot growth

During the experiment, the application of organic Zn sources resulted in a substantial increase in shoot growth of trees. NZn-AAC$_{400}$ showed the highest shoot growth (44.07 cm), whereas, $ZnSO_4$ application showed the lowest value (31.23 cm). Among all other zinc nutrient sources, Zn-Glu$_{400}$, Zn-Gly$_{200}$, and Zn-Glu$_{200}$ had statistically similar effects, with corresponding values of 34.61, 33.69 and 33.39 cm. When compared to $ZnSO_4$ application, NZn-AAC$_{400}$ application indicated the highest per cent increase of 41.1, followed by NZn-AAC$_{200}$ (29.3%), Zn-AAC$_{400}$ (25.7%) and Zn-AAC$_{200}$ (18.6%).

4.1.1.1.5 Leaf area

The data presented in Table 1 indicated that leaf area varied among different Zn bio-organic nutrient sources, which ranged between 26.25 and 39.70 cm^2. Leaf area was maximum in NZn-AAC$_{400}$ (39.70 cm^2), whereas, it was minimum (26.25 cm^2) in $ZnSO_4$. The treatment application of NZn-AAC$_{400}$ exceeded all other treatments, which demonstrates statistically significant effects with a remarkable 51.2 per cent increase compared to control. However, $ZnSO_4$ (control) showed statistically similar results with Zn-Glu$_{200}$ (27.19 cm^2).

4.1.1.1.6 Leaf area index

A cursory glance at the data highlighted the significant effect of Zn organic sources on leaf area index (LAI) which varied from 1.54 to 1.83. Highest LAI (1.83) was recorded in NZn-AAC$_{400}$, which was statistically analogous to NZn-AAC$_{200}$ (1.80). The lowest LAI (1.54) was noticed in $ZnSO_4$ application. Among different Zn sources, LAI was observed in the order of NZn-AAC$_{400}$ > NZn-AAC$_{200}$ > Zn-AAC$_{400}$ > Zn-AAC$_{200}$ > Zn-Gly$_{400}$ > Zn-Glu$_{400}$. The treatment of NZn-AAC$_{400}$ exhibited 1.2 times increase in LAI compared to control.

4.1.1.2 GENERATIVE TRAITS

Data highlighting the effect of different bio-organic Zn nutrient sources on generative traits of apple trees *viz.*, trunk cross-sectional area, canopy volume and canopy area is presented in Table 1.

4.1.1.2.1 Trunk cross-sectional area (TCSA)

The data depicted in Table 1 revealed that different Zn organic sources exerted a noticeable effect on TCSA of apple trees. Treatment NZn-AAC$_{400}$ exhibited the highest TCSA of 12.06 cm^2, which was statistically at par with NZn-AAC$_{200}$ (11.16 cm^2) and Zn-AAC$_{400}$ (10.73 cm^2). However, minimum TCSA (5.91 cm^2) was reported in the treatment ZnSO$_4$. The treatments application of Zn-Glu$_{400}$, Zn-Gly$_{200}$ and Zn-Glu$_{200}$ also demonstrated similar effects of Zn sources in relation to TCSA with corresponding values of 7.73, 6.95 and 6.44 cm^2, respectively. The treatment NZn-AAC$_{400}$ also recorded 2.0 times increase of TCSA compared to control.

4.1.1.2.2 Canopy volume (CV)

Among different Zn nutrient sources, CV of apple trees ranged between 1.08 and 2.50 m^3. Maximum CV (2.50 m^3) was recorded in NZn-AAC$_{400}$, which was statistically significant compared to all other treatments. Similarly, statistically similar results were observed for treatments Zn-AAC$_{400}$ and Zn-AAC$_{200}$ which showed corresponding values of 2.01 and 1.90 m^3. However, treatment ZnSO$_4$ exhibited the lowest CV (1.08 m^3).

4.1.1.2.3 Canopy area (CA)

When compared to the control, the organic Zn sources had a favorable effect on the canopy area of apple trees. The treatment NZn-AAC$_{400}$ registered the maximum canopy area (1.53 m^2) compared to all other Zn treatments. The treatments namely, NZn-AAC$_{200}$, Zn-AAC$_{400}$, Zn-AAC$_{200}$, Zn-Gly$_{400}$ and Zn-Glu$_{400}$ showed the corresponding values of CA in the order of 1.40, 1.32, 1.26, 1.13 and 1.02 m^2. It was however, the lowest (0.80 m^2) in ZnSO$_4$. The treatment application of NZn-AAC$_{400}$ also showed 1.9 times increase in canopy area over control.

In the current study, contrast to ZnSO$_4$, foliar application of organic Zn combinations indicated a significant increase in the vegetative characteristics of apples including plant height, trunk girth, canopy diameter, shoot growth, leaf area, and leaf area index. The vital effect of zinc in cell elongation, cell membrane function, and protein synthesis is associated with the increase in vegetative growth characteristics of apple (Bala et al., 2019; Yeboah et al., 2021). Moreover, higher vegetative growth indices might have resulted from substantial hormonal stimulation carried out by the organic chelates coupled to Zn (Marschner, 2012). Our findings are in line with studies of Foroutan et al. (2015) and Jalali (2020), which concluded that Zn and amino acid chelates had significant effects on plant growth and development processes. In addition, the corresponding amino acids might be able to promote plant growth, which is how Zn amino acid compounds are preferred to $ZnSO_4$ (El-Bassiouny et al., 2008). Jie et al. (2008) reported that the use of amino acid chelates resulted in two-fold increase in growth parameters compared to $ZnSO_4$.

Our study also documented the significant effects of nano Zn amino acid chelate on vegetative growth parameters of apple trees. The increase in plant height through nano Zn amino acid chelate application might be due to the involvement of Zn in tryptophan synthesis or synergistic effects of Zn and N on the catalytic and metabolic processes within plant (Cakmak et al., 2010; Hasan et al., 2023). According to Alzreejawi and Al-Juthery (2021), nanoparticles have a greater ability to bind with protein carriers, such as amino acids, thereby making it easier for them to pass through cell walls (Rossi et al., 2018) and thereby had stimulated growth of trees. Similarly, increase in plant height and canopy diameter might have been related to the potential effect of zinc amino acid chelate on the dynamics of overall plant growth and distribution of dry matter (Amanullah, 2016). According to Barker and Pilbeam (2010), zinc acts as an activator for various enzymes in plants, which affects the production of growth regulators such as auxins, and thereby this might have contributed to the significant growth of shoots (Ashraf et al., 2013). It further attributed to better absorption and improved translocation efficiency of nano scale amino acid chelates (Ge et al., 2009; Garcia et al., 2011; Ghoname et al., 2012; Ghasemi et al., 2014; Asri et al., 2015). Ortiz-Lopez et al. (2000) believed that the growth-stimulating characteristics of glycine could be responsible to increase shoot growth which ascribed to Zn glycinate application. Earlier published work by Ghasemi et al. (2013), El-Sayed et al. (2014) and Mosa et al. (2020) provided further support for this hypothesis. Moreover, experimental evidence has also shown that zinc glycinate exceeded zinc gluconate compared to $ZnSO_4$ due to its higher bioavailability in the plant system (Gandia et al., 2007; Xu et al., 2022).

Grzyb et al. (2012) have confirmed that amino acids could boost the height and girth of the apple trees. Furthermore, Sun et al. (2018) have proposed that the addition of auxiliary carriers, such as amino acids, to micronutrients sprays could improve the solution’s effectiveness in stimulating plant development. In addition, an adequate supply of nutrients, available N (22-27%) and plant growth hormones even under stress conditions contributed to amino acid chelates’ capacity to perform better than inorganic Zn fertilizers (Ge et al., 2009; He et al., 2013; Ghasemi et al., 2014). Increased Zn penetration through leaves, bio-stimulation, and greater absorption processes have been suggested by Mohammadipour and Souri (2019) and Shooshtari et al. (2020). Moghaddasi et al. (2017)

and Chattha et al. (2022) highlighted the superior efficacy of nano formulations over the alternatives formulations, emphasizing the importance of smaller particle size, larger surface area, and quicker stomata penetration. Auxin synthesis, which is dependent on Zn availability for tryptophan synthesis (Souri and Hatamian, 2019) has a significant impact on leaf area of plants (Rafie et al., 2017). The increased metabolic processes (Al-Toki et al., 2021), meristematic activity (Al-Alusi and Mahmoud, 2009), photosynthesis (Ajeng et al., 2020; Alabdallah and Alzahrani, 2020; Alzreejawi and Al-Juthery, 2020), and the production of carbohydrates and proteins (Thumma et al., 2001) might be related to increased leaf area induced by the application of nano Zn amino acid chelate. The significant association between photosynthesis and vegetative growth also corroborate the results of this investigation (Aydi et al., 2023). Further, an increase in the leaf area index could have resulted from the increase in leaf area (Bashir et al., 2021). Dawood (2000) provided evidence for increased amount of photosynthates translocation attributed to the higher K uptake in the leaves upon fertilization with nano Zn amino acid chelate. Increase in trunk cross-sectional area, canopy volume, and canopy area indicated the critical effect that nano Zn amino acid chelate contributed to improve plant height and canopy diameter of apple trees. The increased uptake of nutrients and accumulation of photosynthates might be related to better generative characteristics (Chandra et al., 2015). The findings align with the research conducted by Moreira et al. (2015), which also demonstrated the positive effects of foliar application of zinc and amino acids on plant growth.

4.1.1.3 FLOWERING ONTOGENY

The data related to effect of bio-organic Zn amendments on the flowering parameters namely, date of pink bud emergence, initiation of flowering, date of full bloom, duration of flowering, days from full bloom to harvest, length of flowering shoot and spur density of apple has been presented in Table 2 and Fig. 1.

4.1.1.3.1 Date of pink bud emergence

Application of Zn organic sources directly showed significant effects on the date of pink bud emergence in apple trees both during 2022 and 2023, which varied from 12 to 18 March and 9 to 16 March, respectively (Fig. 1). Treatment application of $ZnSO_4$ was the last (18 March and 16 March) that observed the emergence of pink buds, whereas, NZn-AAC_{400} treatment indicated the earliest to flower (12 March and 9 March). The hierarchy of Zn sources in promoting pink bud emergence showed a trailing sequence of treatments *viz.*, NZn-AAC_{400} > NZn-AAC_{200} > Zn-AAC_{400} > Zn-AAC_{200} > Zn-Gly_{400} > Zn-Glu_{400}.

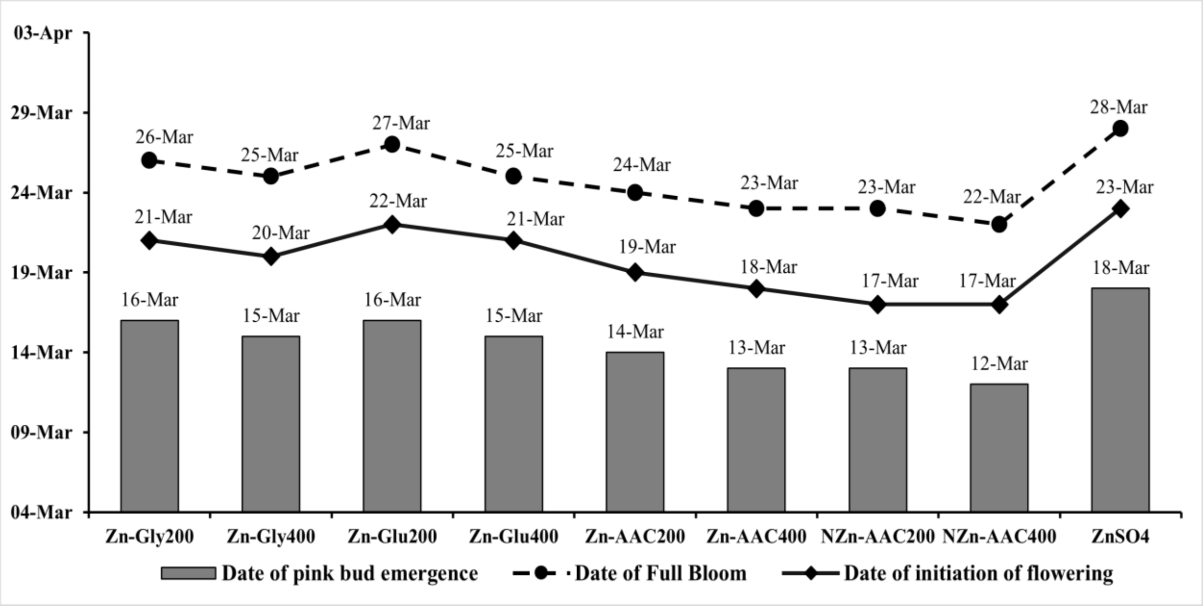


Fig. 2: Date of pink bud emergence, date of full bloom and date of initiation of flowering influenced by organic Zn fertilizers

4.1.1.3.2 Initiation of flowering

The data shown in Fig. 1 depicted that the application of organic Zn nutrition sources had a significant effect on the date of flowering onset. The beginning of flowering for both seasons of 2022 and 2023 was March 17 to March 23 and March 13 to March 21, respectively. Treatment NZn-AAC_{400} was the earliest to observe flowering (17 March and 13 March), however, the treatment $ZnSO_4$ was the last (23 March and 21 March) to show flower initiation in the trees.

4.1.1.3.3 Date of full bloom

The results showed that the date of full bloom varied depending on the organic Zn source (Fig. 1). In 2022, the full bloom period occurred between March 22 and March 28, and in 2023, it was between March 17 and March 25. Application of NZn-AAC_{400} attained the earliest bloom (22 March and 17 March), however, treatment $ZnSO_4$ was the last (28 March and 25 March). The sequence of Zn sources in stimulating full bloom showed a declining trend with NZn-AAC_{400} demonstrating the highest efficacy, succeeded by NZn-AAC_{200} > Zn-AAC_{400} > Zn-AAC_{200} > Zn-Gly_{400} > Zn-Glu_{400}.

4.1.1.3.4 Duration of flowering

The analysis of the data suggested that organic Zn sources had a positive impact on the duration of flowering (Table 2). The longest duration of flowering (13.67 days) was observed in NZn-AAC_{400}, which was statistically similar to that of NZn-AAC_{200} (13.17 days) and Zn-AAC_{400} (12.67 days). On the other hand, treatment $ZnSO_4$ showed the shortest duration of flowering (10.17

days). The per cent increment of 34.4 was observed in NZn-AAC_{200} compared to control. The consistent statistical effects were also exhibited among various Zn treatments in the order of Zn-AAC_{200} (12.19 days), Zn-Gly_{400} (12.17 days), Zn-Glu_{400} (11.83 days), and Zn-Gly_{200} (11.67 days).

4.1.1.3.5 Days from full bloom to harvest

The days from full bloom to harvest varied from 115.17 days to 122.83 days regardless of which zinc source was used. NZn-AAC_{400} showed the greatest number of days (122.83 days) from full bloom to harvest. This was followed by the treatments NZn-AAC_{200}, Zn-AAC_{400}, Zn-AAC_{200}, Zn-Gly_{400}, and Zn-Glu_{400}, with corresponding values of 122.17, 121.67, 119.67, 118.50 and 118.00 days. However, application of $ZnSO_4$ (control) achieved the minimum of 122.83 days from full bloom to harvest. In addition, NZn-AAC_{400} and Zn-AAC_{400} (121.67 days) exhibited statistical equivalency with NZn-AAC_{200} (122.17 days). The progression of days from full bloom to harvest under varied Zn sources unfolded as NZn-AAC_{400} > NZn-AAC_{200} > Zn-AAC_{400} > Zn-AAC_{200} > Zn-Gly_{400} > Zn-Glu_{400}. NZn-AAC_{400} also exhibited 1.1 times increase in days from full bloom to harvest over control.

Table 2. Effect of foliar bio-organic Zn sources on flowering and spur density of apple

Zinc nutrition	Duration of flowering (days)	Days from full bloom to harvest	Length of flowering shoot (cm)	Spur density (number/cm shoot length)
Zn-Gly_{200}	11.67	117.50	35.90	0.17
Zn-Gly_{400}	12.17	118.50	36.69	0.20
Zn-Glu_{200}	10.83	116.33	35.36	0.17
Zn-Glu_{400}	11.83	118.00	36.38	0.19
Zn-AAC_{200}	12.19	119.67	37.58	0.21
Zn-AAC_{400}	12.67	121.67	42.94	0.22
NZn-AAC_{200}	13.17	122.17	43.56	0.23
NZn-AAC_{400}	13.67	122.83	44.69	0.25
$ZnSO_4$	10.17	115.17	34.84	0.13
$CD_{0.05}$	1.03	1.32	1.05	0.02

4.1.1.3.6 Length of flowering shoot

The data showed that the length of the flowering shoot was considerably affected by the application of different Zn sources. Average length of flowering shoots varied from 34.84 cm to 44.69 cm. When compared to all other treatments, NZn-AAC_{400} showed statistically superior effects, while, the treatment NZn-AAC_{200} had identical affects with that of Zn-AAC_{400}. On the other hand, treatment $ZnSO_4$ showed a minimum length of flowering shoots (34.84 cm), which also showed statistically equivalent results to Zn-Glu_{200} (35.36 cm). The hierarchical arrangement of various Zn sources in terms of length of flowering shoot was NZn-AAC$_{400}$ > NZn-AAC$_{200}$ > Zn-AAC$_{400}$ > Zn-AAC$_{200}$ > Zn-Gly$_{400}$ > Zn-Glu$_{400}$. In addition, NZn-AAC$_{400}$ displayed an increase of 28.3 per cent followed by NZn-AAC$_{200}$ (25.0%) and Zn-AAC$_{400}$ (23.2%) over control.

4.1.1.3.7 Spur density

Application of different bio-organic Zn sources resulted in significant increase in spur density in apple trees throughout the cropping period (Table 2). Spur density showed an average variation between 0.13 and 0.25. NZn-AAC$_{400}$ treatment exhibited maximum spur density (0.25), which was statistically similar to that of NZn-AAC$_{200}$ (0.23). However, it was minimum (0.13) in treatment $ZnSO_4$. Similar effects were also obtained by Zn-AAC$_{400}$ and Zn-AAC$_{200}$ treatments with corresponding values of 0.22 and 0.21. Zn organic sources also scored spur density in terms of NZn-AAC$_{400}$ > NZn-AAC$_{200}$ > Zn-AAC$_{400}$ > Zn-AAC$_{200}$ > Zn-Gly$_{400}$ > Zn-Glu$_{400}$. In addition, treatment NZn-AAC$_{400}$ showed a 31.6 per cent increase in spur density over control.

The present study demonstrated that organic Zn sources had a positive effect on apple flowering characteristics. Nano zinc amino acid chelate showed the most promising outcome with respect to flowering ontogeny. This could be explained by the fact that nanoparticles are readily soluble (Naderi and Danesh-Shahraki, 2013) and that Zn has a role in pollen production and reproductive growth (Rahman et al., 2016). Further, amino acid chelates exhibit better nutrient stability and slow release of nutrients, that might correspond with the crop's gradual absorption. This could contribute to better flowering attributes by optimizing the application of nutrients (Niu et al., 2020). Amino acid chelates enable the quicker entry of Zn molecules through the leaf surface when conjugated with nanoparticles, and thereby contributed to induce earlier flowering (Prasad et al., 2012). In order to ensure that nitrogen (N) is available for growth across various plant tissues (Marschner, 2012; Kolota et al., 2013), amino acids are essential for N assimilation in plants. These substances additionally contributed to protein synthesis (Wona et al., 2011) as well as efficient N translocation through the phloem (Calvo et al., 2014) and thereby improved flowering traits (Alloway, 2008; Al-Said and Kamal, 2008; Abbass et al., 2020). The results of Sun et al. (2018) concluded that integrating amino acids with micronutrients can effectively improve their ability to function. Likewise, it is hypothesized that the action of Zn on increased carbohydrate accumulation corresponds to the higher spur density in trees treated to nano Zn amino acid chelate (Amiri et al., 2016).

4.1.2 YIELD CONTRIBUTING PARAMETERS

The data on the effect of Zn organic sources on the yield contributing parameters of apple is illustrated in Table 3 and Fig. 2, 3.

4.1.2.1 Number of flowers/shoot

Application of organic Zn sources resulted in a marked increase in the number of flowers per shoot recorded during the course of investigation (Table 3; Plate 1). Maximum number of flowers per shoot (36.98) was exhibited by NZn-AAC$_{400}$, which was statistically equivalent to NZn-AAC$_{200}$ (36.20), whereas, it was least in $ZnSO_4$ (26.52). Among all the applied Zn nutrient sources, statistically similar results were also recorded in Zn-Gly$_{200}$ and Zn-Glu$_{200}$ with the respective values of 29.07 and 28.24. The treatment of NZn-AAC$_{400}$ also showed the per cent increase of 39.4, followed by NZn-AAC$_{200}$ (36.5%), Zn-AAC$_{400}$ (32.5%) and Zn-AAC$_{200}$ (28.2%) compared to control.

4.1.2.2 Number of fruits/shoot

Number of fruits per shoot ranged from 12.95 to 21.81 (Plate 2). $ZnSO_4$ showed the lowest number of fruits per shoot (12.95), while, NZn-AAC$_{400}$ treatment showed the highest value (21.81). The treatment NZn-AAC$_{400}$ showed statistical significance over all other Zn nutrient sources applied. The treatment NZn-AAC$_{400}$ also showed 68.4 per cent increase over control with respect to number of fruits per shoot.

Table 3. Yield contributing traits of apple influenced by foliar feeding of bio-organic Zn sources

Zinc nutrition	Number of flowers/shoot	Number of fruits/shoot	Fruit set (%)	Fruit yield (kg/tree)
Zn-Gly$_{200}$	29.07	15.97	46.69	4.78
Zn-Gly$_{400}$	32.33	17.81	52.36	5.11
Zn-Glu$_{200}$	28.24	13.96	45.06	4.65
Zn-Glu$_{400}$	30.87	17.10	49.34	4.89
Zn-AAC$_{200}$	34.00	18.63	55.21	5.20
Zn-AAC$_{400}$	35.15	20.34	57.74	5.32
NZn-AAC$_{200}$	36.20	21.16	60.51	5.49
NZn-AAC$_{400}$	36.98	21.81	62.90	5.62
$ZnSO_4$	26.52	12.95	42.73	4.41
CD$_{0.05}$	0.91	0.48	2.99	0.17

4.1.2.3 Fruit set

A cursory glance at the data highlighted the significant effect on fruit set in apple trees with different bio-organic Zn sources supplemented (Fig. 2). Fruit set varied from 42.73 to 62.90 per cent (Table 3). Highest fruit set (62.90%) was recorded in NZn-AAC$_{400}$, which

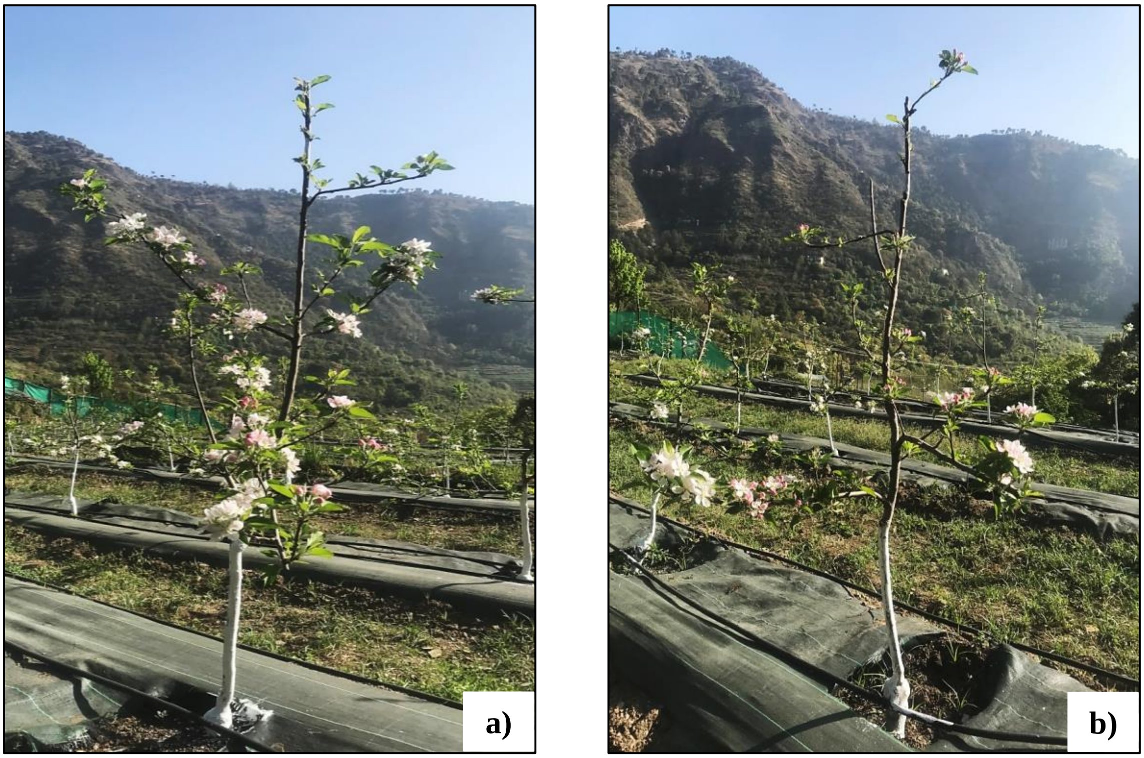


Plate 1: Flowering in apple trees a) NZn-AAC$_{400}$ b) ZnSO$_4$ (control)

Plate 2: Fruit development stage in apple a) NZn-AAC$_{400}$ b) ZnSO$_4$ (control)

was statistically analogous to NZn-AAC$_{200}$ (60.51%). The lowest fruit set (42.73%) was observed in ZnSO$_4$, which was statistically at par with Zn-Glu$_{200}$ (45.06%). Among different bio-organic Zn sources, the order of fruit set was observed as NZn-AAC$_{400}$ > NZn-AAC$_{200}$ > Zn-AAC$_{400}$ > Zn-AAC$_{200}$ > Zn-Gly$_{400}$ > Zn-Glu$_{400}$. The treatment of NZn-AAC$_{400}$ exhibited 1.5 times increase compared to control.

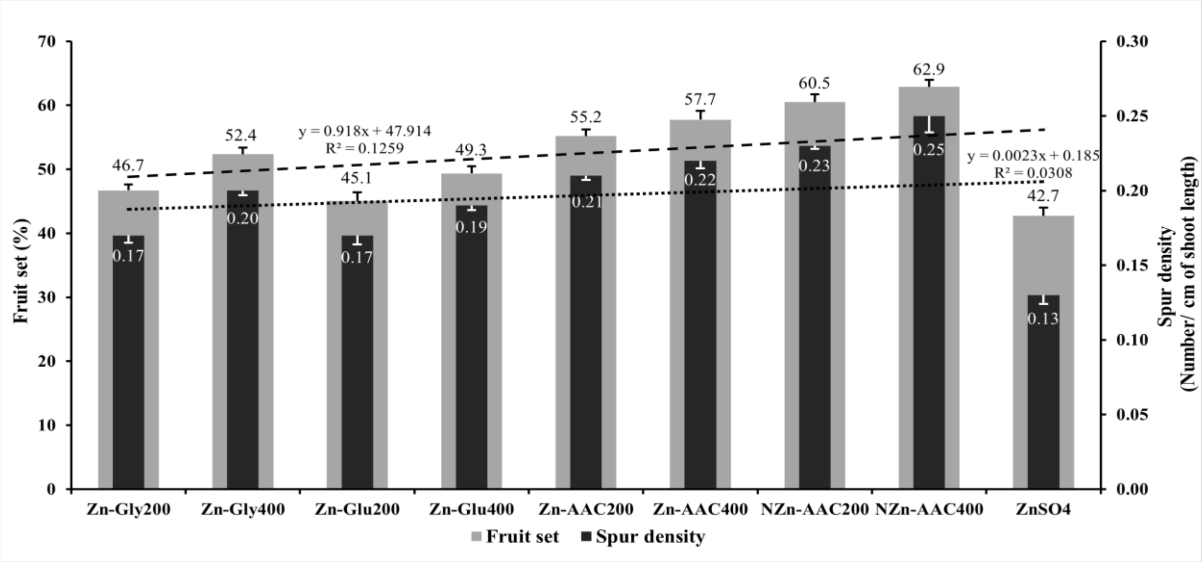


Fig. 3: Relationship studies on fruit set and spur density in apple under bio-organic Zn nutrition

4.1.2.4 Fruit yield

Application of treatment NZn-AAC$_{400}$ recorded the maximum fruit yield (5.62 kg/tree), with marked significance over all other Zn nutrient sources (Table 3). However, it was minimum (4.41 kg/tree) in ZnSO$_4$ treatment. Among all the applied treatments statistically significant results were observed in NZn-AAC$_{200}$ and Zn-AAC$_{400}$ treatments with corresponding values of 5.49 and 5.32 kg/tree. Also, Zn organic sources ranked in term of fruit yield as NZn-AAC$_{400}$ > NZn-AAC$_{200}$ > Zn-AAC$_{400}$ > Zn-AAC$_{200}$ > Zn-Gly$_{400}$ > Zn-Glu$_{400}$. The treatment NZn-AAC$_{400}$ also recorded 1.3 times increase when compared to control.

4.1.2.5 Yield efficiency

A non-significant difference in yield efficiency in terms of leaf area (LA) and TCSA was recorded between various foliar Zn treatments. Among different Zn nutrient sources, yield efficiency of trees in terms of LA ranged between 0.14 and 0.17 kg/cm^2 (Fig. 3). Maximum yield efficiency of 0.17 kg/cm^2 LA was recorded in treatment Zn-Glu$_{200}$ and ZnSO$_4$ which was followed by Zn-AAC$_{200}$, Zn-Glu$_{400}$ and Zn-Gly$_{400}$. However, treatment NZn-AAC$_{400}$ reflected the lowest value of 0.14 kg/cm^2 LA. In terms of TCSA, maximum yield efficiency (0.76 kg/cm^2 of TCSA) was registered by treatment $ZnSO_4$. This treatment was followed by Zn-Glu$_{200}$, Zn-Gly$_{200}$, Zn-Glu$_{400}$, and Zn-Gly$_{400}$, exhibiting respective values of 0.74, 0.70, 0.65, and 0.63 kg/cm^2 of TCSA. The data depicted in Fig. 3 further revealed that yield efficiency as CV ranged between 2.28 to 4.12 kg/m^3 of CV. Application of $ZnSO_4$ exhibited maximum yield efficiency (4.12 kg/m^3 of CV) which has also showed statistical superiority over all other applied treatments. Similarly, yield efficiency as kg/m^2 of CA recorded between 3.69 to 5.51 kg/m^2. Treatment $ZnSO_4$ attained maximum yield efficiency of 5.51 kg/m^2 of CA, whereas, NZn-AAC$_{400}$ recorded the least (3.69 kg/m^2 of CA). The sequence of applied Zn sources to improve yield efficiency showed a declining trend with $ZnSO_4$ demonstrating the highest values, followed by Zn-Glu$_{200}$, Zn-Gly$_{200}$, Zn-Glu$_{400}$ and Zn-Gly$_{400}$.

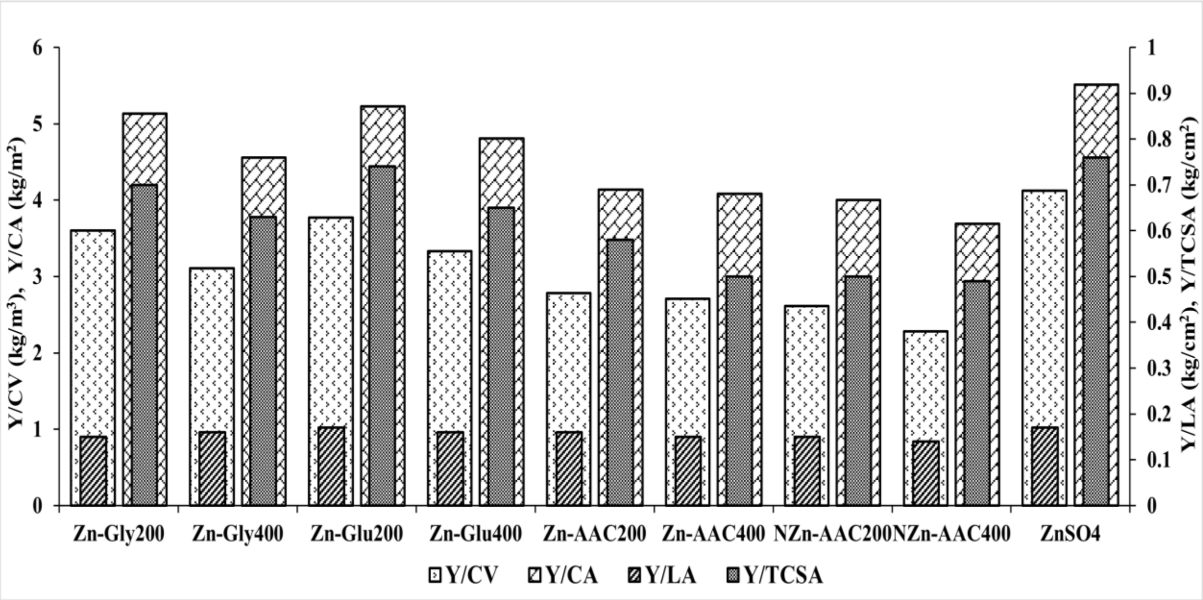


Fig. 4: Foliar bio-organic Zn nutrition effects on yield efficiency of apple

Amino acids are integral in the synthesis of plant hormones (Marschner, 2011), strengthening cell membrane resilience (Gondal et al., 2021), improving photosynthetic efficiency (Areche et al., 2023), facilitating mRNA transcription (Nassar et al., 2003; Ghasemi et al., 2013), and optimizing metabolic functions (Sadak et al., 2015). The type of amino acid applied influenced the extent to which Zn amino acid complexes performed in apple trees. Among all the Zn sources, nano Zn amino acid chelates were the most effective at enhancing yield indicators (Table 3). The increased number of flowers observed after application of nano Zn amino acid could be explained by the significance of Zn in the synthesis of tryptophan, which is a precursor to indole acetic acid, or by the simultaneous effects of N and Zn on the metabolic pathways of plants (Cakmak et al., 2010). Further, greater nutrient absorption via amino acid-chelated Zn could have been an explanation of apple trees in stimulation of yield-contributing characteristics (Souri and Hatamian, 2019). In addition, the increase could be caused by the positive effect that nanoparticles had on regulating the release of nutrients and preventing excessive losses as a result of their own internalization by the trees (El-Sayed et al., 2017). The findings are further supported by Najizadeh and Khoshgoftarmanesh (2019), which suggested that zinc-containing foliar spray before flowers full bloom allows for elongation of pollen tubes, which in turn promoted better pollination, embryo development and the formation of flower buds.

Numerous physiological processes, including cell division (Ahmed and Abd El-Hameed, 2003), somatic embryogenesis (Sun et al., 2018), and glucose assimilation (Baqir et al., 2019) are facilitated by amino acids. Increased diversions of the metabolites to the sink with the application of organic Zn chelates provide the evidence for an increase in the number of fruits (Gourkhede et al., 2020). Past investigations on fruit crops have demonstrated the significance of Zn and N during particular plant phenological stages, especially fruit set (Morshedi, 2001). In addition, more photosynthesis and decreased fruit abscission might have resulted from the increased leaf area due to better zinc and amino acid absorption (Kamiab and Zamanibahramabadi, 2016). These results suggest that the use of nano Zn amino acid chelates might have contributed to the increase in fruit set and decrease in fruit abscission. Higher nutritional bioavailability is the main factor driving higher fruit yield when nano amino acid chelates are applied (Yeboah et al., 2021).

According to Kandil et al. (2016), amino acids optimize the activity of certain enzymes involved in the synthesis of carbohydrates, increase biomass production which was directly correlated with higher crop yields. In a previous study, Arabloo et al. (2017) correlated the benefits of foliar application of amino acids to higher fruit set and yield in Granny Smith and Golden Delicious apple cultivars by delayed ovule senescence and improved pollen tube penetration. Also, the stimulatory effects of Zn amino acid chelate resulted in higher vegetative growth and fruit retention, which in turn improved fruit yield (Datir et al., 2010). It could be associated to the additional simulated effect that occurs when N is added to amino acid complexes to supply Zn (Rafie et al., 2017). Zinc has a favourable effect on yield, which is consistent with the findings of Mousavi et al. (2012), who proposed that Zn activates enzymes associated to nitrogen and carbohydrates metabolism. It is well known that foliar fertilizers increase nutrient use efficiency, which in turn increased fruit yield (Kandil and Eman, 2017). These results are consistent with those findings of Ghasemi et al. (2013), Gourkhede et al. (2020), Tabesh et al. (2020) and Yeboah et al. (2021), who also proposed that Zn amino acid chelates were superior to Zn sulphate.

4.1.3 FRUIT QUALITY PARAMETERS

4.1.3.1 PHYSICAL

The data shown in Table 4 illustrates the effects of foliar organic Zn sources on the physical parameters of fruit samples of apple.

4.1.3.1.1 Fruit length

Foliar application of Zn organic sources depicted significant influence on length of fruit samples which varied from 49.57 mm to 57.13 mm (Table 4). Treatment NZn-AAC$_{400}$ registered the maximum fruit length (57.13 mm), which was statistically superior to treatment NZn-AAC$_{200}$ (55.47 mm). However, it was minimum (49.57 mm) in treatment $ZnSO_4$. Also, Zn organic sources ranked in term of length of fruit samples as NZn-AAC$_{400}$ > NZn-AAC$_{200}$ > Zn-AAC$_{400}$ > Zn-AAC$_{200}$ > Zn-Gly$_{400}$ > Zn-Glu$_{400}$.

Table 4. Physical attributes of apple fruit samples affected by foliar feeding of bio-organic Zn sources

Zinc nutrition	Fruit length (mm)	Fruit breadth (mm)	Shape index	Fruit weight (g)	Fruit volume (cm^3)	Specific gravity	Fruit firmness (kg/cm^2)
Zn-Gly$_{200}$	51.99	61.11	0.85	117.20	131.67	0.89	5.63
Zn-Gly$_{400}$	53.94	63.40	0.85	120.21	138.33	0.87	5.53
Zn-Glu$_{200}$	50.00	60.65	0.82	106.37	116.67	0.91	5.87
Zn-Glu$_{400}$	52.54	62.53	0.84	117.67	135.03	0.87	5.61
Zn-AAC$_{200}$	54.32	64.10	0.85	122.15	140.02	0.87	5.46
Zn-AAC$_{400}$	54.66	64.59	0.84	123.99	142.50	0.87	5.20
NZn-AAC$_{200}$	55.47	64.98	0.85	129.01	151.51	0.85	4.95
NZn-AAC$_{400}$	57.13	66.03	0.86	141.32	153.34	0.94	4.64
$ZnSO_4$	49.57	57.07	0.87	99.86	110.06	0.91	6.19
CD$_{0.05}$	2.22	2.95	NS	6.00	9.57	NS	0.15

NS, Non-significant

4.1.3.1.2 Fruit breadth

The analysis of the data indicated that organic Zn sources had a positive impact on breadth of apple fruits. Fruit breadth at the maximum (66.03 mm) noticed for treatment NZn-AAC$_{400}$ was statistically superior to NZn-AAC$_{200}$ (64.98 mm), Zn-AAC$_{400}$ (64.59 mm) and Zn-AAC$_{200}$ (64.10 mm). However, the treatment $ZnSO_4$ showed the lowest fruit breadth (57.07 mm). The treatments Zn-AAC$_{200}$ (64.10 mm), Zn-Gly$_{400}$ (63.40 mm), and Zn-Glu$_{400}$ (62.53 mm) showed comparable statistical effects. Furthermore, application of NZn-AAC$_{400}$ recorded an increase of 15.7 per cent of fruit breadth over the control.

4.1.3.1.3 Shape index

Shape index of apple fruits showed non-significant effects among the different foliar Zn treatments applied. The variations however, ranged from 0.82 to 0.87. Treatment $ZnSO_4$

recorded highest (0.87) shape index, whereas, lowest (0.82) values were observed for Zn-Glu_{200}.

4.1.3.1.4 Fruit weight

Among different Zn nutrient sources, fruit weight ranged between 99.86 g to 141.32 g. The treatment NZn-AAC_{400} recorded the maximum fruit weight (141.32 g), which was statistically superior to all other applied treatments. Moreover, treatments Zn-AAC_{400}, Zn-AAC_{200} and Zn-Gly_{400} exhibited statistically similar results with corresponding values of 123.99, 122.15 and 120.21 g. The treatment of Zn-AAC_{400} demonstrated 41.5 per cent increase of fruit weight compared to control.

4.1.3.1.5 Fruit volume

The data in Table 4 elucidates that volume of apple fruit samples was significantly influenced by application of organic Zn sources. Highest fruit volume (153.34 cm^3) was reported in NZn-AAC_{400}, which was statistically similar to NZn-AAC_{200} (151.51 cm^3). However, the lowest fruit volume (110.06 cm^3) was observed in the treatment $ZnSO_4$, which was statistically at par with Zn-Glu_{200} (116.67 cm^3). Moreover, treatment NZn-AAC_{400} exhibited 1.4 times increase of volume of the apple fruits over control.

4.1.3.1.6 Specific gravity

The specific gravity of fruit samples ranged from 0.85 to 0.94 and depicted insignificant effects among the different foliar Zn treatments applied. Maximum specific gravity (0.94) was attained by NZn-AAC_{400}, which also exhibited statistically equivalent results with Zn-Glu_{200} and $ZnSO_4$ (0.91 each). However, the treatment NZn-AAC_{200} demonstrated the minimum (0.85) specific gravity for fruit samples.

4.1.3.1.7 Fruit firmness

Among various Zn nutrient sources, fruit firmness varied from 4.64 to 6.19 kg/cm^2. Maximum (6.19 kg/cm^2) fruit firmness was registered in $ZnSO_4$ which was statistically significant compared to all other applied treatments. The lowest values (4.64 kg/cm^2) were, however, noted in the treatment NZn-AAC_{400}. Among other treatments utilizing Zn organic sources, statistically proportionate effects were recorded under Zn-Gly_{200} (5.63 kg/cm^2), Zn-Glu_{400} (5.61 kg/cm^2) and Zn-Gly_{400} (5.53 kg/cm^2).

Zn amino acid chelates have been shown to improve fruit physical attributes which might be associated to improved photosynthetic activity, which in turn led to increased cell division and elongation (Datir et al., 2010). Pre-anthesis cell division has been recognized as a significant factor influencing the cell number in fleshy fruits (Coombe, 1976). In addition, fruits developed from early flowering tend to be larger compared to late flowers, attributed to the increased number of cells present during anthesis (Argenta et al., 2022). Furthermore, it could be related to the simulating effect of amino acids on cell growth, which in turn produced heavier and bigger fruits (Rai, 2002; Altemimy et al., 2019). The quicker transport of photosynthates from the source to the sink might also be linked to the increase in fruit weight caused by Zn amino acid compounds (Gourkhede et al., 2020). Also, improved absorption and increased translocation to sink (Lin and Xing, 2008), increased fruit weight could be a consequence of the improved bioavailability of nano amino acid chelates due to their smaller size and higher surface area (Prasad et al., 2012). Amino acid chelates exhibited higher absorption because of higher cell membrane permeability when compared to their inorganic analogues (Souri, 2016).

4.1.3.2 BIOCHEMICAL

The data pertaining to biochemical attributes of apple fruits influenced by foliar application of different Zn nutrient sources is presented in Table 5.

4.1.3.2.1 Total soluble solids

Data depicted in Table 5 revealed a significant effect of organic Zn sources on the total soluble solids (TSS) content of apple fruits. Maximum TSS content (13.24 °B) was recorded in treatment NZn-AAC$_{400}$, which showed statistically at par effects with treatment NZn-AAC$_{200}$ (13.01 °B). Analogous results were also exhibited by treatments Zn-AAC$_{200}$ and Zn-Gly$_{400}$ with corresponding values of 12.59 and 12.23 °B. However, it was minimum (10.77 °B) in treatment $ZnSO_4$. The treatment of NZn-AAC$_{400}$ also recorded an increase of 22.9 per cent TSS compared to control.

4.1.3.2.2 Titratable acidity

Titratable acidity of apple fruits varied between 0.40 and 0.61 per cent with statistically non-significant effects among the different foliar Zn treatments applied. Lowest titratable acidity (0.40%) was reported in treatment NZn-AAC$_{400}$, whereas, it was highest (0.61%) in treatment $ZnSO_4$. Moreover, a decrease of 52.5 per cent was also observed for titratable acidity in NZn-AAC$_{400}$ compared to control.

4.1.3.2.3 Reducing sugars

Among different Zn sources applied, the reducing sugars content varied from 6.12 to 7.20 per cent in apple fruits. Maximum reducing sugars content of 7.20 per cent was observed in NZn-AAC$_{400}$ followed by NZn-AAC$_{200}$, Zn-AAC$_{400}$, Zn-AAC$_{200}$, Zn-Gly$_{400}$ and Zn-Glu$_{400}$ with respective values of 7.07, 6.83, 6.73, 6.66 and 6.53 per cent, whereas, it was attained minimum (6.12%) in $ZnSO_4$ treated apple trees. Moreover, NZn-AAC_{400} also recorded 1.2 times increase in reducing sugars content over control. In addition, NZn-AAC_{400} exhibited statistical equivalence with NZn-AAC_{200} (7.07%). The progression of reducing sugars content under varied Zn sources observed in the order of NZn-AAC_{400} > NZn-AAC_{200} > Zn-AAC_{400} > Zn-AAC_{200} > Zn-Gly_{400} > Zn-Glu_{400}.

4.1.3.2.4 Non-reducing sugars

Analysis of data of Table 5 revealed that application of varied Zn sources exerted a significant influence on non-reducing sugars content. The average values for non-reducing sugars ranged from 3.09 to 3.72 per cent. Treatment Zn-AAC_{200} exhibited maximum non-reducing sugars content which also showed statistically similar effects with Zn-AAC_{400} (3.71%), NZn-AAC_{400} (3.65%), NZn-AAC_{200} (3.61%), and Zn-Gly_{400} (3.52%). The treatment of $ZnSO_4$ however, demonstrated minimum non-reducing sugars (3.09%) and was statistically similar to Zn-Glu_{200} (3.16%) and Zn-Gly_{200} (3.21%). The hierarchical arrangement of various Zn sources for non-reducing sugars was Zn-AAC_{200} > Zn-AAC_{400} > NZn-AAC_{400} > NZn-AAC_{200} > Zn-Gly_{400}.

4.1.3.2.5 Total sugars

A perusal of the data illustrated a pronounced increase in total sugars content of fruit samples due to the application of organic Zn sources during the cropping cycle. Total sugars content exhibited an average of 9.37 to 11.04 per cent. Application of NZn-AAC_{400} recorded the maximum (11.04%) total sugars content which was statistically at par with NZn-AAC_{200} (10.88%) and Zn-AAC_{400} (10.73%). The lowest value (9.37%) however, was recorded in $ZnSO_4$. Treatments of Zn-AAC_{200} and Zn-Gly_{400} also elicited uniform effects with corresponding values of 10.65 and 10.36 per cent. Treatment NZn-AAC_{400} also experienced an upsurge of 17.8 per cent in total sugars content over control. In terms of total sugars, foliar application of Zn organic sources ranked in the order of NZn-AAC_{400} > NZn-AAC_{200} > Zn-AAC_{400} > Zn-AAC_{200} > Zn-Gly_{400} > Zn-Glu_{400}.

4.1.3.2.6 Ascorbic acid

Ascorbic acid content was exhibited maximum (4.37 mg/100g) in treatment application of NZn-AAC_{400}, whereas, the lowest values were recorded in $ZnSO_4$ (2.91 mg/100g). Among other Zn nutrient sources, statistically equivalent results were observed in Zn-AAC_{400} and Zn-AAC_{200} with the respective values of 3.93 and 3.77 mg/100g. Among all treatments, NZn-AAC_{400} noticed the maximum of 50.2 per cent increase followed by NZn-AAC_{200} (42.9%), Zn-AAC_{400} (35.1%) and Zn-AAC_{200} (29.6%) over control.

Table 5. Bio-chemical parameters of apple fruit samples influenced by foliar bio-organic Zn sources

Zinc nutrition	TSS (°B)	Titratable acidity (%)	Reducing sugars (%)	Non-reducing sugars (%)	Total sugars (%)	Ascorbic acid (mg/100 g)	Anthocyanins content (mg/100 g)	Total phenols (mg/100 g)
Zn-Gly$_{200}$	11.64	0.58	6.45	3.21	9.82	3.33	9.47	59.39
Zn-Gly$_{400}$	12.23	0.54	6.66	3.52	10.36	3.59	9.73	64.22
Zn-Glu$_{200}$	11.15	0.59	6.30	3.16	9.63	3.12	9.41	56.95
Zn-Glu$_{400}$	11.93	0.57	6.53	3.30	9.99	3.48	9.60	61.69
Zn-AAC$_{200}$	12.59	0.53	6.73	3.72	10.65	3.77	9.97	65.20
Zn-AAC$_{400}$	12.66	0.49	6.83	3.71	10.73	3.93	10.17	67.71
NZn-AAC$_{200}$	13.01	0.44	7.07	3.61	10.88	4.16	10.34	72.00
NZn-AAC$_{400}$	13.24	0.40	7.20	3.65	11.04	4.37	10.75	73.34
ZnSO$_4$	10.77	0.61	6.12	3.09	9.37	2.91	9.22	53.96
CD$_{0.05}$	0.30	NS	0.20	0.24	0.31	0.19	0.23	2.15

TSS, Total soluble solids; NS, non-significant

4.1.3.2.7 Anthocyanins content

The data presented in Table 5 indicate that anthocyanins content varied from 9.22 to 10.75 mg/100g among different Zn nutrient sources applied. Minimum anthocyanins (9.22 mg/100g) content was recorded in $ZnSO_4$, whereas, the treatment NZn-AAC_{400} exhibited maximum (10.75 mg/100g) value. The treatment NZn-AAC_{400} exceeded all other Zn treatments, demonstrating statistical significance in terms of anthocyanins content. Treatment NZn-AAC_{400} also demonstrated a remarkable 16.6 per cent increase compared to control. However, the treatment $ZnSO_4$ showed statistically at par effects with Zn-Glu_{200} which exhibited the corresponding value of 9.41 mg/100g.

4.1.3.2.8 Total phenols

A cursory glance at the data highlighted the significant effect of foliar Zn organic sources on total phenols which varied from 53.96 to 73.34 mg/100g in fruit samples. Highest phenols (73.34 mg/100g) were recorded in the treatment NZn-AAC_{400}, which was statistically analogous to NZn-AAC_{200} (72.00 mg/100g), whereas, it was lowest (53.96 mg/100g) in the treatment $ZnSO_4$, which marked a distinctly lower significance compared to all other treatments. The treatment of NZn-AAC_{400} exhibited 1.4 times increase compared to control. The trend in total phenols content in fruit samples was observed as NZn-AAC_{400} > NZn-AAC_{200} > Zn-AAC_{400} > Zn-AAC_{200} > Zn-Gly_{400} > Zn-Glu_{400}.

The results of the present investigation showed that among different Zn nutrient sources, nano Zn amino acid chelate depicted the most influential effect on biochemical parameters of fruit samples (Table 5). Zinc is known to be an essential cofactor for several enzymes, including aldolase and carbonic anhydrase, which have a beneficial effect on carbohydrates metabolism (Najizadeh and Khoshgoftarmanesh, 2019; Hassan et al., 2020; Shalan, 2020; Kandil et al., 2022). Further, it is an essential component of nucleic acid metabolism (Marschner, 2012) and photosynthetic activity (Yruela, 2013). Earlier studies on apple (El Seginy et al., 2003), plum (Hassan et al., 2010), peach (Ali et al., 2014), olive (Saadati et al., 2016), and apricot (El-Badawy, 2019) have shown that zinc has positive effects on fruit quality indicators. Moreover, increased TSS, total sugars, anthocyanins content and reduced titratable acidity might have resulted from the acceleration of the photosynthetic process and the conversion of complex carbohydrates to simple sugars and their subsequent translocation from leaf to fruits (Al-Ali, 2006; Shalan, 2020). Owing to organic nature, trace mineral elements present in amino acid chelates are readily chelated and split in the plant system (Areche et al., 2023) which potentially resulted in improved quality parameters.

It could also be ascribed to the nano-particle size of amino chelates for better penetration through stomata (Amin et al., 2022). Similar results through the application of amino acids have been documented by Keutgen and Pawelzik (2008), Rashad et al. (2003) and Nassar et al. (2003). Because zinc is involved in the synthesis of tryptophan, its effect on the production of auxins caused an increase in leaf area (Al-Jubori, 2023) which in turn improved the accumulation of photosynthates and the quality of the fruit (Candar et al., 2019). Also, it was shown that increased auxins production led to a greater ascorbic acid buildup (Nawaz et al., 2008). Additionally, the synergistic effect of foliar amino acid application on increased Zn absorption and assimilation in plant cells associated with the improved fruit quality traits (Santi et al., 2017). The application of amino chelates in fertilization to increase TSS and ascorbic acid content has also been reported by Keutgen and Pawelzik (2008) and Fahimi et al. (2016). According to Song et al. (2015), photosynthesis and the metabolism of sugars and phenols are closely associated. The results of Tavallali et al. (2018) further supported the increased total phenols in fruits with the application of nano Zn amino acid complexes. Previous research has also indicated that plants with low zinc levels observed a decrease in phenol content since Zn is directly involved in excretion of phenols in plant system (Zheng and Wang, 2001).

4.1.4 SOIL PROPERTIES

4.1.4.1 CHEMICAL

The data on the effect of organic Zn sources on the soil chemical properties of apple is illustrated in Table 6, 7 and Fig. 4.

4.1.4.1.1 pH

There was not a significant difference in pH of soil between various foliar Zn treatments (Table 6). However, the soil pH which was highest (7.35) followed $ZnSO_4$ treatment. This was followed by Zn-Glu$_{200}$ (7.30), Zn-Gly$_{200}$ (7.22), and Zn-Glu$_{400}$ (7.20). The treatment of NZn-AAC$_{400}$ recorded the minimum values (7.01). Further, NZn-AAC$_{200}$ treatment showed a 4.9 per cent decrease when compared to the control.

4.1.4.1.2 Electrical conductivity

The results obtained on the electrical conductivity (EC), which ranged from 0.22 to 0.30 dS/m in soils of apple, indicated non-significant effects among the various Zn treatments (Table 6). The treatment $ZnSO_4$ exhibited the highest EC (0.30 dS/m), whereas, NZn-AAC$_{400}$ had the lowest value (0.22 dS/m).

4.1.4.1.3 Organic carbon

Analysis of the data showed that soil organic carbon (SOC) content varied between 2.49 and 2.71 per cent among various Zn nutrient sources. The treatment of NZn-AAC$_{400}$ exhibited the highest (2.71%) SOC which was statistically at par to the treatments Zn-AAC$_{400}$ (2.66%) and NZn-AAC$_{200}$ (2.68%). In contrast, Zn-AAC$_{200}$, Zn-Gly$_{400}$, and Zn-Glu$_{400}$ treatments showed statistically similar results with corresponding values of 2.63, 2.59 and 2.57 per cent. However, treatment ZnSO$_4$ showed the lowest (2.49%) organic carbon content.

Table 6. Post harvest chemical properties of soil of apple influenced by foliar bio-organic Zn sources

Zinc nutrition	pH	EC (dS/m)	OC (%)	Available N (kg/ha)	Available P (kg/ha)	Available K (kg/ha)
Zn-Gly$_{200}$	7.22	0.28	2.55	315.02	118.91	268.98
Zn-Gly$_{400}$	7.18	0.27	2.59	324.18	128.85	278.03
Zn-Glu$_{200}$	7.30	0.29	2.53	309.60	116.01	264.91
Zn-Glu$_{400}$	7.20	0.28	2.57	317.40	124.84	274.44
Zn-AAC$_{200}$	7.15	0.26	2.63	327.15	133.37	280.39
Zn-AAC$_{400}$	7.09	0.25	2.66	335.29	138.06	285.94
NZn-AAC$_{200}$	7.04	0.24	2.68	339.59	140.39	290.57
NZn-AAC$_{400}$	7.01	0.22	2.71	341.89	142.37	292.80
ZnSO$_4$	7.35	0.30	2.49	303.50	111.05	259.95
CD$_{0.05}$	NS	NS	0.07	2.20	1.32	0.98

EC, Electrical conductivity; OC, organic carbon; NS, non-significant

4.1.4.1.4 Available N

A perusal of the data on available N content illustrated the positive influence of the organic Zn sources on apple soils. Treatment of NZn-AAC$_{400}$ registered the maximum available N (341.89 kg/ha) and showed statistical significance over all other applied treatments. The treatment of NZn-AAC$_{400}$ was followed by NZn-AAC$_{200}$, Zn-AAC$_{400}$, Zn-AAC$_{200}$, Zn-Gly$_{400}$ and Zn-Glu$_{400}$ with respective values of 339.59, 335.29, 327.15, 324.18 and 317.40 kg/ha, whereas, it was least (303.50 kg/ha) in ZnSO$_4$. This treatment observed 1.1 times increase in available N content compared to control.

4.1.4.1.5 Available P

Foliar application of Zn organic sources depicted marked influence on available P content of soil which varied from 111.05 to 142.37 kg/ha (Table 6). Application of NZn-AAC$_{400}$ registered the maximum available P (142.37 kg/ha) content, whereas, it was minimum (110.05 kg/ha) in ZnSO$_4$. The hierarchy of Zn sources regarding available P content showed a sequence of NZn-AAC$_{400}$ > NZn-AAC$_{200}$ > Zn-AAC$_{400}$ > Zn-AAC$_{200}$ > Zn-Gly$_{400}$ > Zn-Glu$_{400}$.

4.1.4.1.6 Available K

A perusal of the data depicted the positive effect of organic Zn sources on available K content of apple soils. Treatment of NZn-AAC$_{400}$ observed maximum (292.80 kg/ha) available K content, which was statistically higher than all other Zn nutrient sources. Following the superior treatment, the subsequent treatments of NZn-AAC$_{200}$, Zn-AAC$_{400}$, Zn-AAC$_{200}$ and Zn-Gly$_{400}$ showed decrease in available K content with the corresponding values of 290.57, 285.94, 280.39 and 278.03 kg/ha. Whereas, it was minimum (259.95 kg/ha) and recorded an increment of 12.6 per cent compared to ZnSO$_4$.

4.1.4.1.7 Exchangeable Ca

The data presented in Table 7 indicates that exchangeable Ca content varied from 1513.53 to 1564.17 mg/kg. The treatment of NZn-AAC$_{400}$ exhibited maximum (1564.17 mg/kg) exchangeable Ca content. However, the treatment ZnSO$_4$ recorded the lowest (1513.53 mg/kg) exchangeable Ca content. Moreover, the treatment of Zn-AAC$_{200}$ and Zn-Gly$_{400}$ showed statistically similar results with the corresponding values of 1537.73 and 1536.06 mg/kg. Application of treatment NZn-AAC$_{400}$ also demonstrated an increment of 3.3 per cent compared to control.

Table 7. Foliar feeding of bio-organic Zn sources affects meso-nutrients and micronutrient cations at 0-15 cm depth of apple rhizosphere

Zinc nutrition	Meso-nutrients (mg/kg)		DTPA extractable micronutrient cations (mg/kg)			
	Exchangeable Ca	Exchangeable Mg	Zn	Fe	Cu	Mn
Zn-Gly$_{200}$	1525.17	550.63	23.08	67.95	10.43	29.95
Zn-Gly$_{400}$	1536.06	559.31	24.03	69.15	11.20	33.93
Zn-Glu$_{200}$	1519.89	546.01	22.70	67.55	10.12	28.50
Zn-Glu$_{400}$	1530.94	555.58	23.46	68.44	10.84	31.29
Zn-AAC$_{200}$	1537.73	564.73	24.53	70.82	11.92	34.38
Zn-AAC$_{400}$	1549.48	569.94	24.98	71.17	12.39	35.34
NZn-AAC$_{200}$	1557.38	574.17	25.50	71.56	12.72	36.17
NZn-AAC$_{400}$	1564.17	577.43	26.04	74.24	12.94	36.76
ZnSO$_4$	1513.53	540.10	21.99	65.31	9.66	25.95
CD$_{0.05}$	2.87	2.65	0.75	1.07	0.27	0.99

4.1.4.1.8 Exchangeable Mg

The data revealed that the treatment NZn-AAC$_{400}$ recorded the maximum exchangeable Mg (577.43 mg/kg) content, which was statistically superior to all other applied treatments, with minimum values in the treatment ZnSO$_4$ (540.10 mg/kg). The superior treatment also showed 6.9

per cent increase compared to control. Moreover, all other applied organic Zn treatments displayed statistical non-significant effects with each other.

4.1.4.1.9 Zinc

Highest DTPA extractable Zn (26.04 mg/kg) content was reported in the treatment NZn-AAC$_{400}$ which was statistically at par to treatment NZn-AAC$_{200}$ (25.50 mg/kg). Whereas, it was lowest (21.99 mg/kg) in the treatment $ZnSO_4$, which showed statistically similar results with Zn-Glu$_{200}$ (22.70 mg/kg). Moreover, a significant increase of 1.2 times was observed with NZn-AAC$_{400}$ treatment compared to the control.

4.1.4.1.10 Iron

DTPA extractable Fe content in apple soils is significantly affected by different organic Zn nutrients (Table 7). Among the various Zn nutrients, DTPA extractable Fe content ranged from 65.31 to 74.24 mg/kg. NZn-AAC$_{400}$ treatment recorded the highest DTPA extractable Fe content in apple soil. The treatment of $ZnSO_4$ however, demonstrated the minimum Fe content. The treatments of Zn-Glu$_{400}$, Zn-Gly$_{200}$ and Zn-Glu$_{200}$ were statistically at par with each other recording corresponding values of 68.44, 67.95 and 67.55 mg/kg.

4.1.4.1.11 Copper

Maximum DTPA extractable Cu (12.94 mg/kg) content was registered in treatment NZn-AAC$_{400}$ which was statistically equivalent with the values recorded in NZn-AAC$_{200}$ (12.72 mg/kg). The lowest values (9.66 mg/kg) were, however, noted in the treatment $ZnSO_4$. Similarly, the treatments of NZn-AAC$_{200}$, Zn-AAC$_{400}$, Zn-AAC$_{200}$, Zn-Gly$_{400}$ and Zn-Glu$_{400}$ following the superior treatment depicted decreasing efficacy with respective values of 12.72, 12.39, 11.92, 11.20 and 10.84 mg/kg.

4.1.4.1.12 Manganese

Data showed that organic Zn sources had a positive effect on DTPA extractable Mn content (Table 7). Highest Mn content (36.76 mg/kg) was observed in the NZn-AAC$_{400}$ treatment, showing statistically similar results to the NZn-AAC$_{200}$ treatment (36.17 mg/kg). Zn-AAC$_{200}$ and Zn-Gly$_{400}$ treatments also showed comparable results, with the values of 34.38 and 33.93 mg/kg, respectively. However, treatment $ZnSO_4$ indicated a minimum Mn content of 25.95 mg/kg. A further 41.7 per cent increase was observed in the superior treatment of NZn-AAC$_{400}$ when compared to control.

According to the current study, the influence of amino acid chelates to increase soil microbial activity explains the significant effect of foliar application of Zn amino acid complexes on chemical properties of soil (Areche et al., 2023). Moreover, higher microbial activity in the soil induced organic matter to decompose rapidly, transforming organic minerals into forms which were readily available (Anitha, 2020). The rapid breakdown of organic matter might also be the reason for increased SOC and other available nutrients in the soils (Babu and Sharma, 2005; Shashidhar et al., 2009). Moreover, the application of the recommended dose of FYM might have contributed to the significant shift (Saini et al., 2021). Two to three ionic forms found in chemical fertilisers are frequently susceptible to negative interactions in the soil, such as precipitation and fixation (Kumar et al., 2019). On the other hand, since amino chelates are organic, can prevent these kinds of reactions and boost the availability of nutrients in the soil (Souri and Hatamian, 2019; Dolev et al., 2020). In addition, based on the pH of the soil, the molecular structure of amino acids enabled them to behave as bases or acids, which improved the nutrient status of the soil (Souri, 2016). Also, it could be associated to the positive interaction between biological minerals and nano chelates, which enhanced the chemical and physical characteristics of soil (Liu et al., 2006).

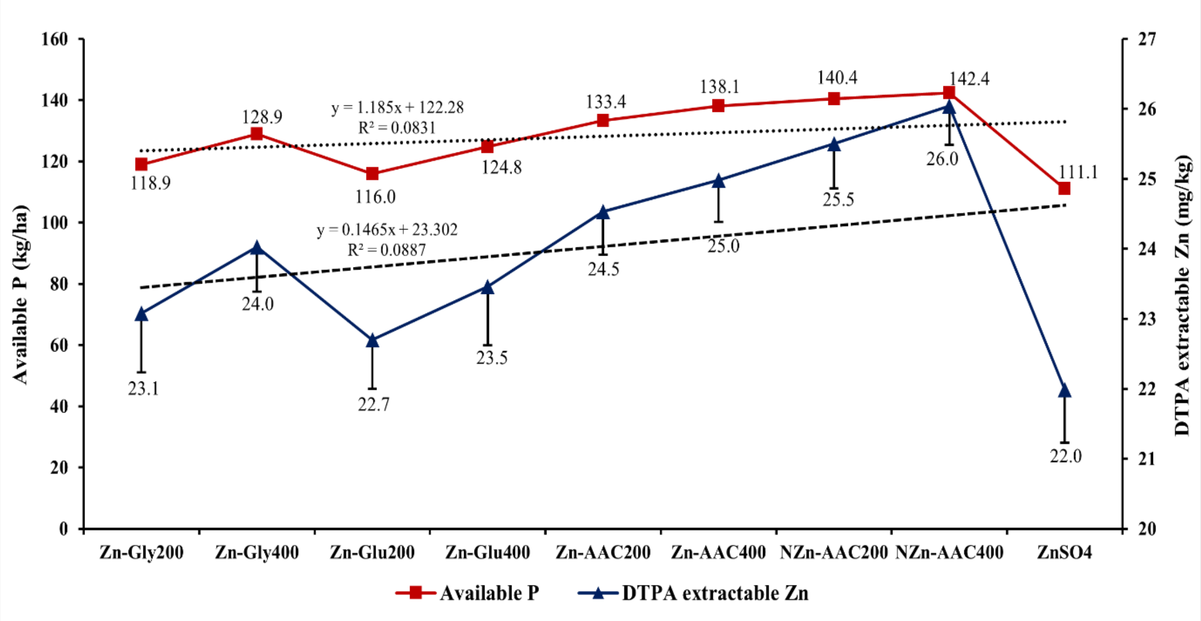


Fig. 5: Correlation between available P and DTPA extractable Zn in soils of apple under bio-organic Zn nutrition

The breakdown and chelation action of amino acids in the apple rhizosphere could also be responsible for the increase in macro and micronutrient content of soil (Wei-Hong et al., 2007). Further, increased biogeochemical processes, such as nitrification by the addition of nano fertilizers, are associated with enhanced available N in soil (Khardia et al., 2022). In addition, slow-release nanoparticles showed better cation exchange capability and more accessible N than their traditional counterparts (Rajonee et al., 2016). Lonergan et al.

(2009), Wu et al. (2011) and Ghoneim (2016) have also reported the beneficial effects of Zn fertilization for available soil N, P, and K in fruit crops. Habashy et al. (2006) observed increased soil micronutrient (Zn, Fe, Mn, and Cu) concentration following the application of Zn amino acid chelates. According to Sidhu and Sharma (2010), this increase was also attributed to the application of zinc, which improved the organic carbon content of soil. Furthermore, Regar et al. (2022) reported that the formation of soluble complexes between zinc and soil organic matter contributed for the increased DTPA extractable Zn concentration (Fig. 4). Improved nitrogen in the soil is further enhanced by reduced nutrient fixation, microbial transformation, leaching, and environmental losses by nano Zn (Thirunavukkarasu and Subramanian, 2015).

4.1.4.2 MICROBIOLOGICAL

Data highlighting the effect of different bio-organic Zn nutrient sources on the soil microbiological properties is presented in Table 8, 9 and Fig. 5.

4.1.4.2.1 Total bacterial count

The data depicted that the total number of bacteria in the rhizosphere of apple trees varied between 1.22 × 10^6 and 1.50 × 10^6 cfu/g of soil ($\log_{10}$ transformed). The treatment of NZn-AAC$_{400}$ had the highest recorded total bacterial count (1.50 × 10^6 cfu/g), whereas, the treatment ZnSO$_4$ had the lowest (1.22 × 10^6 cfu/g). Total bacterial count in treatment of NZn-AAC$_{400}$ however, was increased by 22.9 per cent over control (Table 8; Plate 3). Similar results were also demonstrated by Zn-Gly$_{200}$ and Zn-Glu$_{200}$ treatments, obtaining the values of 1.27 × 10^6 cfu/g, respectively.

Table 8. Total viable microbial population ($\log_{10}$ transformed) at 0-15 cm depth affected by foliar bio-organic Zn sources in apple rhizosphere

Zinc nutrition	Total bacterial count (× 10^6 cfu/g)	Soil fungi (× 10^3 cfu/g)	Actinobacterial count (× 10^4 cfu/g)
Zn-Gly$_{200}$	1.27 (18.74)	0.91 (8.21)	0.97 99.28)
Zn-Gly$_{400}$	1.36 (22.66)	0.95 (8.86)	1.02 (10.49)
Zn-Glu$_{200}$	1.27 (18.44)	0.89 (7.81)	0.95 (8.84)
Zn-Glu$_{400}$	1.32 (21.00)	0.93 (8.50)	1.00 (10.07)
Zn-AAC$_{200}$	1.40 (24.94)	0.98 (9.50)	1.04 (10.90)
Zn-AAC$_{400}$	1.42 (26.51)	0.99 (9.80)	1.06 (11.38)
NZn-AAC$_{200}$	1.48 (29.91)	1.01 (10.34)	1.08 (12.11)
NZn-AAC$_{400}$	1.50 (31.42)	1.03 (10.71)	1.09 (12.27)
ZnSO$_4$	1.22 (16.46)	0.76 (5.78)	0.85 (7.01)

Figures in parentheses are actual values; cfu, colony forming units

4.1.4.2.2 Soil fungi

Among different Zn bio-organic sources applied, maximum soil fungal population (1.03×10^3 cfu/g) was demonstrated by NZn-AAC$_{400}$ which was followed by NZn-AAC$_{200}$, Zn-AAC$_{400}$, Zn-AAC$_{200}$, and Zn-Gly$_{400}$ with respective values of 1.01, 0.99, 0.98 and 0.95 $\times 10^3$ cfu/g (log$_{10}$ transformed). Minimum soil fungal (0.76×10^3 cfu/g) population was, however, recorded in the treatment ZnSO$_4$ (Plate 4). The progression of soil fungal population under varied Zn sources unfolded as NZn-AAC$_{400}$ > NZn-AAC$_{200}$ > Zn-AAC$_{400}$ > Zn-AAC$_{200}$ > Zn-Gly$_{400}$ > Zn-Glu$_{400}$. NZn-AAC$_{400}$ also exhibited 1.4 times increase in soil fungal population over control.

4.1.4.2.3 Actinobacterial count

Analysis of data presented in Table 8 (log$_{10}$ transformed) revealed that application of varied Zn sources exerted a significant influence on actinobacterial count in apple rhizosphere (Plate 5). Treatment of NZn-AAC$_{400}$ exhibited maximum (1.09×10^4 cfu/g) actinobacterial count and also showed statistically identical effects with NZn-AAC$_{200}$ (1.08×10^4 cfu/g). However, the treatment ZnSO$_4$ demonstrated minimum of 0.85×10^4 cfu/g actinobacterial count of soil. The hierarchical arrangement of various Zn sources for soil actinobacterial population was NZn-AAC$_{400}$ > NZn-AAC$_{200}$ > Zn-AAC$_{400}$ > Zn-AAC$_{200}$ > Zn-Gly$_{400}$ > Zn-Glu$_{400}$.

4.1.4.2.4 Phosphorus solubilizing bacteria

Different bio-organic Zn sources resulted in a significant increase in the number of phosphorus solubilizing bacteria (PSB) in rhizosphere (log$_{10}$ transformed). Maximum PSB count of 0.96×10^4 cfu/g was exhibited by the treatment NZn-AAC$_{400}$, which was statistically superior to all other applied treatments (Table 9; Plate 6). However, treatment ZnSO$_4$ showed the lowest count (0.59×10^4 cfu/g). Different bio-organic Zn sources obtained PSB count in the order of NZn-AAC$_{400}$ > NZn-AAC$_{200}$ > Zn-AAC$_{400}$ > Zn-AAC$_{200}$ > Zn-Gly$_{400}$ > Zn-Glu$_{400}$.

4.1.4.2.5 Potassium solubilizing bacteria

Application of organic Zn sources resulted in a marked increase in the potassium solubilizing bacteria (KSB) count recorded during the course of investigation (log$_{10}$ transformed). KSB count was maximum (0.89×10^5 cfu/g) in the treatment NZn-AAC$_{400}$, whereas, it was least in ZnSO$_4$ (0.68×10^5 cfu/g). Among other Zn nutrient sources, statistically equivalent results were reported in Zn-Gly$_{200}$ and Zn-Glu$_{200}$ with the respective

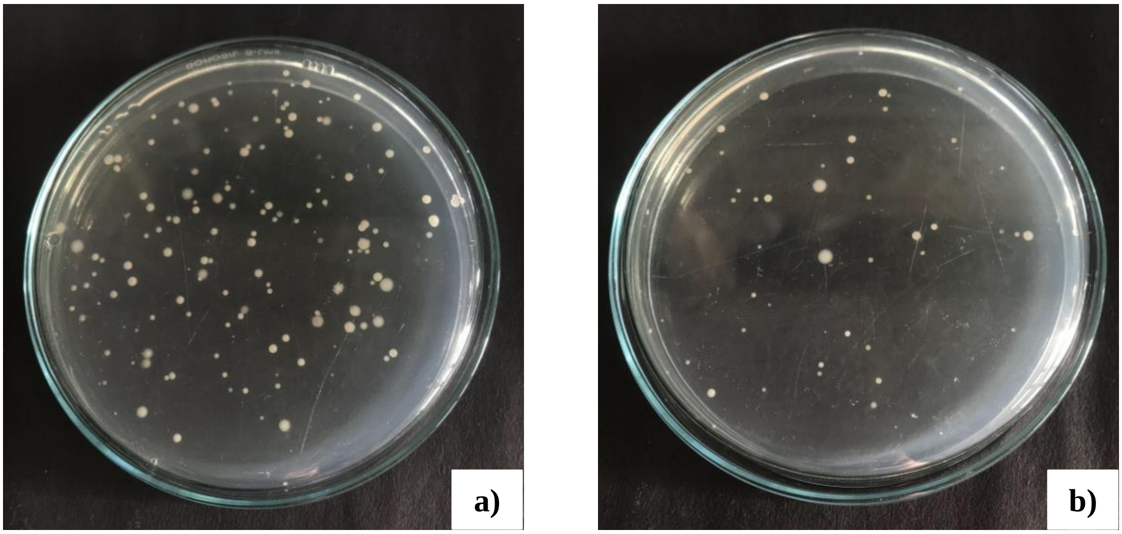


Plate 3: Total bacterial population (× 10^6 cfu/g) in rhizosphere of apple influenced by a) NZn-AAC$_{400}$ b) ZnSO$_4$ (control)

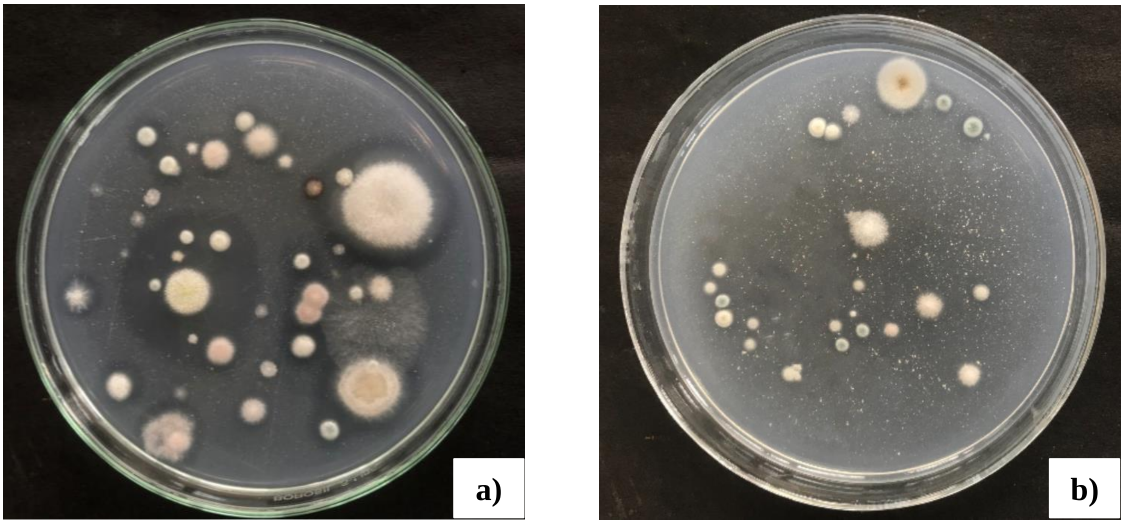


Plate 4: Soil fungal population (× 10^3 cfu/g) in rhizosphere of apple influenced by a) NZn-AAC$_{400}$ b) ZnSO$_4$ (control)

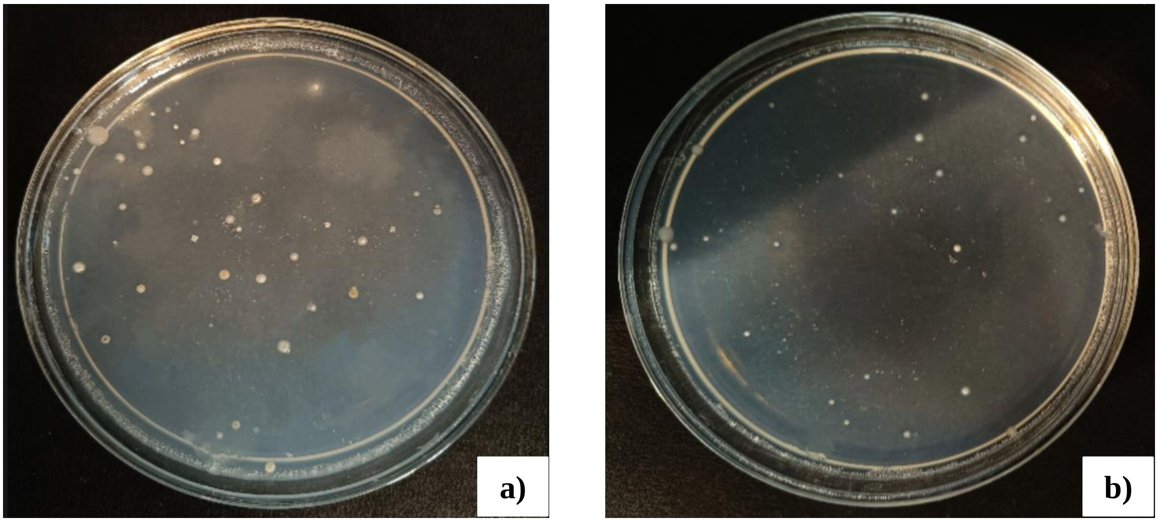


Plate 5: Actinobacterial count (× 10^4 cfu/g) in rhizosphere of apple influenced by a) NZn-AAC$_{400}$ b) ZnSO$_4$ (control)

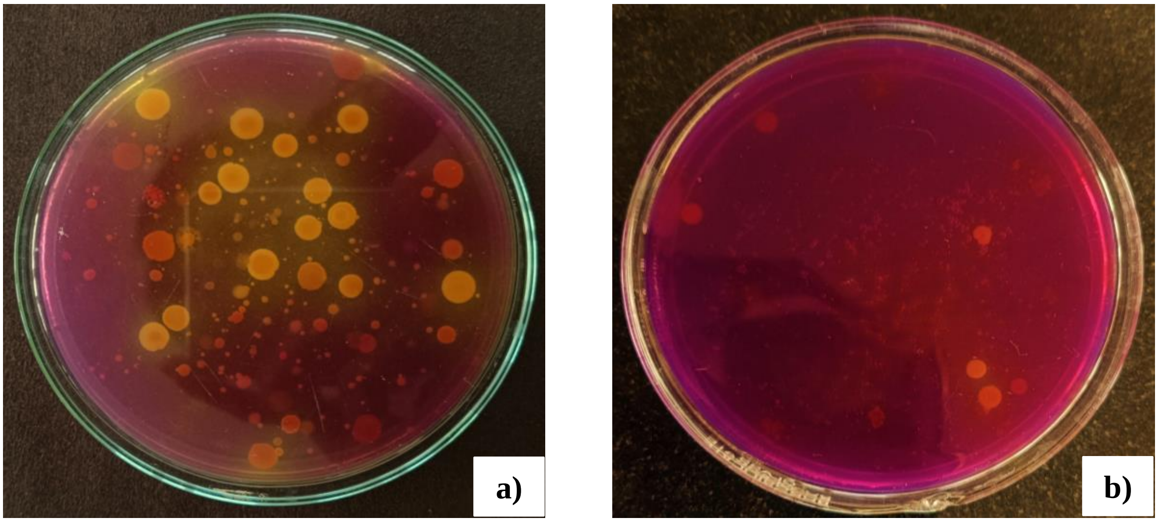


Plate 6: Phosphorus solubilizing bacteria (× 10^4 cfu/g) in rhizosphere of apple influenced by a) NZn-AAC$_{400}$ b) ZnSO$_4$ (control)

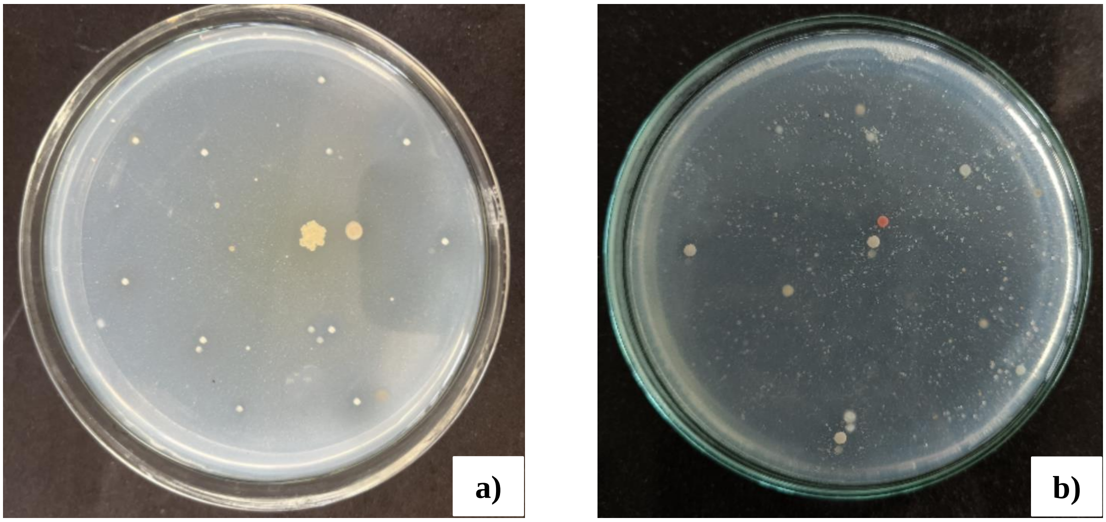


Plate 7: Zinc solubilizing bacteria (× 10^5 cfu/g) in rhizosphere of apple influenced by a) NZn-AAC$_{400}$ b) ZnSO$_4$ (control)

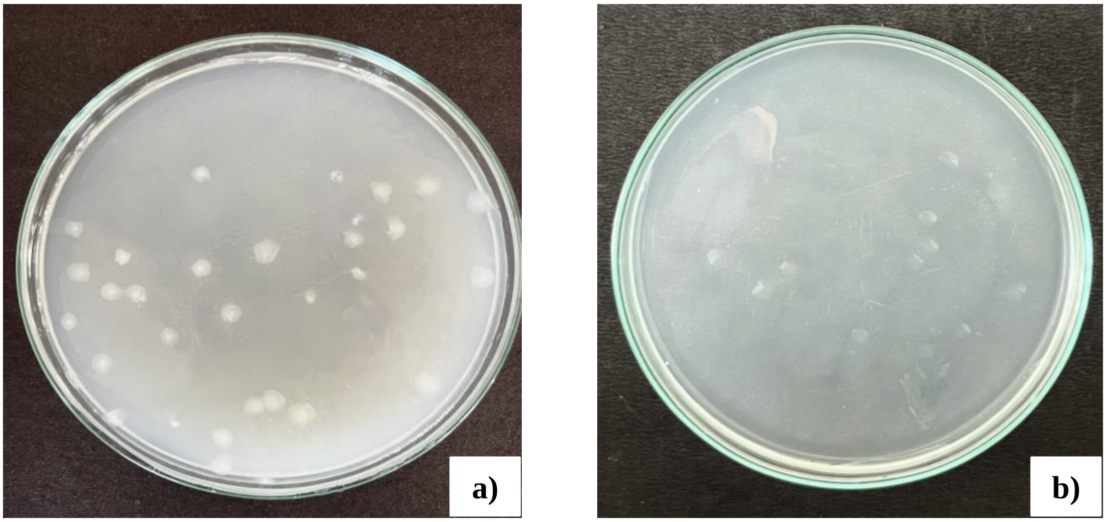


Plate 8: *Azotobacter* count (× 10^5 cfu/g) in rhizosphere of apple influenced by a) NZn-AAC$_{400}$ b) ZnSO$_4$ (control)

values of 0.77 × 10^5 cfu/g and 0.76 × 10^5 cfu/g. Further, NZn-AAC$_{400}$ displayed the maximum per cent increase of 30.8, followed by NZn-AAC$_{200}$ (29.4%), Zn-AAC$_{400}$ (27.9%) and Zn-AAC$_{200}$ (25.0%) over control.

Table 9. Viable microbial count ($\log_{10}$ transformed) of P, K, Zn solubilizers and N_2-fixers in apple rhizosphere at 0-15 cm depth

Zinc nutrition	PSB (× 10^4 cfu/g)	KSB (× 10^5 cfu/g)	ZSB (× 10^5 cfu/g)	*Azotobacter* count (× 10^5 cfu/g)
Zn-Gly$_{200}$	0.78 (6.06)	0.77 (5.87)	0.82 (6.62)	1.14 (13.83)
Zn-Gly$_{400}$	0.83 (6.82)	0.83 (6.78)	0.90 (7.97)	1.18 (15.27)
Zn-Glu$_{200}$	0.75 (5.61)	0.76 (5.76)	0.79 (6.11)	1.13 (13.62)
Zn-Glu$_{400}$	0.82 (6.55)	0.80 (6.37)	0.85 (7.00)	1.16 (14.58)
Zn-AAC$_{200}$	0.86 (7.21)	0.85 (7.09)	0.91 (8.22)	1.21 (16.17)
Zn-AAC$_{400}$	0.90 (7.99)	0.87 (7.39)	0.94 (8.70)	1.23 (17.14)
NZn-AAC$_{200}$	0.93 (8.47)	0.88 (7.63)	0.95 (9.01)	1.25 (17.73)
NZn-AAC$_{400}$	0.96 (9.02)	0.89 (7.80)	0.99 (9.68)	1.27 (18.47)
$ZnSO_4$	0.59 (3.87)	0.68 (4.83)	0.70 (5.01)	1.04 (11.07)

Figures in parentheses are actual values; PSB, phosphorus solubilizing bacteria; KSB, potassium solubilizing bacteria; ZSB, zinc solubilizing bacteria; cfu, colony forming units

4.1.4.2.6 Zinc solubilizing bacteria

The population of zinc solubilizing bacteria (ZSB) varied between 0.70 × 10^5 and 0.99 × 10^5 cfu/g in rhizosphere of apple ($\log_{10}$ transformed). $ZnSO_4$ showed the lowest ZSB count (0.70 × 10^5 cfu/g), whereas, NZn-AAC$_{400}$ treatment showed the highest (0.99 × 10^5 cfu/g). NZn-AAC$_{400}$ treatment exceeded all other treatments, indicating statistical significance with 41.4 per cent increase over control (Table 9; Plate 7).

4.1.4.2.7 *Azotobacter* count

A cursory glance at the data ($\log_{10}$ transformed) highlighted the significant impact of Zn organic sources on *Azotobacter* count which varied from 1.04 to 1.27 (× 10^5 cfu/g). Highest *Azotobacter* count (1.27 × 10^5 cfu/g) was recorded in NZn-AAC$_{400}$, which was statistically superior to all other treatments. The lowest *Azotobacter* count (1.04 × 10^5 cfu/g) was noticed in $ZnSO_4$ treated trees, which marked a distinctly lower significance compared to all other treatments (Plate 8). The treatment of NZn-AAC$_{400}$ also exhibited 1.2 times increase compared to control.

4.1.4.2.8 AM fungi spore count

Different Zn organic sources exerted a significant impact on the AM fungal spore count in the rhizosphere of apple trees (Fig. 5). Treatment of NZn-AAC$_{400}$ exhibited the highest AM fungal spore count (127.11 spores/50 g of soil), which was statistically at par with NZn-AAC_{200} (124.45 spores/50 g of soil). However, it was minimum (97.18 spores/50 g of soil) in the treatment $ZnSO_4$. Application of treatments namely, Zn-Glu_{400}, Zn-Gly_{200} and Zn-Glu_{200} also demonstrated similar effects in relation to AM fungal spore count with corresponding values of 105.65, 103.85 and 101.09 spores/50 g of soil, respectively. The treatment NZn-AAC_{400} however, also recorded 1.3 times increase in AM fungal spore population compared to control.

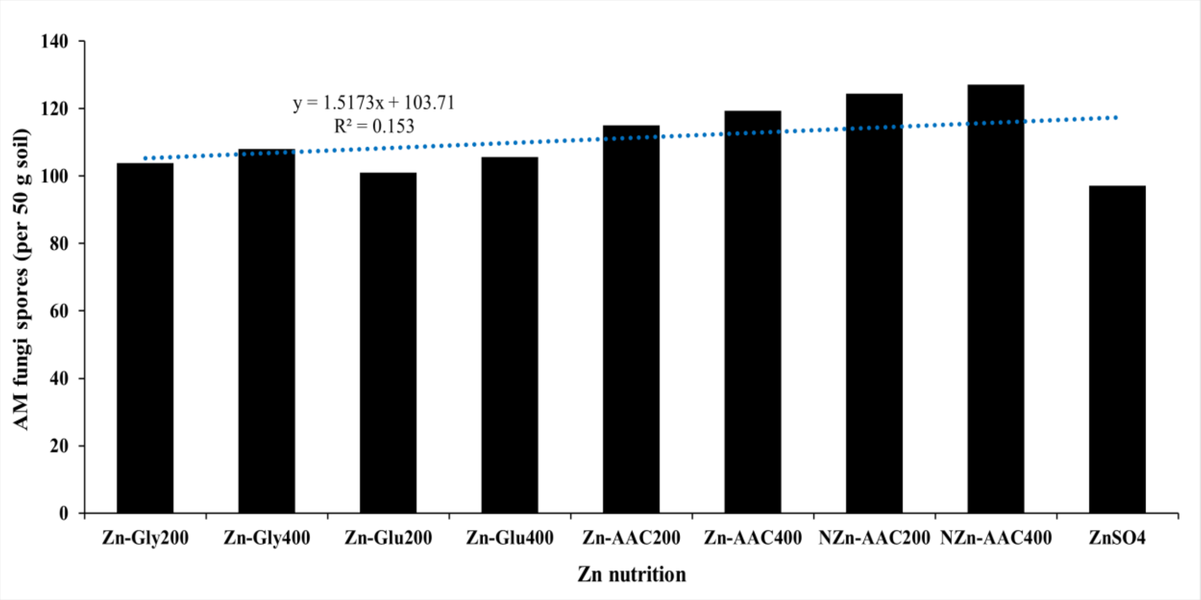


Fig. 6: AM fungi spore count in apple rhizosphere (0-15 cm depth) as affected by bio-organic Zn sources

4.1.4.3 SOIL ENZYMES

The data on the effect of Zn organic sources on the enzymatic activity in the rhizosphere of apple is illustrated in Table 10, Fig. 6-8.

4.1.4.3.1 Acid phosphatase

Among various Zn nutrition sources, the variability of acid phosphatase enzymatic activity was ranged from 104.35 to 135.37 µmole PNP/h/g of soil in apple rhizosphere (Table 10; Fig. 6). Treatment NZn-AAC_{400} exhibited the highest acid phosphatase activity (135.37 µmole PNP/h/g of soil), which was statistically significant when compared to all other applied treatments. A consistent pattern of NZn-AAC_{400} > NZn-AAC_{200} > Zn-AAC_{400} > Zn-AAC_{200} > Zn-Gly_{400} > Zn-Glu_{400} was observed for various Zn nutrition sources. Considering acid phosphatase activity, treatment $ZnSO_4$ had the lowest value (104.35 µmole PNP/h/g of soil).

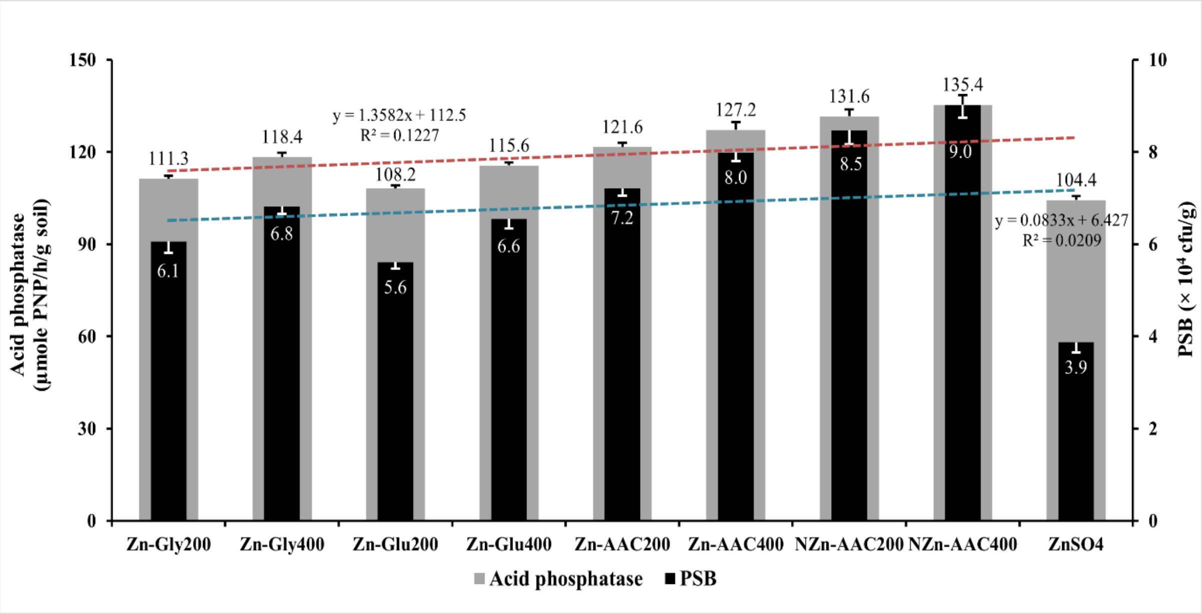


Fig. 7: Relationship studies on acid phosphatase and PSB in soils of apple under bio-organic Zn nutrition

4.1.4.3.2 Alkaline phosphatase

When compared to control, the organic Zn sources showed a positive effect on alkaline phosphatase activity in the rhizosphere of apple (Fig. 7). Highest alkaline phosphatase activity was recorded by treatment NZn-AAC$_{400}$ (159.63 µmole PNP/h/g of soil), exhibiting statistically similar results to treatment NZn-AAC$_{200}$ (157.08 µmole PNP/h/g of soil). Treatments Zn-AAC$_{400}$, Zn-AAC$_{200}$ and Zn-Gly$_{400}$ subsequently showed the corresponding values of 153.27, 146.24 and 143.21 µmole PNP/h/g of soil. Nevertheless, it was minimum (126.13 µmole PNP/h/g soil) in treatment ZnSO$_4$. The results also showed that the treatment of Zn-AAC$_{400}$ resulted in 1.3 times increase in alkaline phosphatase activity in the rhizosphere over the control.

Table 10. Soil enzymatic activities in apple rhizosphere (0-15 cm depth) influenced by foliar bio-organic Zn sources

Zinc nutrition	Acid phosphatase (µmole PNP/h/g soil)	Alkaline phosphatase (µmole PNP/h/g soil)	Dehydrogenase (µg TPF/h/g soil)
Zn-Gly$_{200}$	111.33	136.06	8.84
Zn-Gly$_{400}$	118.36	143.21	10.00
Zn-Glu$_{200}$	108.21	130.83	8.58
Zn-Glu$_{400}$	115.59	139.94	9.60
Zn-AAC$_{200}$	121.61	146.24	10.99
Zn-AAC$_{400}$	127.19	153.27	11.56
NZn-AAC$_{200}$	131.60	157.08	12.87
NZn-AAC$_{400}$	135.37	159.63	13.18
ZnSO$_4$	104.35	126.13	8.75
CD$_{0.05}$	2.50	3.76	1.22

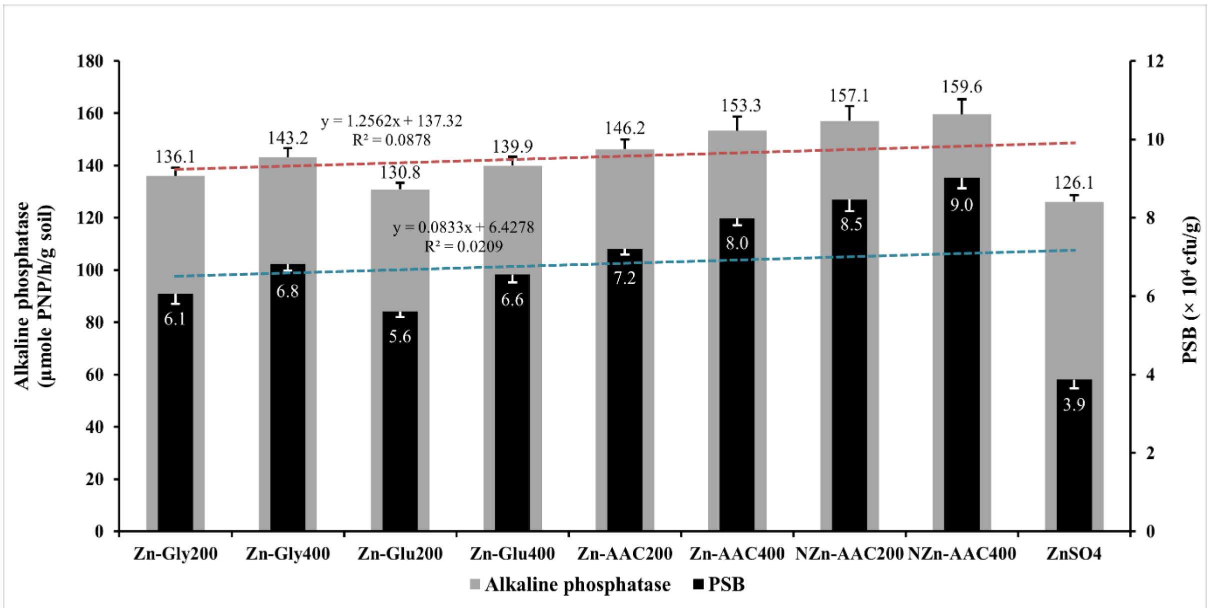


Fig. 8: Correlation between alkaline phosphatase and PSB in rhizosphere of apple under bio-organic Zn fertilization

4.1.4.3.3 Dehydrogenase

The rhizosphere of apple trees showed substantial fluctuations in soil dehydrogenase activity, which ranged from 8.75 to 13.18 µg TPF/h/g of soil (Table 10; Fig. 8). Highest dehydrogenase activity was recorded by treatment NZn-AAC$_{400}$ (13.18 µg TPF/h/g of soil). Also, the treatment of NZn-AAC$_{200}$ showed statistically significant results (12.87 µg TPF/h/g soil). However, $ZnSO_4$ treated apple trees recorded minimal values of 8.75 µg TPF/h/g of soil. In terms of dehydrogenase activity in rhizosphere, different bio-organic Zn sources were ranked in descending order of NZn-AAC$_{400}$ > NZn-AAC$_{200}$ > Zn-AAC$_{400}$ > Zn-AAC$_{200}$ > Zn-Gly$_{400}$.

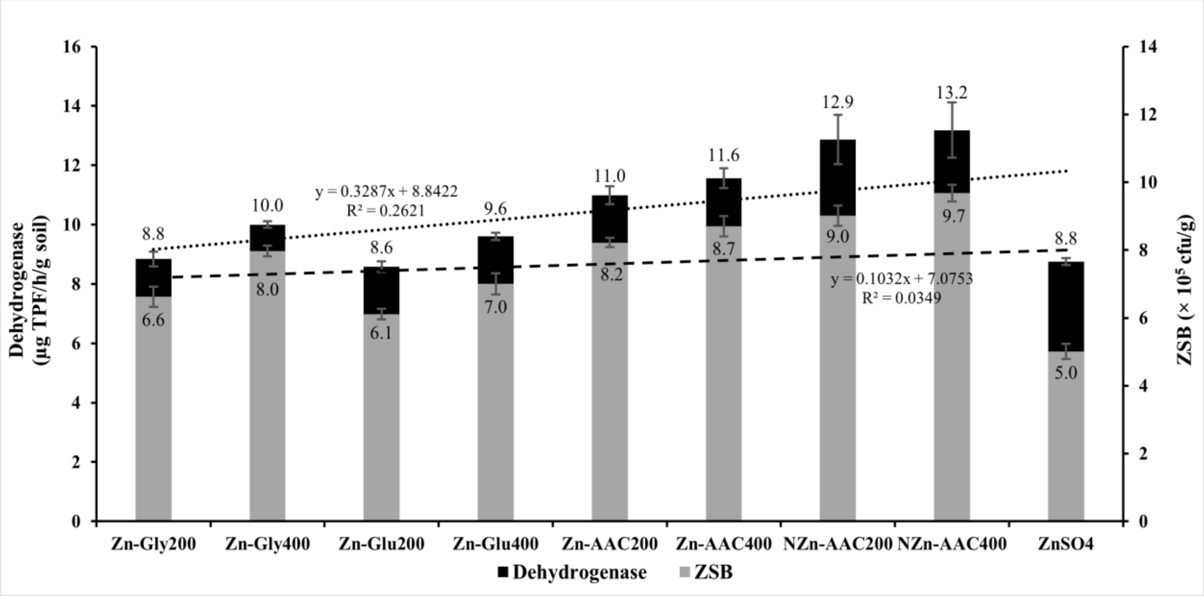


Fig. 9: Relationship between dehydrogenase and ZSB in soils of apple under bio-organic Zn nutrition

The findings showed that the soil microbial attributes were the most significantly affected by nano Zn amino acid chelates. This effect could be explained by the fact that amino acids are considered the most desirable compounds/substances for soil microbes (Souri, 2015). Also, Baghaie and Keshavarzi (2021) observed that microbial respiration is an important component contributing to the significant impact of Zn amino acid complexes on increased microbial activity in rhizosphere. In addition, Zhan et al. (2018) found that application of bio-organic sources enhanced the soil microbial community, in contrast to chemical fertilizers that had a negative impact on microbial population of soil. When compared to $ZnSO_4$, zinc amino acid chelates exhibited better bioavailability, which increased nutrient availability and, in turn, benefited the soil microbial community (Raza et al., 2023). Moreover, the application of Zn amino acid complexes led to an increase in the deposition of organic acids, which acidified the soil and had an additional effect on the bacterial and fungal population (Upadhayay et al., 2022). The slow release of nanofertilizers release humic acid and root exudates, increase C and N in the soil while providing food for the microbes in the soil (Solanki et al., 2015; Nandy et al., 2023). It could also be explained by the fact that zinc become more soluble in the soil, increasing the amount of zinc that the soil's bacterial population can access (Srithaworn et al., 2023). Previous study has also shown that actinomycetes, bacteria, fungus, and acid phosphatase activity in soil were significantly affected by nano Zn chelates (Raliya and Tarafdar, 2013; Khardia et al., 2022).

In addition, the microbial community expanded as a result of the improvement of photosynthesis with nano Zn (Thomas et al., 2017). Similar findings have also been observed by Raliya and Tarafdar (2013) and Bala et al. (2019), who reported that foliar application of nano Zn increased the number of microorganisms and enzymatic activity in soil. In addition, better chemical interactions within soil ascribed to enhanced Zn availability in nano form led to higher soil dehydrogenase activity (Vijayalaxmi et al., 2013; Nandy et al., 2024). Wyszkowska et al. (2013) reported the catalytic and regulatory properties of nano Zn to boost microbial and dehydrogenase activity in rhizosphere.

4.1.5 PHYSIOLOGICAL PARAMETERS

The data depicting the influence of bio-organic Zn supplements on the physiological parameters of apple in high density plantation has been presented in Table 11 and Table 12.

4.1.5.1 Total chlorophylls

Among various bio-organic Zn sources, the amount of chlorophylls in leaves of apple trees was ranged from 2.32 to 3.80 mg/g. The treatment of NZn-AAC_{400} obtained the maximum leaf chlorophyll content (3.80 mg/g), whereas, it was the lowest (2.32 mg/g) in the treatment $ZnSO_4$. Application of Zn-AAC_{400} was the most effective zinc source in increasing leaf chlorophyll content, followed by NZn-AAC_{200}, Zn-AAC_{400}, Zn-AAC_{200} and Zn-Gly_{400} treatments.

4.1.5.2 Photosynthetic efficiency

A perusal of the data presented in Table 11 showed that the trees treated with NZn-AAC_{400} attained maximum (9.18 µmol CO_2 m^2/s) photosynthetic efficiency which was statistically comparable to that of NZn-AAC_{200} (9.05 µmol CO_2 m^2/s). But under the $ZnSO_4$ treatment, it was at a minimum (6.64 µmol CO_2 m^2/s). Furthermore, an increase of 38.3 per cent in NZn-AAC_{400} was observed when compared to the control. Treatments Zn-Gly_{200} (7.07 µmol CO_2 m^2/s) and Zn-Glu_{200} (6.90 µmol CO_2 m^2/s) demonstrated consistent statistical effects in terms of photosynthetic efficiency of apple trees.

Table 11. Physiological parameters of apple trees influenced by foliar bio-organic Zn sources

Zinc nutrition	Total chlorophylls (mg/g)	Photosynthetic efficiency (µmol CO_2 m^2/s)	Transpiration rate (mmol H_2O m^2/s)	Stomatal conductance (mmol H_2O m^2/s)
Zn-Gly_{200}	2.83	7.07	2.16	0.122
Zn-Gly_{400}	3.22	7.93	2.52	0.127
Zn-Glu_{200}	2.60	6.90	2.07	0.120
Zn-Glu_{400}	2.98	7.54	2.37	0.125
Zn-AAC_{200}	3.32	8.30	2.62	0.131
Zn-AAC_{400}	3.53	8.79	2.69	0.132
NZn-AAC_{200}	3.75	9.05	2.82	0.134
NZn-AAC_{400}	3.80	9.18	2.92	0.138
$ZnSO_4$	2.32	6.64	2.01	0.117
$CD_{0.05}$	0.11	0.24	0.08	NS

NS, Non-significant

4.1.5.3 Transpiration rate

Apple trees showed differences in transpiration rate among different Zn nutrition sources, with values ranging from 2.01 to 2.92 mmol H_2O m^2/s. The treatment of NZn-AAC_{400} demonstrated the highest rate of transpiration (2.92 mmol H_2O m^2/s), compared to all other applied treatments. On the other hand, the treatment $ZnSO_4$ exhibited the lowest transpiration rate (2.01 mmol H_2O m^2/s). Treatments Zn-AAC_{400}, Zn-AAC_{200} and Zn-Gly_{400} exhibited congruent statistical findings with corresponding values of 2.69, 2.62 and 2.52 mmol H_2O m^2/s. The superior treatment demonstrated a 45.3 per cent increase compared to control.

4.1.5.4 Stomatal conductance

The data in Table 11 elucidates that stomatal conductance of apple registered non-significant results through application of organic Zn sources. Highest stomatal conductance of 0.138 mmol H_2O m^2/s was reported in NZn-AAC_{400} treatment, which was statistically similar to NZn-AAC_{200} (0.134 mmol H_2O m^2/s). However, the lowest stomatal conductance (0.117 mmol H_2O m^2/s) was observed in the treatment $ZnSO_4$, which also showed statistical equivalence to Zn-Glu_{200} (0.120 mmol H_2O m^2/s). Moreover, a significant increase of 1.2 times was observed with the superior treatment in contrast to the control.

4.1.5.5 Total carbohydrate content

4.1.5.5.1 Fruiting shoots

Among various bio-organic Zn nutrient sources, the carbohydrate content of fruiting shoots of apple trees ranged from 61.00 to 73.71 mg/g dry weight (Table 12). Maximum (73.71 mg/g dry weight) carbohydrate content of fruiting shoots was exhibited by NZn-AAC_{400}, which showed statistical superiority over all other Zn nutrient sources. However, the treatment $ZnSO_4$ demonstrated the minimum (61.00 mg/g dry weight) values for carbohydrate content of fruiting shoots. Statistical equivalence was also displayed among treatments namely, Zn-AAC_{200}, Zn-Gly_{400} and Zn-Glu_{400} with corresponding values of 67.77, 67.24 and 66.09 mg/g dry weight.

4.1.5.5.2 Non-fruiting shoots

The carbohydrate content of non-fruiting shoots of apple ranged from 93.34 to 105.69 mg/g dry weight. Maximum (105.69 mg/g dry weight) carbohydrate content of non-fruiting shoots was observed in treatment NZn-AAC_{400} which was statistically significant compared to all other applied treatments. The lowest value (93.34 mg/g dry weight) however, was recorded in the treatment $ZnSO_4$. Among other Zn organic sources, statistically proportionate effects on carbohydrate content of non-fruiting shoots were recorded in the order of Zn-AAC_{400} (99.44 mg/g dry weight) > Zn-AAC_{200} (99.24 mg/g dry weight) > Zn-Gly_{400} (97.91 mg/g dry weight).

4.1.5.5.3 Leaves

Highest amount of carbohydrates in leaves (39.76 mg/g dry weight) was found in the NZn-AAC_{400} treatment, which showed statistically similar results to the NZn-AAC_{200} treatment (38.64 mg/g dry weight). Treatments Zn-Gly_{400} and Zn-Glu_{400} exhibited comparable results, with corresponding values of 35.76 and 34.64 mg/g dry weight. However, minimal (28.46 mg/g dry weight) carbohydrate content of leaves was observed with treatment $ZnSO_4$. In addition, the superior treatment of NZn-AAC_{400} showed a 39.7 per cent increase over control.

Table 12. Foliar application of bio-organic Zn sources affects total carbohydrate content of apple

Zinc nutrition	Total carbohydrate content (mg/g dry weight)			
	Fruiting shoots	Non-fruiting shoots	Leaves	Fruit
Zn-Gly$_{200}$	65.10	95.27	31.95	91.25
Zn-Gly$_{400}$	67.24	97.91	35.76	99.62
Zn-Glu$_{200}$	63.11	94.41	30.20	88.53
Zn-Glu$_{400}$	66.09	96.04	34.64	95.41
Zn-AAC$_{200}$	67.77	99.24	36.39	100.64
Zn-AAC$_{400}$	69.77	99.44	38.05	102.12
NZn-AAC$_{200}$	71.14	102.30	38.64	107.03
NZn-AAC$_{400}$	73.71	105.69	39.76	110.43
ZnSO$_4$	61.00	93.34	28.46	86.41
CD$_{0.05}$	2.09	2.22	1.15	1.78

4.1.5.5.4 Fruits

Analysis of the data showed that the variation of carbohydrate content in apple fruits was 86.41 to 110.43 mg/g dry weight (Table 12; Fig. 9). The treatment NZn-AAC$_{400}$ exhibited the highest fruit carbohydrate content (110.43 mg/g dry weight), whereas, the treatment ZnSO$_4$ showed the lowest value (86.41 mg/g dry weight). Furthermore, it was noticed that the fruit carbohydrate content in NZn-AAC$_{400}$ was 27.8 per cent higher than control. Similar results, with corresponding values of 100.64 and 99.62 mg/g dry weight were obtained with treatments Zn-AAC$_{200}$ and Zn-Gly$_{400}$.

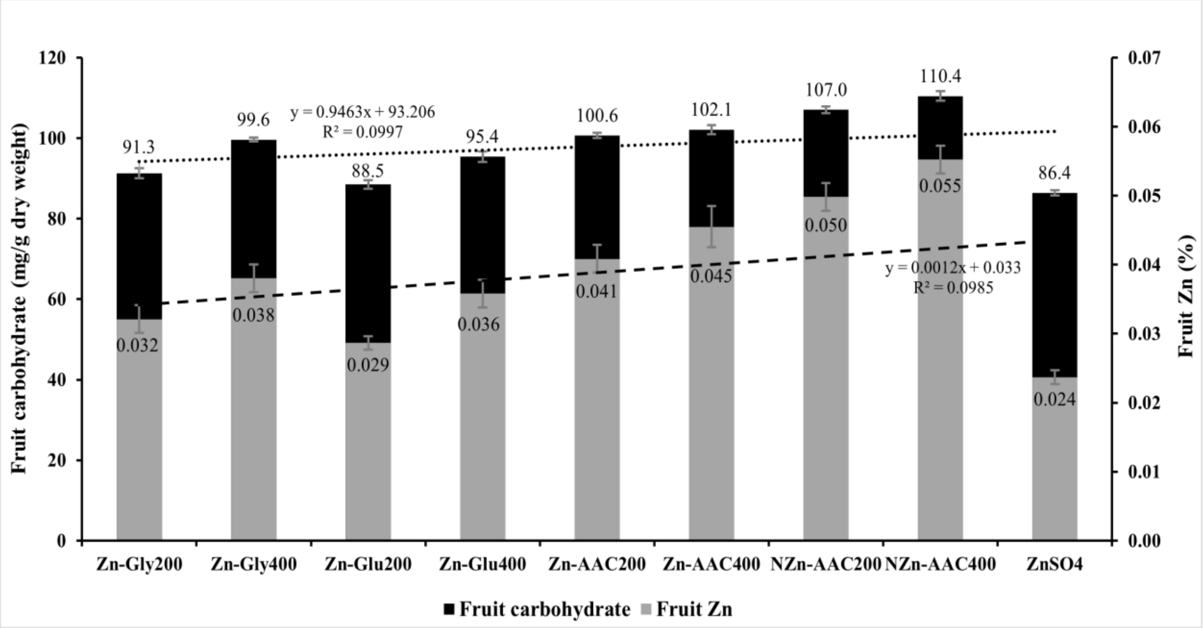


Fig. 10: Relationship studies on fruit carbohydrate and fruit Zn content in apple under bio-organic Zn nutrition

In the current study, the use of Zn amino acid complexes led to a notable improvement in the physiological parameters of apple. The positive impact of amino acid complexes is linked to the direct assimilation of amino acids within plants, thereby resulting in the enhancement of vital physiological processes (Nasholm et al., 2009). Increased leaf chlorophyll content might be ascribed to the dual effect of amino acids i.e., stimulating chlorophyll biosynthesis and concurrently reducing chlorophyll degradation (Fahimi et al., 2016; Souri et al., 2017; Noroozlo et al., 2019b). Similar effects of amino acids chelates on chlorophyll have been reported by Amin et al. (2011), Garcia et al. (2011) and Yadavi et al. (2014). Besides, it is ascribed to the role of Zn as an integral component of proteins which in turn influenced the biosynthesis of pigments (Mirbolook et al., 2020). In addition, role of Zn as a cofactor for carbonic anhydrase is vital in the biosynthetic pathway of chlorophyll (Kamiab and Zamanibahramabadi, 2016). The enhanced efficacy of nano Zn amino acid chelates is credited to the facilitated movement of nanoparticles through the Casparian strip within inner cortical cells (Larue et al., 2012), thereby effectively boosting chlorophyll biosynthetic pathways (Ruttkay-Nedecky et al., 2017; Yousuf and Abbass, 2023). Zn glycinate also increased total chlorophyll content compared to Zn sulphate due to the combined effect of Zn and glycine on maintenance of photosynthetic pigments (Anbu and Maseeha, 2019). The findings align with those of Mosa et al. (2020), who similarly observed increased chlorophyll content in apple through application of glycine.

Chlorophyll content is a crucial factor influencing plant photosynthetic activity (Keutgen and Pawelzik, 2008; Datir et al., 2012). Amino acids exert a stimulatory effect on chlorophyll synthesis, consequently elevating the photosynthetic efficiency (Zeid, 2009; Amin et al., 2011). Apart from this, Rehman et al. (2018) have documented the role of Zn in gene regulation, photosynthesis and carbohydrate metabolism. It might also be ascribed to the catalytic role of Zn in enzymatic reactions and role of amino acids in optimization of plant cellular metabolism (Marschner, 2011). Besides, a positive relationship between N and Zn has been demonstrated in the study by Wang et al. (2017). The increased canopy area facilitated by nano Zn amino acid chelate considerably influenced the crop’s microclimate, leading to enhanced photosynthetic efficiency (Fageria et al., 2011; Yeboah et al., 2017). Xu and Rothstein (2018) underscored the importance of anthocyanin in the regulation of reactive oxygen species and the maintenance of photosynthetic efficiency. Additionally, the increase in anthocyanin content mediated by nano Zn protected photosynthetic system and thus improved the photosynthetic activity (Chattha et al., 2022). The role of Zn on increasing stomatal conductance is also recorded by Yeboah et al. (2021). Similarly, Pradhan et al. (2014) also noted improved photosynthetic activity, transpiration rate and stomatal conductance through Zn amino acid chelate in contrast to Zn sulphate.

In addition, zinc has been shown to boost total carbohydrate content due to its function in increasing leaf K content, which increases leaf area and CO_2 assimilation, both of which improve assimilate translocation (Dawood, 2000). This could also be related to the extra advantage of simpler amino acid chelate molecule translocation from phloem to sink (Ge et al., 2009; Fahimi et al., 2016). Although sink competition has a major impact on the subsequent distribution of carbohydrates, photosynthesis is thought to be the main driver of carbohydrate synthesis (Ryan et al., 2018). Lauzike et al. (2021) also found that during fruit harvesting, fruiting shoots exhibited lower carbohydrate content than non-fruiting shoots. Furthermore, less carbohydrate accumulation was observed in leaves as compared to fruits, which might be associated to the existence of a strong sink in the form of fruits (Tartachnyk and Blanke, 2004). Moreover, the transfer of carbohydrates from the leaves through the fruit-bearing shoots could be responsible for the higher total carbohydrate content in fruits (Aprea et al., 2017; Li et al., 2018).

4.1.6 LEAF NUTRIENTS

4.1.6.1 Nitrogen (N)

Apple trees exhibited leaf N content varied from 1.82 to 2.38 per cent (Table 13). NZn-AAC_{400} showed highest leaf N content (2.38%), followed by NZn-AAC_{200}, Zn-AAC_{400}, Zn-AAC_{200}, Zn-Gly_{400}, and Zn-Glu_{400} with respective values of 2.33, 2.19, 2.10, 2.06 and 1.98 per cent. However, the $ZnSO_4$ treatment obtained the lowest leaf N content (1.82%). Among different bio-organic Zn sources, leaf N content progressed as follows: NZn-AAC_{400} > NZn-AAC_{200} > Zn-AAC_{400} > Zn-AAC_{200} > Zn-Gly_{400} > Zn-Glu_{400}. In addition, NZn-AAC_{400} showed 1.3 times increase in leaf N compared to the control.

4.1.6.2 Phosphorus (P)

Mean values for leaf P content ranged from 0.17 to 0.27 per cent. The treatment NZn-AAC_{400} exhibited maximum leaf P (0.27%) content however, treatment $ZnSO_4$ demonstrated minimum leaf P (0.17%) content. The hierarchical arrangement of various Zn sources for leaf P content was NZn-AAC_{400} > NZn-AAC_{200} > Zn-AAC_{400} > Zn-AAC_{200} > Zn-Gly_{400} > Zn-Glu_{400}. In addition, NZn-AAC_{400} recorded an increase of 58.8 per cent of leaf P content, followed by NZn-AAC_{200} (47.1%), Zn-AAC_{400} (41.2%) and Zn-AAC_{200} (29.4%) over control.

4.1.6.3 Potassium (K)

Data in Table 13 illustrated a pronounced increase in leaf K content over the cropping period due to the application of organic Zn sources. Leaf K content exhibited an average range spanning from 1.41 to 1.71 per cent. The treatment NZn-AAC_{400} demonstrated maximum

(1.71%) leaf K content. The lowest values (1.41%) were, however, recorded in treatment $ZnSO_4$. In terms of leaf K content, the Zn organic sources ranked as NZn-AAC_{400} > NZn-AAC_{200} > Zn-AAC_{400} > Zn-AAC_{200} > Zn-Gly_{400} > Zn-Glu_{400}. Treatment NZn-AAC_{400} also experienced an upsurge of 21.2 per cent in leaf K content over control.

Table 13. Leaf nutrient content of apple under foliar feeding of different bio-organic Zn sources

Zinc nutrition	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Zn (mg/kg)
Zn-Gly_{200}	1.91	0.18	1.48	1.64	0.28	45.51
Zn-Gly_{400}	2.06	0.22	1.56	1.70	0.30	54.09
Zn-Glu_{200}	1.86	0.18	1.44	1.63	0.27	41.90
Zn-Glu_{400}	1.98	0.20	1.51	1.66	0.29	48.51
Zn-AAC_{200}	2.10	0.22	1.59	1.74	0.31	56.93
Zn-AAC_{400}	2.19	0.24	1.64	1.77	0.32	69.41
NZn-AAC_{200}	2.33	0.25	1.67	1.80	0.34	74.35
NZn-AAC_{400}	2.38	0.27	1.71	1.82	0.35	79.19
$ZnSO_4$	1.82	0.17	1.41	1.57	0.26	36.16
$CD_{0.05}$	NS	NS	NS	NS	NS	2.66

NS, Non-significant

4.1.6.4 Calcium (Ca)

The treatment NZn-AAC_{400} showed the highest leaf Ca content (1.82%), whereas, it was minimum in $ZnSO_4$ (1.57%). The superior treatment was followed by NZn-AAC_{200}, Zn-AAC_{400}, Zn-AAC_{200} and Zn-Gly_{400} treatments which demonstrated the corresponding values of 1.80, 1.77, 1.74 and 1.70 per cent. NZn-AAC_{400} showed the highest per cent increase of 15.9 compared to all other Zn treatments, followed by NZn-AAC_{200} (14.6%), Zn-AAC_{400} (12.7%), and Zn-AAC_{200} (10.8%), all of which were superior to the control.

4.1.6.5 Magnesium (Mg)

Leaf Mg content ranged from 0.26 to 0.35 per cent. NZn-AAC_{400} treatment showed the highest value (0.35%), while, it was least in $ZnSO_4$ (0.26%). This was followed by the NZn-AAC_{200}, Zn-AAC_{400}, Zn-AAC_{200}, Zn-Gly_{400} and Zn-Glu_{400} with respective values of 0.34, 0.32, 0.31, 0.30 and 0.29 per cent. Furthermore, treatment NZn-AAC_{400} showed 34.6 per cent increase in leaf Mg content over control.

4.1.6.6 Zinc (Zn)

Perusal of the data revealed that leaf Zn concentration, which ranged from 36.16 to 79.19 mg/kg was significantly affected by Zn organic sources (Table 13). The treatment

NZn-AAC_{400} showed the highest leaf Zn content (79.19 mg/kg), which was statistically superior to all other applied Zn treatments. The treatment $ZnSO_4$ exhibited the lowest leaf Zn content (36.16 mg/kg), however, it was less significant than the other treatments. The progression in leaf Zn concentration among Zn sources was determined to be NZn-AAC_{400} > NZn-AAC_{200} > Zn-AAC_{400} > Zn-AAC_{200} > Zn-Gly_{400} > Zn-Glu_{400}. The NZn-AAC_{400} treatment was superior, indicating 2.1 times increase in leaf Zn content compared to the control.

The findings on leaf nutrient content demonstrated the remarkable effects of organic Zn complexes compared to Zn sulphate. Due to the potential of nanoparticles to bind with protein carriers, which increases nutrient uptake by enhancing their penetration through cell walls, thereby, nano Zn amino acid chelate showed the highest leaf nutrient content by the crop (Gonzalez-Melendi et al., 2008; Kutman et al., 2011; Alzreejawi and Al-Juthery, 2021). Similar results were also reported by Prasad et al. (2012), who observed that nano Zn complexes showed higher uptake and translocation within leaves than Zn sulphate. Additionally, it has been shown that application of amino acids complexes directly improves the efficiency of nutrient uptake, which in turn affects physiological processes in plants to maximize the uptake of macro and micronutrients by the trees (Irshad et al., 2002; Nasholm et al., 2009; Anitha, 2020). Zn-chelated amino acids improved the uptake and transport of micronutrients in plants in a synergistic way (Zhou et al., 2007; Souri, 2016). Similarly, Zn primarily forms bonds with organic compounds that have benefits favourable to plant nutritional status (Soliman et al., 2023). It could also be attributed to increased permeability of cell membranes and the manner in which chelated amino acids boost hormonal actions (Haydon and Cobbett, 2007; Shafie et al., 2021). Furthermore, amino acids can start signaling pathways in a variety of physiological mechanisms, which stimulates plants to assimilate N more easily (Gioseffi et al., 2012; Calvo et al., 2014; Santi et al., 2017). Research by Weiland et al. (2016), Khan et al. (2019) and Souri and Hatamian (2019) showed that addition of amino acid supplements increased root development through promoting N fixation and expanding the root surface for better nutrient uptake. Radkowski et al. (2018) observed that amino acids have a positive effect on the N, P, and K content of leaves.

Moreover, better nitrogen cycling is associated with enhanced soil enzymatic activity (Neemisha and Sharma, 2022). By stimulating the root system and activating soil enzymes, nano Zn amino acid chelate improves the absorption of nutrients and improves the levels of N, P, and K in leaves (Alzreejawi and Al-Juthery, 2021). Jamtgard et al. (2008), Nasholm et al. (2009) and Tida et al. (2009) presented evidence about the direct absorption of amino acids, despite the normal absorption of nitrate and NH_4^+ ions. Similar results have also been observed by Ghasemi et al. (2013), who demonstrated that application of Zn amino acid chelate increased the amount of N in leaves. Also, the transfer of amino acids through phloem and xylem promotes the ongoing transfer between roots and shoots and accelerates faster redistribution of N especially which remain stationary within the plant system (Owen and Jones, 2001). In addition, higher leaf Zn and Fe contents demonstrated the promotion of plant growth via Zn amino acid complexes (Zhao et al., 2019; Baghaie and Keshavarzi, 2021). Zn-glycinate showed less foliar phytotoxicity than $ZnSO_4$, which enhanced plants' ability to absorb Zn (Xu et al., 2022). According to Nasholm et al. (2009), glycine increases the efficiency of Zn uptake by acting as a source of N for plants.

4.1.7 FRUIT NUTRIENTS

4.1.7.1 Flesh

4.1.7.1.1 Phosphorus

The data indicated that different Zn organic sources exerted a non-significant impact on P content of apple flesh (Table 14). Treatments NZn-AAC_{400}, NZn-AAC_{200}, Zn-AAC_{400} and Zn-AAC_{200} exhibited the highest P (0.03%) content in flesh of fruit samples. However, it was recorded minimum (0.02%) in the treatments Zn-Gly_{200}, Zn-Gly_{400}, Zn-Glu_{200}, Zn-Glu_{400} and $ZnSO_4$. All the applied treatments demonstrated statistically similar effects in relation to P content in flesh. The superior treatment NZn-AAC_{400} also recorded 1.8 times more P content of fruit flesh compared to control.

4.1.7.1.2 Potassium

Among different Zn nutrient sources, K content of fruit flesh exhibited insignificant results which ranged between 0.40 and 0.56 per cent. Maximum flesh K (0.56%) content was recorded in treatment NZn-AAC_{400}. Statistically uniform results were observed among all the applied Zn treatments. However, treatment $ZnSO_4$ reflected the lowest value (0.40%) in terms of flesh K content.

4.1.7.1.3 Calcium

Data analysis in relation to Ca content of fruit flesh illustrated that Ca content in apple flesh was not influenced significantly by bio-organic Zn sources compared to control. However, treatment NZn-AAC_{400} registered the maximum flesh Ca (0.30%). This treatment was followed by NZn-AAC_{200}, Zn-AAC_{400}, Zn-AAC_{200}, Zn-Gly_{400} and Zn-Glu_{400} exhibiting the corresponding values of 0.28, 0.27, 0.27, 0.24 and 0.23 per cent. It was however, recorded minimum (0.19%) in treatment $ZnSO_4$. The results also reflected 1.6 times increase in Ca content of apple flesh through superior treatment of NZn-AAC_{400} over control.

4.1.7.1.4 Magnesium

Foliar application of Zn organic sources depicted insignificant effects on Mg content of apple flesh which varied from 0.05 to 0.08 per cent (Table 14). Treatments NZn-AAC_{400}, NZn-AAC_{200} and Zn-AAC_{400} registered maximum Mg (0.08%) content in fruit flesh. However, the minimum value of 0.05 per cent was registered by the treatment $ZnSO_4$. The hierarchy of Zn sources in promoting Mg content in fruit flesh showed an order of NZn-AAC_{400} = NZn-AAC_{200} = Zn-AAC_{400} > Zn-AAC_{200} = Zn-Gly_{400} = Zn-Glu_{400} > Zn-Gly_{200}.

4.1.7.1.5 Zinc

The recorded data exhibited variation in Zn content of apple flesh subjected to different organic Zn sources. There was not a significant difference in Zn content of apple flesh between various foliar Zn treatments. Zn content in fruit flesh ranged between 0.01 to 0.02 per cent. Treatments NZn-AAC_{400}, NZn-AAC_{200}, Zn-AAC_{400}, Zn-AAC_{200}, Zn-Gly_{400} and Zn-Glu_{400} attained the highest Zn (0.02%) content in fruit flesh, however, treatments $ZnSO_4$, Zn-Glu_{200} and Zn-Gly_{200} observed the lowest (0.01%) values.

4.1.7.2 Peel

4.1.7.2.1 Phosphorus

Perusal of the data inferred that P content of peel of fruit samples improved from the application of organic Zn sources (Table 14). However, all the applied treatments showed statistically insignificant effects in terms of P content in peel of fruit samples. Treatment application of Zn-AAC_{400} recorded maximum P content in peel of fruits (0.06%). However, treatments $ZnSO_4$, Zn-Glu_{200}, and Zn-Gly_{200} showed the lowest (0.04%) peel P content. This superior treatment showed 54.1 per cent more peel P content in comparison to the control.

4.1.7.2.2 Potassium

The current study has revealed insignificant differences in K content of apple peel amongst the various Zn nutritional sources, with values ranging from 0.48 to 0.82 per cent. The treatment of NZn-AAC_{400} showed the highest K content (0.82%) in fruit peel. On the other hand, the treatment $ZnSO_4$ has the lowest K content (0.48%) in peel. The treatments of NZn-AAC_{400} also showed 1.7 times increase in peel K content as compared to control.

Table 14. Nutrient content of apple fruit samples influenced by foliar bio-organic Zn sources

Zinc nutrition	P (%)		K (%)		Ca (%)		Mg (%)		Zn (%)	
	Flesh	Peel	Flesh	Peel	Flesh	Peel	Flesh	Peel	Flesh	Peel
Zn-Gly$_{200}$	0.02	0.04	0.43	0.60	0.22	0.49	0.06	0.13	0.01	0.02
Zn-Gly$_{400}$	0.02	0.05	0.46	0.68	0.24	0.54	0.07	0.15	0.02	0.02
Zn-Glu$_{200}$	0.02	0.04	0.42	0.56	0.21	0.48	0.06	0.13	0.01	0.02
Zn-Glu$_{400}$	0.02	0.05	0.45	0.65	0.23	0.50	0.07	0.14	0.02	0.02
Zn-AAC$_{200}$	0.03	0.05	0.47	0.70	0.27	0.55	0.07	0.16	0.02	0.02
Zn-AAC$_{400}$	0.03	0.05	0.51	0.75	0.27	0.56	0.08	0.16	0.02	0.03
NZn-AAC$_{200}$	0.03	0.05	0.52	0.77	0.28	0.59	0.08	0.16	0.02	0.03
NZn-AAC$_{400}$	0.03	0.06	0.56	0.82	0.30	0.61	0.08	0.17	0.02	0.03
ZnSO$_4$	0.02	0.04	0.40	0.48	0.19	0.44	0.05	0.13	0.01	0.01
CD$_{0.05}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS, Non-significant

4.1.7.2.3 Calcium

The data presented in Table 14 revealed that Ca content of apple peel was not-significantly influenced through application of organic Zn sources. However, highest Ca content of 0.61 per cent in fruit peel was observed in NZn-AAC$_{400}$, and it was least (0.44%) in $ZnSO_4$. Moreover, a significant increase of 1.4 times of peel Ca content was observed with NZn-AAC$_{400}$ treatment in contrast to the control.

4.1.7.2.4 Magnesium

Apple peel Mg content depicted insignificant effects through application of organic Zn nutrient sources. Among various Zn nutrient sources, the Mg content of fruit peel ranged from 0.13 to 0.17 per cent. Maximum Mg content (0.17%) in fruit peel was recorded in NZn-AAC$_{400}$. However, the treatments $ZnSO_4$, Zn-Glu$_{200}$ and Zn-Gly$_{200}$ demonstrated the minimum Mg content (0.13%) for apple peel.

4.1.7.2.5 Zinc

Among different bio-organic sources, Zn content of apple peel ranged from 0.01 to 0.03 per cent and showed non-significant effects among the different foliar Zn treatments applied (Table 14). Maximum Zn content (0.03%) in fruit peel was registered in treatments NZn-AAC$_{400}$, NZn-AAC$_{200}$ and Zn-AAC$_{400}$. The lowest values (0.01%) were, however, noticed in $ZnSO_4$.

4.1.8 FRUIT NUTRIENTS RATIO

4.1.8.1 Flesh

4.1.8.1.1 K/Ca

The analysis of the data in Table 15 revealed significant relationship between bio-organic Zn sources and flesh K/Ca ratio of apple fruit samples. K/Ca ratio of fruit flesh was recorded maximum (2.12) in treatment $ZnSO_4$, which did not show statistical equivalence with all other applied treatments. Analogous results were exhibited by treatments Zn-Glu$_{200}$, Zn-Gly$_{200}$, Zn-Gly$_{400}$, Zn-Glu$_{400}$ and NZn-AAC$_{400}$ with corresponding values of 1.98, 1.97, 1.94, 1.93, and 1.88 respectively. However, minimum (1.73) values were observed in treatment Zn-AAC$_{200}$.

4.1.8.1.2 (K+Mg)/Ca

It is evident from the data that (K+Mg)/Ca ratio of apple flesh varied between 1.99 and 2.41. Lowest (K+Mg)/Ca (1.99) ratio of fruit flesh was reported in Zn-AAC$_{200}$, whereas, it was maximum (2.41) in treatment $ZnSO_4$. However, statistically similar results were observed in treatments of Zn-Glu$_{200}$ (2.27), Zn-Gly$_{200}$ (2.27), Zn-Gly$_{400}$ (2.23), Zn-Glu$_{400}$ (2.22) and NZn-AAC$_{400}$ (2.16).

Table 15. Influence of foliar bio-organic Zn sources on K/Ca and (K+Mg)/Ca ratio in flesh and peel of apple fruit samples

Zinc nutrition	Flesh		Peel	
	K/Ca	(K+Mg)/Ca	K/Ca	(K+Mg)/Ca
Zn-Gly$_{200}$	1.97	2.27	1.23	1.50
Zn-Gly$_{400}$	1.94	2.23	1.26	1.53
Zn-Glu$_{200}$	1.98	2.27	1.15	1.43
Zn-Glu$_{400}$	1.93	2.22	1.28	1.56
Zn-AAC$_{200}$	1.73	1.99	1.26	1.55
Zn-AAC$_{400}$	1.83	2.11	1.33	1.61
NZn-AAC$_{200}$	1.85	2.14	1.29	1.57
NZn-AAC$_{400}$	1.88	2.16	1.34	1.62
ZnSO$_4$	2.12	2.41	1.05	1.33
CD$_{0.05}$	0.11	0.11	NS	NS

NS, Non-significant

4.1.8.2 Peel

4.1.8.2.1 K/Ca

Among different bio-organic Zn sources, the K/Ca ratio of fruit peel ranged from 1.05 to 1.34 (Table 15). Maximum K/Ca ratio in fruit peel (1.34) was observed in NZn-AAC$_{400}$, followed by Zn-AAC$_{400}$, NZn-AAC$_{200}$, and Zn-Glu$_{400}$ with respective values of 1.33, 1.29 and 1.28. Minimum (1.05) K/Ca ratio of fruit peel was, however, attained by the treatment ZnSO$_4$. All the applied treatments exhibited statistically insignificant effects with each other. The progression of K/Ca ratio of fruit peel under varied Zn sources unfolded as NZn-AAC$_{400}$ > Zn-AAC$_{200}$ > NZn-AAC$_{200}$ > Zn-Glu$_{400}$ > Zn-Gly$_{400}$ = Zn-AAC$_{200}$. NZn-AAC$_{400}$ also exhibited 1.3 times increase of fruit peel K/Ca ratio over control.

4.1.8.2.2 (K+Mg)/Ca

Application of varied Zn sources exerted a non-significant influence on (K+Mg)/Ca ratio of apple peel. The average values for (K+Mg)/Ca ratio of fruit peel ranged from 1.33 to 1.62. The treatment NZn-AAC$_{400}$ exhibited maximum (1.62) (K+Mg)/Ca ratio of fruit peel. However, treatment ZnSO$_4$ demonstrated minimum (K+Mg)/Ca ratio of fruit peel (1.33). The hierarchical arrangement (K+Mg)/Ca ratio of fruit peel among various Zn sources reported as NZn-AAC$_{400}$ > Zn-AAC$_{200}$ > NZn-AAC$_{200}$ > Zn-Glu$_{400}$ > Zn-AAC$_{200}$ > Zn-Gly$_{400}$. In addition, NZn-AAC$_{400}$ also exhibited 21.8 per cent increase of (K+Mg)/Ca ratio in fruit peel followed by Zn-AAC$_{400}$ (21.1%), NZn-AAC$_{200}$ (18.0%) and Zn-Glu$_{400}$ (17.3%) over control.

In the current investigation, the improvement in fruit nutrient content by foliar application of nano Zn amino acid chelate which ascribed to the fact that amino acids facilitated translocation of Zn from the leaves to the fruits (Anitha, 2020; Tabesh et al., 2020). Amino acids as potent chelators of nutrients, profoundly enhance the efficacy of nutrient uptake and their subsequent distribution within the plant system (Ge et al., 2009; Garcia et al., 2011; Souri and Hatamian, 2019). Moreover, Sharma et al. (2014b) documented that nano Zn increased nutrient absorption and sugar accumulation in fruit samples. Ghasemi et al. (2013) reported enhanced fruit nutrient content through Zn amino acid chelate compared to zinc sulphate. This could be explained by the fact that Zn amino acid chelate has a lower molecular weight (Wei et al., 2012) and a higher availability (Chen et al., 2022) than $ZnSO_4$ (Colle et al., 2009), which renders it easier to pass through leaves and reach the fruits. The current findings were in agreement with those of Wei et al. (2012) and Ghasemi et al. (2013), who also suggested that foliar application of zinc amino acid chelate decreased the level of phytic acid and increased the bioavailability of zinc, hence increasing the nutrient content of fruit. Moreover, the increased water solubility of organic zinc chelates is responsible for an increase in zinc utilization rate (Souri, 2016).

The quick absorption and transfer of nutrients through foliar application could also be associated with the increase in fruit nutrient content (Wei et al., 2012; Al-Juthery et al., 2019). Similar findings were reported by Garcia-Lopez et al. (2019), who observed that following foliar application of nano zinc, Zn was transferred via phloem from leaves to fruits during fruit growth and maturity stages. Similarly, application of Zn amino acid chelate resulted in higher Zn content of fruit samples (Ning et al. 2021). Furthermore, since glycine transforms quickly into amide and ammonium compounds, thereby increased Zn content of fruit samples in Zn glycinate treated apple trees compared to $ZnSO_4$ (Cakmak et al., 2010; Recena et al., 2021). Also, Zn amino acid chelates are environmentally friendly substitutes for inorganic Zn (Areche et al., 2023), which could additionally have led to the production of nutrient rich fruits (Dolev et al., 2020). Earlier literature revealed that peel of apple fruits accumulates higher concentration of P, Ca, Mg and Zn than apple flesh (Henriquez et al., 2010; Manzoor et al., 2012; Michailidis et al., 2021).

4.1.9 ANTIOXIDANTS ACTIVITY

4.1.9.1 Phytic acid

Data in Table 16 illustrated a pronounced increase in phytic acid content over the cropping period due to the application of bio-organic Zn sources in apple trees. Phytic acid content ranged between 1.09 and 1.60 per cent (Fig. 10). The treatment NZn-AAC$_{400}$ demonstrated the minimum phytic acid content (1.09%) which was statistically similar to NZn-AAC$_{200}$ (1.12%). Whereas, it was maximum (1.60%) in $ZnSO_4$ treated trees. Treatments application of Zn-Gly$_{400}$, Zn-AAC$_{200}$ and Zn-AAC$_{400}$ also elicited uniform effects with corresponding values of 1.39, 1.32 and 1.27 per cent. Treatment of NZn-AAC$_{400}$ experienced 46.7 per cent decrease in phytic acid content in apple fruit samples compared to control.

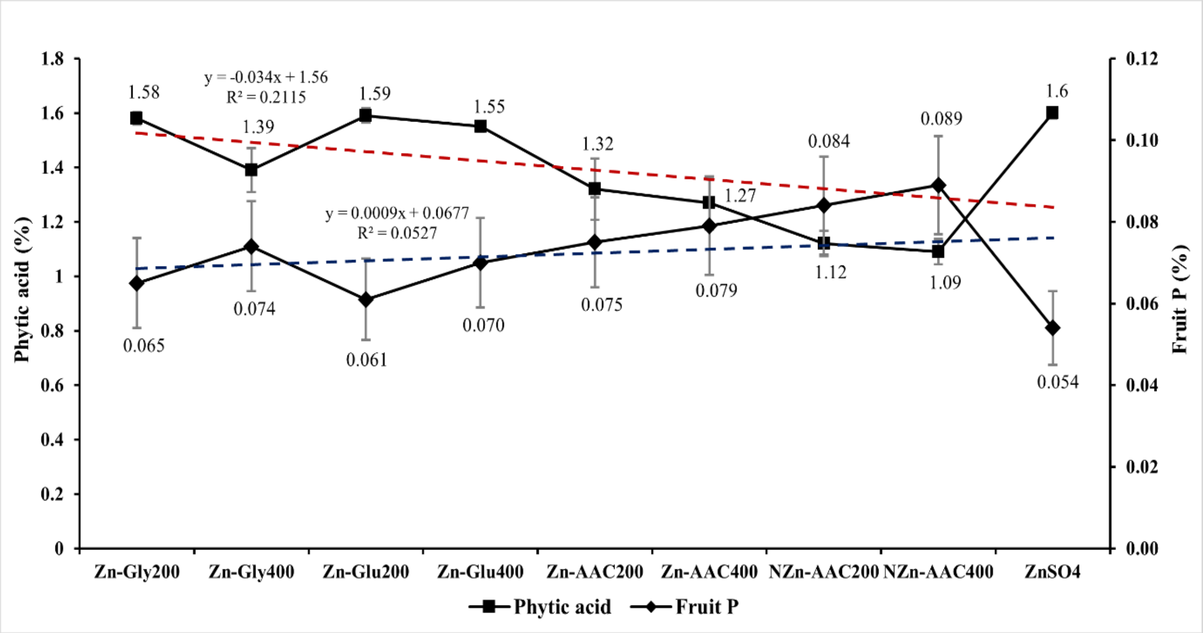


Fig. 11: Correlation between phytic acid and fruit P content in apple under bio-organic Zn fertilization

4.1.9.2 Phytic acid/ Fruit nutrients

4.1.9.2.1 PA: Ca

Application of organic Zn sources resulted in a marked increase in phytic acid to nutrient ratio in fruit samples (Table 16). Fruit PA: Ca ratio was exhibited maximum (2.59) in $ZnSO_4$, whereas, the least values were recorded in NZn-AAC$_{400}$ (1.21). Among other Zn nutrient sources, statistically equivalent results were reported in Zn-Gly$_{200}$ and Zn-Glu$_{400}$ with the respective values of 2.27 and 2.15. Among all treatments, NZn-AAC$_{400}$ displayed the maximum per cent decrease of 114.0, followed by NZn-AAC$_{200}$ (99.2%), Zn-AAC$_{400}$ (68.1%) and Zn-AAC$_{200}$ (58.9%) over control.

4.1.9.2.2 PA: Mg

Fruit PA: Mg ratio varied among different Zn nutrient sources, which ranged between 4.36 and 8.95. Minimum fruit PA: Mg ratio (4.36) was recorded in NZn-AAC$_{400}$, whereas, the treatment $ZnSO_4$ exhibited the maximum value of 8.95. The treatment of NZn-AAC_{400} also demonstrated statistical significance with NZn-AAC_{200} (4.63). However, the treatment $ZnSO_4$ showed statistically superiority over all other applied Zn treatments. Treatment NZn-AAC_{400} also demonstrated a remarkable 105.3 per cent decrease compared to control.

Table 16. Phytic acid and Ursolic acid content of apple affected by foliar application of different bio-organic Zn sources

Zinc nutrition	Phytic acid (%)	PA: Ca	PA: Mg	PA: Zn	Ursolic acid (µg/mL)
Zn-Gly_{200}	1.58	2.27	8.04	50.37	211.74
Zn-Gly_{400}	1.39	1.82	6.54	37.92	239.15
Zn-Glu_{200}	1.59	2.36	8.38	56.28	204.01
Zn-Glu_{400}	1.55	2.15	7.52	44.20	235.05
Zn-AAC_{200}	1.32	1.63	5.89	33.42	240.88
Zn-AAC_{400}	1.27	1.54	5.40	28.96	248.73
NZn-AAC_{200}	1.12	1.30	4.63	22.95	258.01
NZn-AAC_{400}	1.09	1.21	4.36	19.96	273.95
$ZnSO_4$	1.60	2.59	8.95	68.98	199.24
$CD_{0.05}$	0.12	0.13	0.46	3.70	2.99

4.1.9.2.3 PA: Zn

A cursory glance at the data highlighted the significant impact of Zn organic sources on fruit PA: Zn content which varied from 19.96 to 68.98. Highest fruit PA: Zn content (68.98) was recorded in $ZnSO_4$, which marked a distinctly higher significance compared to all other treatments. The lowest fruit PA: Zn content (19.96) however, was observed in NZn-AAC_{400}, which was statistically similar to NZn-AAC_{200} (22.95). The descending trend of fruit PA: Zn content among different bio-organic Zn treatments recorded as NZn-AAC_{400} > NZn-AAC_{200} > Zn-AAC_{400} > Zn-AAC_{200} > Zn-Gly_{400} > Zn-Glu_{400}. The treatment of NZn-AAC_{400} found 3.4 times less PA: Zn content in fruit samples compared to control.

4.1.9.3 Ursolic acid

The data displayed in Table 16 indicated that different Zn organic sources exerted a notable impact on the ursolic acid content in apple fruit samples (Table 16; Fig. 11). Treatment NZn-AAC_{400} exhibited the highest ursolic acid (273.95 µg/mL) content, which depicted statistical superiority compared to all other applied Zn treatments. However, it was minimum (199.24 µg/mL) in $ZnSO_4$ treated trees. All the applied treatments were statistically insignificant with each other. The progression of fruit ursolic acid content under varied Zn sources reported in the order of NZn-AAC_{400} > NZn-AAC_{200} > Zn-AAC_{400} > Zn-AAC_{200} > Zn-Gly_{400} > Zn-Glu_{400}.

Moreover, the treatment NZn-AAC$_{400}$ also recorded 1.4 times increase in ursolic acid content compared to control.

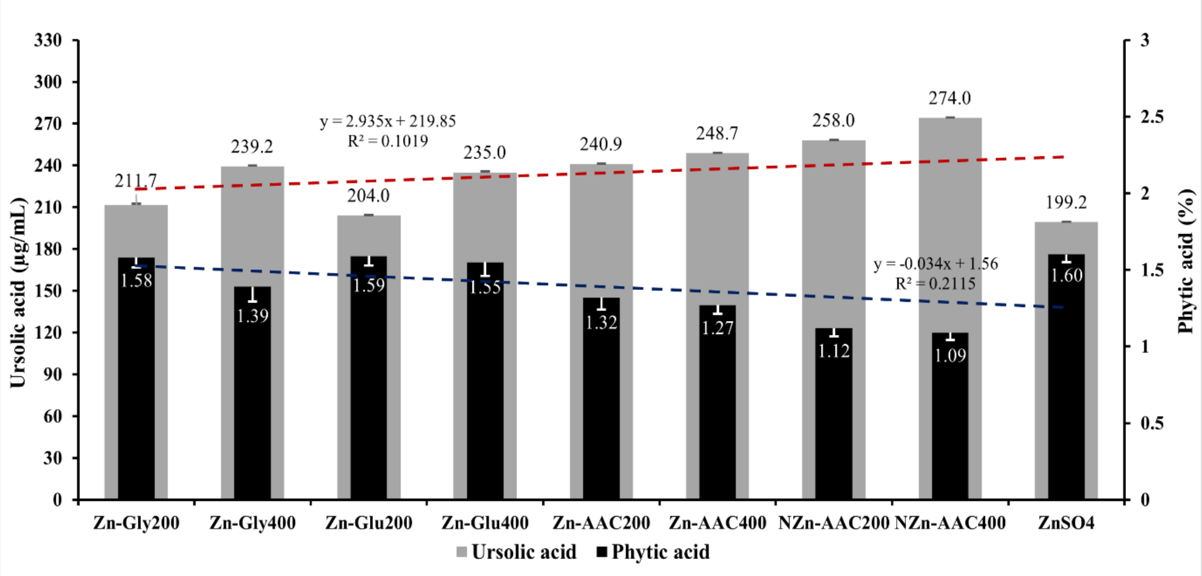


Fig. 12: Relationship between ursolic acid and phytic acid content in apple under bio-organic Zn fertilization

Earlier studies documented the ability of zinc fertilizers to increase bioavailability and lower the PA: Zn ratio in fruits (Raza et al., 2023). The current study showed beneficial effects of organic zinc chelates in improving antioxidant activity of fruit samples. Phosphorous is primarily stored as phytic acid, thus making up 60-80 per cent of the total P content (Gupta et al., 2015). The conjugation of phytic acid with zinc leads to a considerable reduction in the bioavailability of zinc for humans (Saha et al., 2017). By lowering the PA: Zn ratio in fruits, zinc amino acid chelates improve Zn accessibility to plants and increase its bioavailability in human body system (Mirbolook et al., 2020). But when it applies to inorganic zinc as well, humans are unable to absorb zinc due to the binding of zinc sulphate with PA (Schlegel et al., 2013). Through a decrease in PA content and an increase in fruit Zn content in fruit samples emphasizes the potential of Zn amino acid complexes compared to $ZnSO_4$ (Ghasemi et al. 2013; Seddigh et al. 2016). Yang et al. (2021) observed that decrease in PA content could be the result of a decrease in the conversion of inorganic phosphorus to PA, which might be brought about by increased zinc accumulation in fruits (Fig. 12). It might have to do with how amino acids bind PA, which would increase zinc absorption (Sun et al., 2018).

Ursolic acid (UA) is a biologically active pentacyclic tri-terpenoid found in higher concentration in apple peel (Li et al., 2023). In the present investigation, the highest UA content was observed following the application of nano Zn amino chelate, might be due to the involvement of Zn to facilitate antioxidant pathways (Yaish, 2015). Earlier literature documented that UA extracted from fruits possess biological activities that catalyze metabolic pathways, which could improve fruit antioxidants (Jayaprakasam et al., 2006). Moreover, Butkeviciute et al. (2018) revealed that UA constitutes more than 70 per cent of tri-terpenes in apple peel. Higher UA content in the apple peels might be ascribed to the intense red pigmentation induced by anthocyanin (Li et al., 2018). Besides, increase in anthocyanin content of fruits through supplementation of Zn amino acid chelate was also reported by Shalan (2020). Moreover, Farag and Palta (1992) also observed a positive correlation between anthocyanins and UA content of fruit samples (Fig. 13).

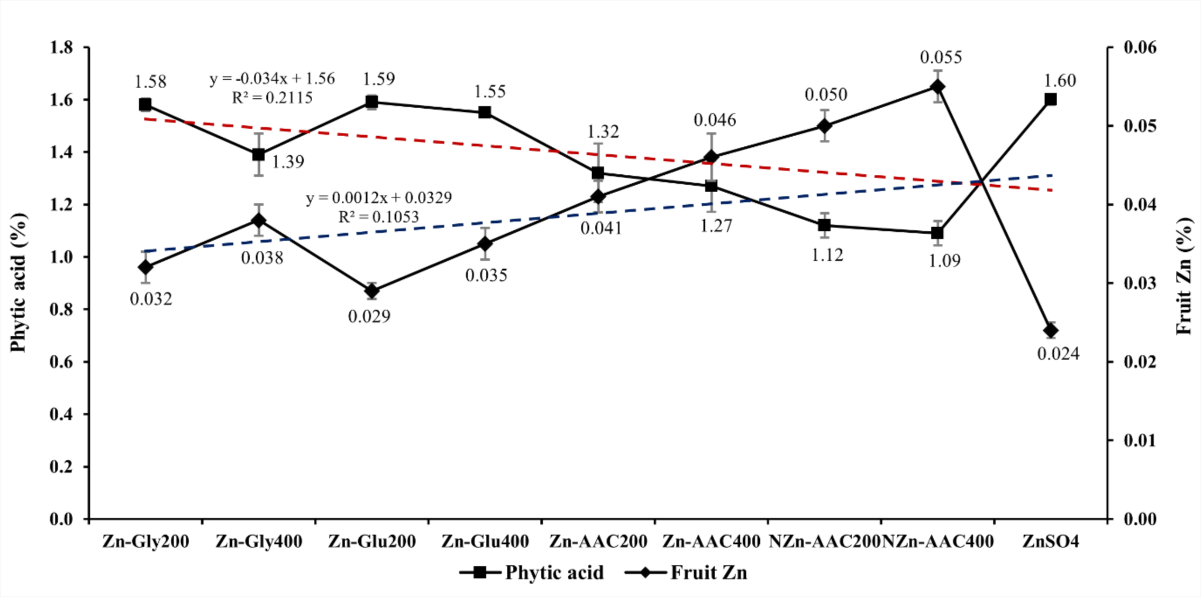


Fig. 13: Relationship studies on phytic acid and fruit Zn content in apple under bio-organic Zn nutrition

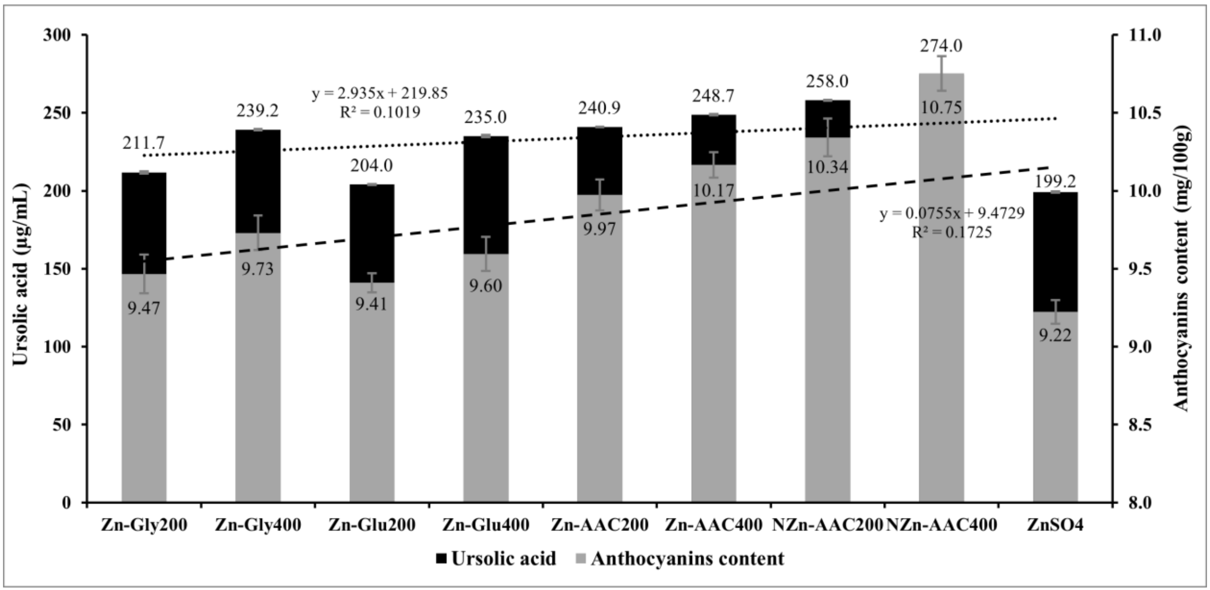


Fig. 14: Correlation between ursolic acid and anthocyanins content in apple under bio-organic Zn nutrition

4.1.10 PRINCIPAL COMPONENT ANALYSIS

4.1.10.1 PCA analysis for growth, yield contributing and fruit quality parameters in apple under bio-organics Zn nutrition

A principal component analysis (PCA) was performed to evaluate the influence of different Zn nutrient sources on growth, yield contributing and fruit quality parameters. The plot summarized the association between the variable and the represented axis of growth traits (leaf area, TCSA, CV, CA), yield contributing parameters (fruit set, fruit yield), and quality parameters (fruit length, fruit breadth, fruit weight, fruit firmness, TSS, titratable acidity, reducing sugars, non-reducing sugars, total sugars, anthocyanins, phytic acid, ursolic acid). The graphical representation of correlation biplot was achieved via constructing *Scree* plot and PCA biplots with the original factors depicted as *Eigen* vectors (Fig. 14). Data that explained at least 10 per cent of variation and had an *Eigen* value >1 were taken into consideration for each component. Based on this, PCA subsequently identified the major components with highest variance, which explained 95.70 per cent (PC1), 97.81 per cent (PC2), 99.11 per cent (PC3), 99.42 per cent (PC4) and 99.63 per cent (PC5) of the cumulative variance (Table 17). Highest cumulative variance in PC5 was observed in traits pertaining to growth, yield contributing and quality parameters of apple. PCA analysis involved variables whose values were equal or higher than two-third of the highest variable value within each principal component. The study also highlighted that PC1, which represented 95.70 per cent of the variance, was significantly impacted by all the parameters except firmness, titratable acidity and phytic acid. The parameters including CA, fruit set, fruit yield, fruit length, fruit breadth, firmness, TSS, titratable acidity, non-reducing sugars, total sugars, phytic acid and ursolic acid were heavily loaded for PC2 with variation of 2.11 per cent. The PC3 highly contributed to the parameters related to leaf area, fruit yield, fruit length, fruit breadth, fruit weight, TSS, titratable acidity, reducing sugars, phytic acid and ursolic acid which accounted for 1.29 per cent of variance. Furthermore, PC4 was strongly correlated with most of the studied parameters. Likewise, PC5 was characterized by the highest positive loadings primarily derived from growth parameters (TCSA, CV, CA), fruit set, fruit yield, fruit breadth, firmness, anthocyanins, phytic acid and ursolic acid. Variables demonstrating factor loadings greater than 0.40 were considered significant among various PCs (Wander and Bolero, 1999). Similarly, PCA has been employed in apple (Kumar et al., 2018) and strawberry (Kumar et al., 2020) for data reduction of important variables.

Table 17: PCA of growth, yield contributing and quality parameters of apple influenced by bio-organic Zn sources

Parameter	Principal Component				
	PC1	PC2	PC3	PC4	PC5
Eigen value	17.23	0.38	0.23	0.06	0.04
Variability (%)	95.70	2.11	1.29	0.31	0.21
Cumulative variance (%)	95.70	97.81	99.11	99.42	99.63

Growth traits and fruit quality parameters	Factor loadings					Eigen vectors				
	F1	F2	F3	F4	F5	F1	F2	F3	F4	F5
Leaf area	0.971	-0.198	0.058	-0.025	-0.062	0.234	-0.320	0.120	-0.105	-0.314
TCSA	0.990	-0.019	-0.100	0.012	0.024	0.239	-0.031	-0.208	0.052	0.122
CV	0.995	-0.024	-0.064	-0.001	0.013	0.240	-0.039	-0.132	-0.006	0.066
CA	0.996	0.029	-0.072	0.007	0.002	0.240	0.046	-0.148	0.029	0.011
Fruit set	0.997	0.025	-0.070	0.008	0.014	0.240	0.041	-0.145	0.034	0.071
Fruit yield	0.997	0.046	0.027	0.037	0.001	0.240	0.075	0.056	0.158	-0.002
Fruit length	0.987	0.038	0.090	-0.114	-0.035	0.238	0.061	0.187	-0.483	-0.178
Fruit breadth	0.951	0.156	0.223	0.135	0.058	0.229	0.253	0.462	0.570	0.294
Fruit weight	0.965	-0.119	0.209	-0.053	-0.051	0.232	-0.193	0.433	-0.222	-0.260
Firmness	-0.978	0.178	-0.080	-0.065	0.024	-0.236	0.288	-0.167	-0.277	0.124
TSS	0.990	0.095	0.082	-0.019	-0.025	0.238	0.155	0.170	-0.080	-0.125
TA	-0.970	0.199	0.129	-0.028	-0.016	-0.234	0.322	0.268	-0.117	-0.079
RS	0.995	-0.067	0.034	0.021	-0.005	0.240	-0.108	0.070	0.089	-0.028
NRS	0.901	0.421	-0.077	-0.018	-0.048	0.217	0.683	-0.159	-0.076	-0.246
TS	0.988	0.149	-0.022	0.010	-0.026	0.238	0.241	-0.045	0.042	-0.135
Anthocyanins	0.984	-0.110	-0.089	0.023	0.019	0.237	-0.178	-0.185	0.098	0.097
PA	-0.968	0.024	0.233	0.001	0.019	-0.233	0.038	0.484	0.006	0.095
UA	0.981	0.008	0.056	-0.113	0.146	0.236	0.012	0.115	-0.480	0.746

PC1, Principal component 1; PC2, Principal component 2; PC3, Principal component 3; PC4 Principal component 4; PC5, Principal component 5; F1, factor-1; F2, factor-2; F3, factor-3, F4, factor-4; F5, factor-5; TCSA, trunk cross sectional area; CV, canopy volume; CA, canopy area; TSS, total soluble solids; TA, titratable acidity; RS, reducing sugars; NRS, non-reducing sugars; TS, total sugars; PA, phytic acid; UA, ursolic acid

Factor scores					
Treatment (T)	**F1**	**F2**	**F3**	**F4**	**F5**
Zn-Gly$_{200}$	-3.028	-0.472	0.685	0.011	-0.425
Zn-Gly$_{400}$	0.089	0.611	0.265	-0.163	0.087
Zn-Glu$_{200}$	-4.676	-0.164	-0.082	0.536	0.144
Zn-Glu$_{400}$	-1.729	-0.053	0.749	-0.157	0.333
Zn-AAC$_{200}$	1.530	1.171	-0.086	-0.053	-0.145
Zn-AAC$_{400}$	2.898	0.555	-0.282	0.132	-0.010
NZn-AAC$_{200}$	4.629	-0.372	-0.380	0.144	-0.040
NZn-AAC$_{400}$	6.808	-0.925	0.001	-0.121	0.057
ZnSO$_4$	-6.519	-0.350	-0.869	-0.328	0.001

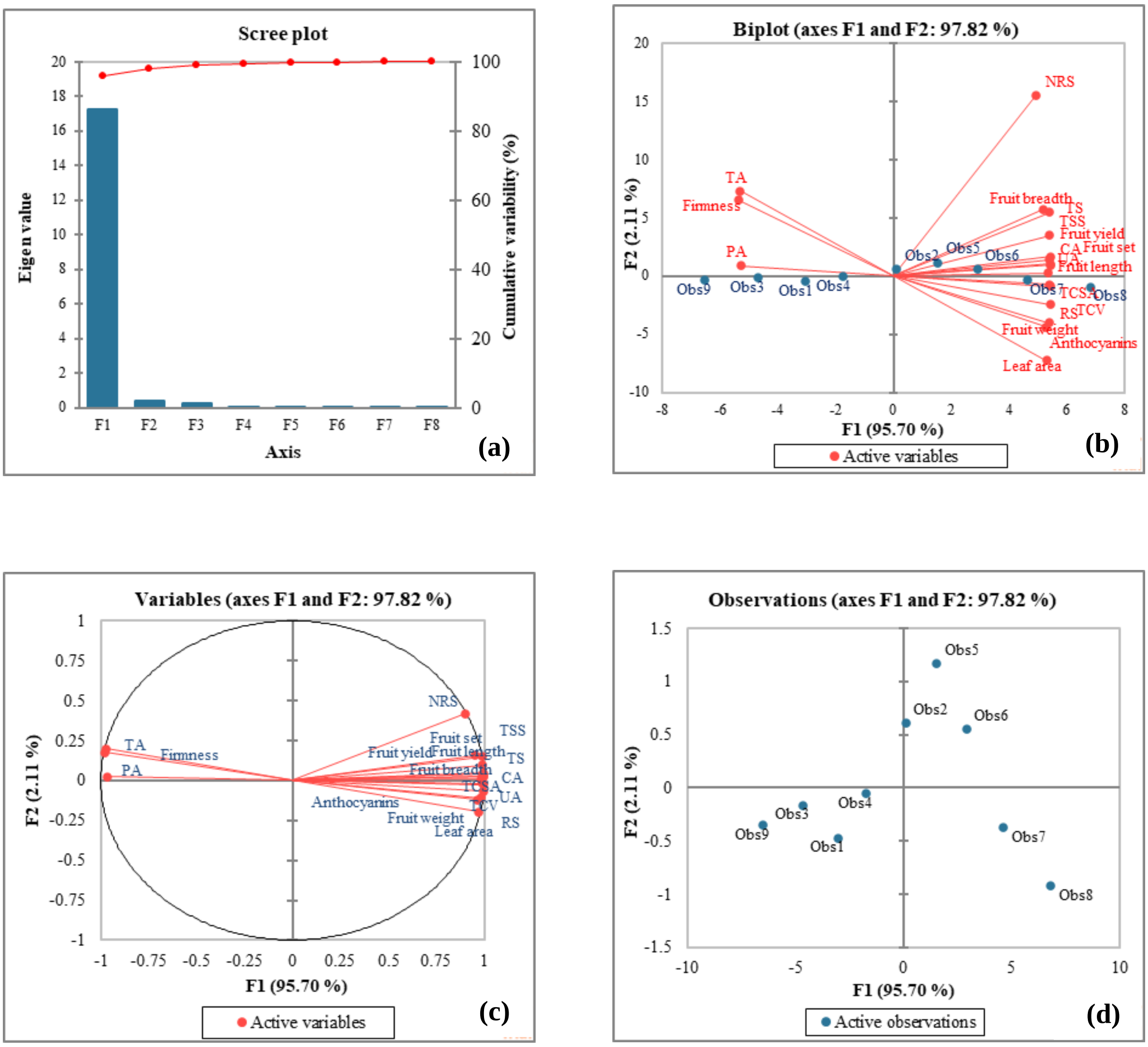


Fig. 15: PCA Scree plot (a) and Correlation biplot (b-d) between growth, yield and quality parameters of apple under bio-organic Zn nutrition

Table 18: PCA of chemical and microbiological properties of soils of apple influenced by bio-organic Zn sources

Parameter	**Principal Component**			
	PC1	**PC2**	**PC3**	**PC4**
Eigen value	21.53	0.28	0.09	0.04
Variability (%)	97.87	1.28	0.39	0.19
Cumulative variance (%)	97.87	99.15	99.54	99.72

Soil chemical and microbiological properties	**Factor loadings**				**Eigen vectors**			
	F1	**F2**	**F3**	**F4**	**F1**	**F2**	**F3**	**F4**
Fruit yield	0.998	-0.019	-0.020	0.023	0.215	-0.036	-0.067	0.113
OC	0.997	0.044	0.034	0.022	0.215	0.083	0.115	0.109
N	0.996	0.033	-0.044	-0.023	0.215	0.062	-0.150	-0.112
P	0.994	0.001	-0.088	0.032	0.214	0.000	-0.301	0.161
K	0.997	0.015	-0.056	-0.034	0.215	0.029	-0.192	-0.167
Ca	0.988	0.105	0.026	-0.072	0.213	0.198	0.089	-0.359
Mg	0.999	0.020	-0.031	0.003	0.215	0.038	-0.104	0.015
Zn	0.999	0.031	0.013	0.026	0.215	0.059	0.043	0.130
Fe	0.977	-0.008	0.180	0.103	0.211	-0.016	0.613	0.510
Cu	0.994	0.065	-0.037	0.034	0.214	0.123	-0.126	0.166
Mn	0.981	-0.135	-0.121	0.058	0.211	-0.254	-0.414	0.286
Bacteria	0.988	0.137	0.024	0.002	0.213	0.258	0.082	0.009
Fungi	0.973	-0.208	0.079	-0.032	0.210	-0.393	0.270	-0.160
Actinomycetes	0.982	-0.171	-0.013	-0.053	0.212	-0.323	-0.043	-0.263
PSB	0.987	-0.127	0.070	-0.073	0.213	-0.240	0.240	-0.361
KSB	0.989	-0.126	-0.047	0.023	0.213	-0.239	-0.159	0.113
ZSB	0.993	-0.086	-0.026	0.056	0.214	-0.163	-0.088	0.279
Azotobacter	0.992	-0.100	0.055	-0.014	0.214	-0.189	0.187	-0.069
AM Fungi	0.987	0.139	0.047	-0.005	0.213	0.262	0.159	-0.023
Acid Ph	0.995	0.088	0.004	-0.028	0.214	0.167	0.015	-0.137
Alkaline Ph	0.997	0.035	-0.035	-0.047	0.215	0.066	-0.119	-0.233
Dehydrogenase	0.961	0.270	-0.011	-0.001	0.207	0.509	-0.036	-0.006

PC1, Principal component 1; PC2, Principal component 2; PC3, Principal component 3; PC4 Principal component 4; F1, factor-1; F2, factor-2; F3, factor-3, F4, factor-4; OC, organic carbon; PSB, phosphorus solubilizing bacteria; KSB, potassium solubilizing bacteria; Acid Ph, acid phosphatase; Alkaline Ph, alkaline phosphatase

Factor scores				
Treatment (T)	**F1**	**F2**	**F3**	**F4**
Zn-Gly$_{200}$	-3.440	-0.420	0.213	-0.137
Zn-Gly$_{400}$	-0.036	-0.570	-0.436	0.116
Zn-Glu$_{200}$	-4.781	-0.367	0.431	-0.031
Zn-Glu$_{400}$	-1.757	-0.394	-0.067	-0.218
Zn-AAC$_{200}$	1.738	-0.242	-0.054	0.415
Zn-AAC$_{400}$	3.685	-0.019	-0.212	-0.018
NZn-AAC$_{200}$	5.463	0.419	-0.198	-0.311
NZn-AAC$_{400}$	6.953	0.457	0.483	0.101
$ZnSO_4$	-7.824	1.136	-0.161	0.084

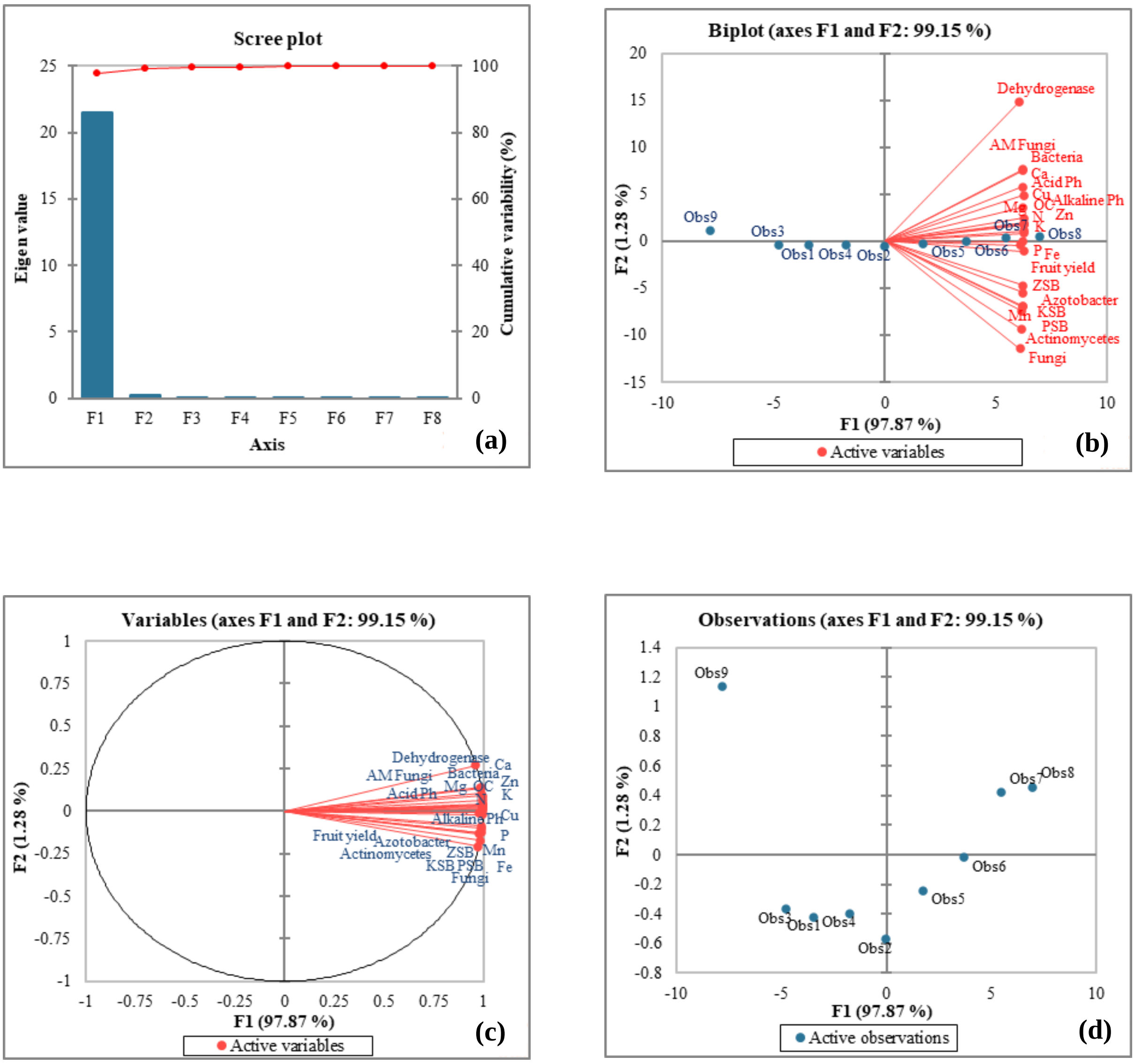


Fig. 16: PCA Scree plot (a) and Correlation biplot (b-d) between chemical and microbiological properties of soils of apple under bio-organic Zn nutrition

4.1.10.2 PCA analysis for soil chemical and microbiological properties in apple under bio-organics Zn nutrition

PCA was also employed to assess the influence of Zn nutrient sources on soil chemical and microbiological properties. PCA analysis identified the primary components with *Eigen* values exceeding one, with PC1 explaining 97.87 per cent, PC2 (99.15%), PC3 (99.54%) and PC4 (99.72%) of the variance observed (Table 18). PC4 accounted for the greatest cumulative variance among soil chemical and microbiological properties. The research findings indicated that PC1 exhibited notably higher loadings with 97.87 per cent of variance from all the studied parameters. Subsequently, PC2 demonstrated a predominant influence from the soil chemical properties (organic carbon, available N, P, K, exchangeable Ca, Mg, DTPA extractable Zn, Cu) and microbiological properties (bacteria, AM fungi, acid phosphatase, alkaline phosphatase and dehydrogenase). PC3 loaded highly for organic carbon, exchangeable Ca, DTPA extractable Zn, Fe, bacteria, fungi, PSB, *Azotobacter*, AM fungi and acid phosphatase, succeeded by PC4 which showed a correlation with most of the studied characters except available N, K, exchangeable Ca, fungi, actinomycetes, PSB, *Azotobacter*, AM fungi, acid phosphatase, alkaline phosphatase and dehydrogenase. The *Scree* plot and PCA biplots featuring original factors represented as *Eigen* vectors demonstrated the correlation between the factor and two axis of soil chemical and microbiological properties (Fig. 15). The findings are in accordance with Kumar et al. (2017) in apple and Kumar et al. (2020) for strawberry.

EXPERIMENT 2:

CONJOINT EFFECT OF ORGANIC Zn AND BIO-ORGANICS FERTILIZATION

The results based on zinc biofortification through conjoint application of organic Zn and bio-organics have been summarized under the following heads and sub-heads:

4.2.1 Cropping behaviour
4.2.2 Yield contributing traits
4.2.3 Fruit quality
4.2.4 Soil properties
4.2.5 Physiological parameters
4.2.6 Leaf nutrients
4.2.7 Fruit nutrients
4.2.8 Fruit nutrients ratio
4.2.9 Antioxidants activity

4.2.1 CROPPING BEHAVIOUR

4.2.1.1 VEGETATIVE GROWTH

The data recorded on the influence of soil application of Zn organic sources and bio-organics on vegetative growth parameters of apple namely, plant height, trunk girth, canopy diameter, shoot growth, leaf area and leaf area index has been presented in Table 19.

4.2.1.1.1 Plant height

The data depicted in Table 19 indicated that conjoint application of organic Zn and bio-organic sources exerted a notable impact on the increase in plant height of apple trees. Treatment application of nano Zn-AAC$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$ (T$_8$) exhibited the maximum increase in plant height (48.81 cm) followed by nano Zn-AAC$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$ (T$_7$), Zn-AAC$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$ + HA$_{120}$ (T$_6$), Zn-AAC$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$ + HA$_{100}$ (T$_5$), Zn-Gly$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$ + HA$_{120}$ (T$_2$) and Zn-Glu$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$ + HA$_{120}$ (T$_4$) with corresponding values of 46.59, 43.90, 40.18, 39.63 and 38.90 cm. However, the minimum height (32.53 cm) was reported in the treatment Inorganic N: P: K @105:53:105 g/plant + ZnSO$_4$ @0.5% (T$_9$). Treatment application of T$_2$ and T$_4$ also observed similar effects in relation to increment in plant height of apple trees. The treatment of T$_8$ also recorded 1.1, 1.2, 1.3 and 1.5 times increase in plant height compared to T$_6$, T$_2$, T$_4$ and T$_9$ treatments, respectively.

4.2.1.1.2 Trunk girth

Data analysis in relation to trunk girth depicted that trunk girth of apple trees was positively influenced by the soil application of organic Zn and bio-organics nutrient sources. Treatment T$_8$ registered the maximum trunk girth (13.90 cm) which was statistically significant over all other treatments applied. The superior treatment was followed by treatments T$_7$, T$_6$, T$_5$, T$_2$ and T$_4$ exhibiting the corresponding values of 12.52, 11.73, 11.41, 11.15 and 10.26 cm. However, it was recorded minimum in treatment T$_9$ (9.77 cm). Treatments Zn-Glu$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$ + HA$_{120}$ (T$_4$), Zn-Gly$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$ + HA$_{100}$ (T$_1$) and Zn-Glu$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$ + HA$_{100}$ (T$_3$) also displayed uniform effects with respective values of 10.26, 10.15 and 10.03 cm. The results also reflected a per cent increase of 18.5, 24.6, 35.5 and 42.3 on girth of trees through treatment T$_8$ compared to T$_6$, T$_2$, T$_4$ and T$_9$, respectively.

4.2.1.1.3 Canopy diameter

Appraisal of the data revealed that among different Zn supplements, the trees canopy diameter ranged between 100.77 and 147.08 cm. Maximum canopy diameter (147.08 cm) was recorded in T_8 which was statistically superior to all other applied treatments. Similarly, statistically uniform results were also observed for T_6, T_5 and T_2 treatments which exhibited the corresponding values of 127.38, 125.55 and 124.36 cm. However, treatment T_9 reflected the lowest value (100.77 cm). The canopy diameter for the organic Zn and bio-organic treatment combinations was followed in the order of $T_8 > T_7 > T_6 > T_5 > T_2 > T_4$.

4.2.1.1.4 Shoot growth

The perusal of data exhibited variation in shoot growth of apple trees subjected to different organic Zn and bio-organics sources (Table 19). The values for shoot growth varied from 48.50 to 61.26 cm. Treatment T_8 recorded maximum shoot growth (61.26 cm) which was statistically superior to all the applied Zn treatments. However, the treatment T_9 recorded minimum (48.50 cm) values. Among other treatments, the treatments T_7 (58.10 cm) and T_6 (57.03 cm) and the treatments T_5 and T_2 were statistically equivalent to each other with corresponding values of 55.21 and 55.01 cm. The order followed pertaining to shoot growth for the organic Zn nutrient and bio-organic treatments applied as $T_8 > T_7 > T_6 > T_5 > T_2 > T_4$.

Table 19. Vegetative growth responses in apple to organic zinc and bio-organic nutrient sources

Treatment	**Increase in plant height (cm)**	**Trunk girth (cm)**	**Canopy diameter (cm)**	**Shoot growth (cm)**	**Leaf area (cm^2)**	**Leaf area index**	**TCSA (cm^2)**	**CV (m^3)**	**CA (m^2)**
T_1	37.36	10.15	107.23	52.49	33.90	1.76	8.21	1.18	0.90
T_2	39.63	11.15	124.36	55.01	35.19	1.88	9.90	1.72	1.22
T_3	35.41	10.03	104.64	50.86	33.07	1.73	8.03	1.09	0.86
T_4	38.90	10.26	116.78	53.72	35.05	1.81	8.39	1.45	1.07
T_5	40.18	11.41	125.55	55.21	35.99	1.93	10.39	1.82	1.24
T_6	43.90	11.73	127.38	57.03	36.38	1.97	10.97	1.95	1.27
T_7	46.59	12.52	140.23	58.10	38.87	2.04	12.54	2.65	1.55
T_8	48.81	13.90	147.08	61.26	42.17	2.11	15.38	2.93	1.70
T_9	32.53	9.77	100.77	48.50	32.20	1.65	7.62	0.96	0.80
$CD_{0.05}$	0.93	0.30	3.41	1.08	0.90	0.06	0.58	0.18	0.07

TCSA, Trunk cross-sectional area; CV, canopy volume; CA, canopy area; T_1: Zn-Gly$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$ + HA$_{100}$; T_2: Zn-Gly$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$ + HA$_{120}$; T_3: Zn-Glu$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$ + HA$_{100}$; T_4: Zn-Glu$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$ + HA$_{120}$; T_5: Zn-AAC$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$ + HA$_{100}$; T_6: Zn-AAC$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$ + HA$_{120}$; T_7: Nano Zn-AAC$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$; T_8: Nano Zn-AAC$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$; T_9: Inorganic N:P:K (105:53:105 g/plant) + $ZnSO_4$ @0.5%

4.2.1.1.5 Leaf area

A perusal of the data showed the positive impact of conjoint application of organic Zn and bio-organics supplements on leaf area of apple trees. Average leaf area ranged between 32.20 and 42.17 cm^2. The treatment of T_8 recorded maximum leaf area (42.17 cm^2) which was statistically higher compared to all other applied treatments. Minimum (32.20 cm^2)

values were however, observed in treatment T_9. The result also depicted that treatments T_6 (36.38 cm^2) and T_5 (35.99 cm^2), T_2 (35.19 cm^2) and T_4 (35.05 cm^2), T_1 (33.90 cm^2) and T_3 (33.07 cm^2) showed statistically similar effects. During the cropping period, an increment of 15.9, 19.8, 20.3 and 30.9 per cent in leaf area was observed in treatment T_8 compared to T_6, T_2, T_4 and T_9 treatments, respectively.

4.2.1.1.6 Leaf area index

The data presented in Table 19 showed notable variations in leaf area index (LAI) through conjoint application of different organic Zn and bio-organic nutrient sources. The treatment T_8 exhibited maximum LAI (2.11) which was statistically superior to all other applied treatments. However, minimum LAI (1.65) was recorded in treatment T_9. Among other Zn treatments, the treatments T_6 and T_5 were also statistically equivalent to each other with the values of 1.97 and 1.93, respectively. The superior treatment also demonstrated a 7.1, 12.2, 16.6 and 27.8 per cent increase compared to T_6, T_2, T_4 and T_9, respectively.

4.2.1.2 GENERATIVE TRAITS

Data related to generative traits of apple namely, trunk cross-sectional area, canopy volume and canopy area affected by conjoint treatment combinations of organic Zn and bio-organics sources has been depicted in Table 19.

4.2.1.2.1 Trunk cross-sectional area

Apple tree trunk cross-sectional area (TCSA) ranged from 7.62 to 15.38 cm^2. Maximum TCSA of 15.38 cm^2 was registered in treatment T_8 which was statistically superior compared to all other applied treatments (Table 19). The lowest value (7.62 cm^2) however, was recorded in the treatment T_9. Among other organic Zn treatments and bio-organic sources, the statistically proportionate effects were also recorded in treatments of T_4 (8.39 cm^2), T_1 (8.21 cm^2) and T_3 (8.03 cm^2). TCSA of apple trees for various Zn nutrient and bio-organic treatments was followed in the order $T_8 > T_7 > T_6 > T_5 > T_2 > T_4$.

4.2.1.2.2 Canopy volume

Data analysis revealed a beneficial influence of Zn nutrient sources and bio-organics on canopy volume (CV) of apple trees. Maximum CV (2.93 m^3) was registered in treatment T_8, followed by T_7, T_6, T_5, T_2 and T_4 with corresponding values of 2.65, 1.95, 1.82, 1.72 and 1.45 m^3. However, minimum CV (0.96 m^3) was observed in treatment T_9. The superior treatment was statistically insignificant to all other applied treatments; however, the statistically similar results were reported among treatments of T_6 (1.95 m^3) and T_5 (1.82

m^3). The treatment application of T_8 also depicted an increase of 1.5, 1.7, 2.0 and 3.0 times compared to T_6, T_2, T_4 and T_9, respectively.

4.2.1.2.3 Canopy area

It is evident from the data that canopy area (CA) of apple trees was significantly influenced with conjoint application of organic Zn and bio-organic sources. Maximum CA (1.70 m^2) was recorded in T_8 treatment. However, it was minimum (0.80 m^2) in T_9 treatment. Moreover, the statistically equivalent results were observed with treatments of T_6, T_5 and T_2 with corresponding values of 1.27, 1.24 and 1.22 m^2. The treatment of T_8 also recorded a per cent increase of 33.8, 39.3, 58.8 and 112.5 m^2 compared to T_6, T_2, T_4 and T_9, respectively.

In the present investigation, the conjoint application of organic Zn and bio-organic supplements significantly affected vegetative growth characteristics of apple trees. This could be attributed to Zn function in maintaining the integrity of cell membranes, regulating phyto-hormones and enzymatic activities associated with the production of proteins and carbohydrates (Marschner, 2012; Hafeez et al., 2013). The increased cell division and elongation attributed to the extra effects of Zn (Bala et al., 2019; Yeboah et al., 2021) and amino acids (El-Bassiouny et al., 2008; Mirbolook et al., 2020) which also contributed to an increase in vegetative growth characteristics of apple. Because of their large molecular weight, inorganic fertilizers were difficult to transport through leaf surfaces (Ahmed et al., 2011; Elakbawy et al., 2021). Furthermore, amino acid chelates' capacity to form stable complexes with micronutrients cations especially Zn (Jacob et al., 2024) which thereby increased absorption rate (Ghasemi et al., 2014; Mirbolook et al., 2020). In addition, the precise distribution of nutrients along with an adequate N content (22-27%) and plant hormones could be responsible for the superiority of amino acid chelates over inorganic fertilizers (Ge et al., 2009; He et al., 2013). Moreover, the increase in vegetative characteristics of trees observed through the application of bio-organics and nano-Zn amino acid chelate can be ascribed to the strong binding affinity between amino acids and nanoparticles (Rossi et al., 2018). Furthermore, because of nanoscale particle size, larger surface area and higher responsiveness, nano-based amino acid chelates also contributed to more Zn absorption and translocation (Garcia et al., 2011; Ghasemi et al., 2014, Asri et al., 2015). According to Barker and Pilbeam (2010), Zn acts as an activator of several enzymes within plant system, which directly affects the production of growth regulators like auxins. This might have contributed to the significant growth of shoots (Ashraf et al., 2013). Also, it could be related to the improved absorption and translocation of amino acid chelates in nano forms (Ge et al., 2009; Garcia et al., 2011; Ghoname et al., 2012; Ghasemi et al., 2014, Asri et al., 2015). Previous research employing Zn amino acid chelate showed higher leaf area due to improved metabolic processes, photosynthetic efficiency and sugar assimilation (Thumma et al., 2001; Ajeng et al., 2020; Alzreejawi and Al-Juthery, 2020; Al-Toki et al., 2021). Similarly, an increase in leaf area might have contributed to increased leaf area index of the trees (Bashir et al., 2021). The improved trunk cross-sectional area, canopy volume and canopy area clearly show the significant effect of nano Zn amino acid chelate to improve plant height and canopy diameter of the trees. Trunk girth might have improved as a result of enhanced absorption of carbohydrates and their subsequent translocation towards the shoots (Chandra et al., 2015).

Zn glycinate and Zn gluconate have also shown better results than Zn sulphate, even with the application of nano-based organic Zn complexes (Baligah et al., 2020; Xu et al., 2022). Because of the greater bioavailability to plants, the potential effects of zinc glycinate over zinc gluconate were also confirmed (Gandia et al., 2007). Furthermore, improvement in generative traits of trees ascribed to increased microbial activity in the soils enriched with bio-organics, which could result to a higher nutrient availability in the rhizosphere. Improved vegetative growth traits were a result of the bio-organic nutrient sources' additional enhancement of the physiological and metabolic processes of plants (Ashwini et al., 2022). The improved microbial activity during the breakdown process of vermicompost enables growth influencing chemicals such as humic acid and plant growth regulators to be more accessible, which could account for the increase in plant height (Atiyeh et al., 2002; Arancon et al., 2004; Singh et al., 2010).

Furthermore, it appears that the increased N uptake is associated with *Azolla* that could boost total chlorophylls (Maswada et al., 2021), which might improve the vegetative growth characteristics. Our findings coincide with those of Ali et al. (2019) who demonstrated that AM fungal colonization increases root area, stimulates root activity and promotes chlorophyll synthesis. This mutualistic relationship increased water and nutrient absorption, enhanced photosynthetic activity and hence, vegetative growth traits of trees. In addition, application of PGPR enhanced vegetative growth characteristics by synthesizing plant growth regulators like auxins, gibberellins and cytokinins (Khalid et al., 2004; Aslantas et al., 2007), leading to increased nutrient uptake (Kohler et al., 2006). Esitken et al. (2006), Aslantas et al. (2007) and Pirlak et al. (2007) have similarly reported increased vegetative growth characteristics following supplementation with PGPR. In addition, the conversion of N and P from insoluble to soluble forms is facilitated by the synthesis of organic acids through the application of PGPR, resulting in improved plant vegetative growth (Richardson et al., 2009; Anli et al., 2020; Kumar et al., 2020). Plant growth indicators could have been improved by the PGPR-induced increase in Zn solubilization rate (Saravanan et al., 2013; Sirohi et al., 2015). Additionally, though enhanced nutrition and water absorption, stress resistance, cell membrane stability, and hormone synthesis, humic acid provided increased vegetative development of the plants (Calvo et al., 2014; Aslam et al., 2016; Al-Saif et al., 2023).

4.2.1.3 FLOWERING

The data related to flowering traits of apple as influenced by conjoint application of organic Zn and bio-organics supplements have been presented in Fig. 16-19.

4.2.1.3.1 Date of pink bud emergence

The data revealed that the date of pink bud emergence varied from 13 to 18 March and 10 to 17 March during 2022 and 2023, respectively (Fig. 16). In T_8 treatment, pink bud emergence (March 13 and March 10) was observed the first, whereas, it appeared the last in T_9 treatment. Combined application of organic Zn and bio-organics sources during both the years observed the pink bud emergence in the order $T_8 > T_7 > T_6 > T_5 > T_2 > T_4$.

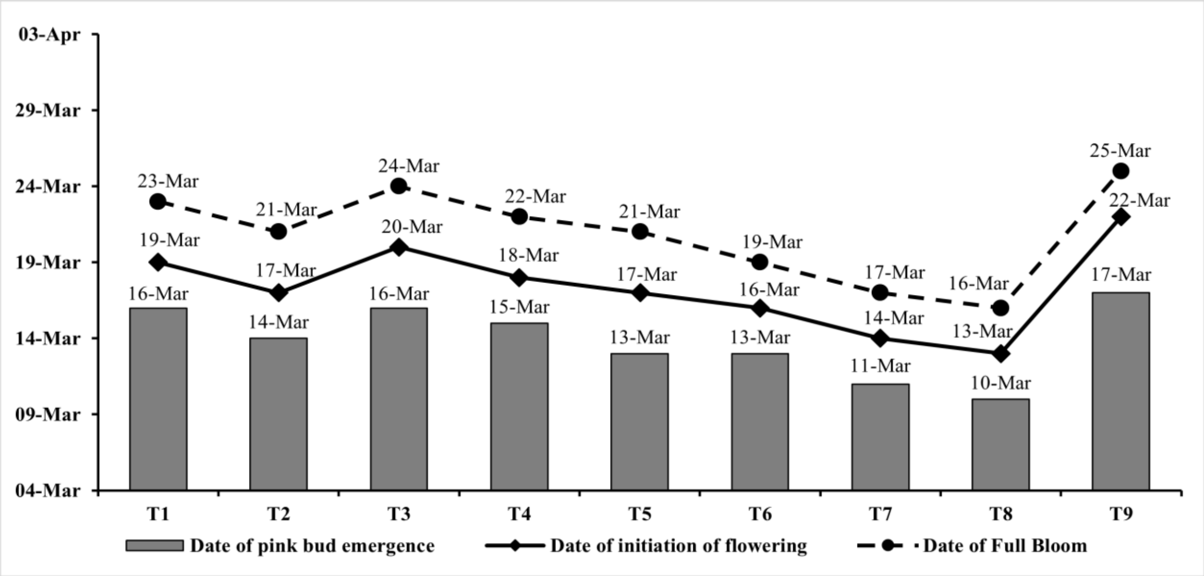


Fig. 17: Date of pink bud emergence, date of full bloom and date of initiation of flowering influenced by organic Zn and bio-organic fertilizers

4.2.1.3.2 Date of initiation of flowering

The date of initiation of flowering varied from 18 March to 24 March and 13 March to 22 March during 2022 and 2023, respectively (Fig. 16). Treatment T_8 had the earliest flower initiation (18 March and 13 March), whereas, treatment T_9 recorded the last (24 March and 22 March). Furthermore, the order pertaining to initiation of flowering in apple trees through the supplementation of organic Zn and bio-organics sources recorded as $T_8 > T_7 > T_6 > T_5 > T_2 > T_4$.

4.2.1.3.3 Date of full bloom

Date of full bloom in apple trees varied from 23 to 29 March in 2022 and 16 to 25 March in 2023 (Fig. 16). The treatment T_8 showed the earliest dates of full bloom (March 23 and March

16), whereas treatment T_9 showed the latest (March 29 and 25) when supplemented with organic Zn and bio-organics nutrient sources.

4.2.1.3.4 Duration of flowering

During the experiment, the application of organic Zn and bio-organics showed a considerable increase in the duration of flowering in apple trees (Fig. 17). Highest flowering duration (12.50 days) was observed in T_8 which was statistically similar to T_7 (11.83 days). On the other hand, T_9 had the shortest flowering duration of 9.50 days. Statistically similar results were also obtained with treatments of T_2, T_4, T_1 and T_3 with corresponding values of 10.83, 10.67, 10.33, and 10.00 days. Treatment T_8 showed a percentage increase of 8.6, 15.4, 17.2, and 31.6 when compared to treatments of T_6, T_2, T_4 and T_9.

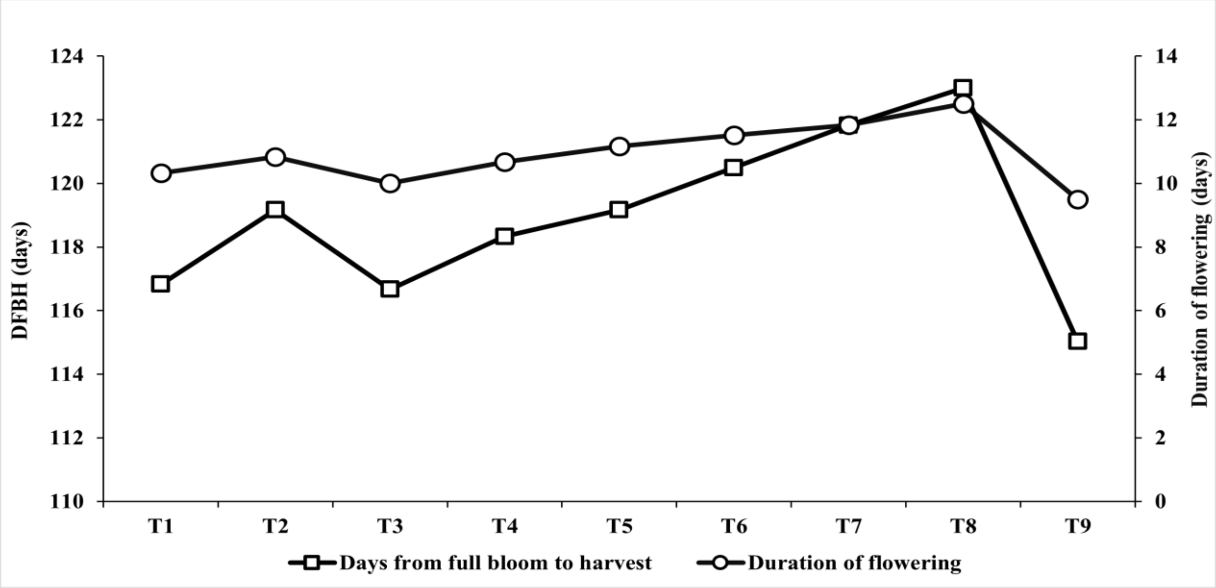


Fig. 18: Impact of conjoint application of organic Zn and bio-organic fertilizers on duration of flowering and days from full bloom to harvest in apple

4.2.1.3.4 Days from full bloom to harvest

The data pertaining to days from full bloom to harvest (DFFBH) showed a positive effect when conjoint application of organic Zn and bio-organics in apple trees was carried out. Treatment of T_8 recorded maximum DFFBH (123.00 days) which was significantly superior to all the other applied treatments. Whereas, the treatment T_9 recorded the minimum DFFBH (115.03 days). Furthermore, the treatment application of T_1 (116.83 days) was statistically at par with T_3 (116.67 days) and also recorded the identical effect among treatments T_2 and T_5 (119.17 days each).

4.2.1.3.5 Length of flowering shoot

A perusal of the data indicated the significant influence of organic Zn and bio-organics application on length of flowering shoot of apple (Fig. 18). Length of flowering shoot varied between 32.88 and 47.64 cm. The treatment T_8 observed maximum (47.64 cm) length of flowering shoot which was statistically superior to all other applied treatments. However, the minimum length of flowering shoot (32.88 cm) was found in treatment T_9. The per cent increase of 19.6, 23.1, 27.4 and 44.9 was reported in treatment T_8 as compared to T_6, T_2, T_4 and T_9 treatments, respectively.

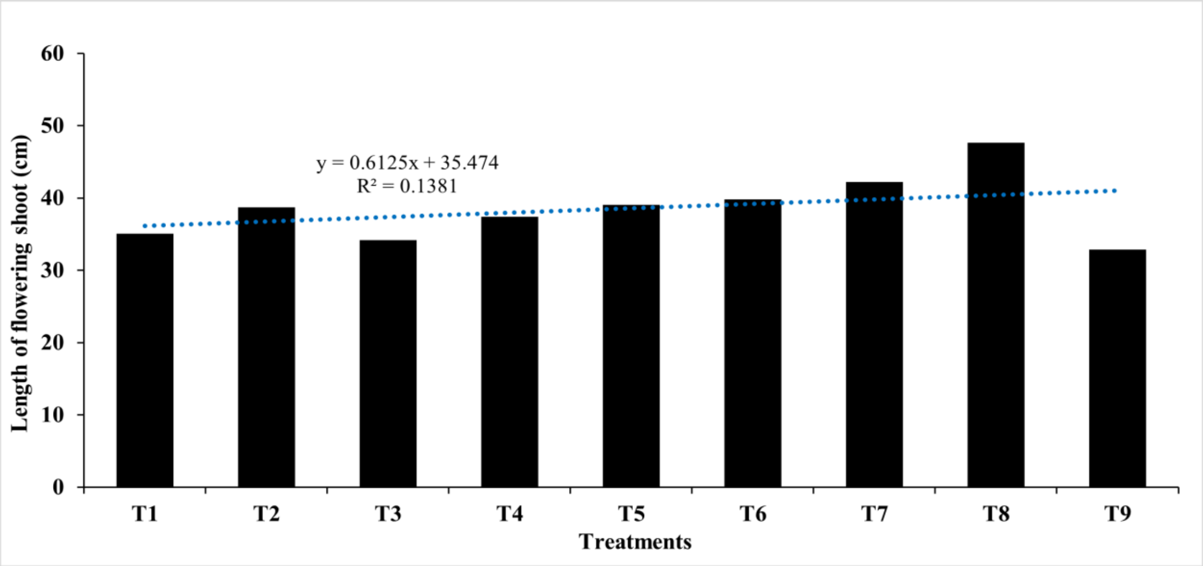


Fig. 19: Length of flowering shoot affected by organic Zn and bio-organic fertilizers in apple

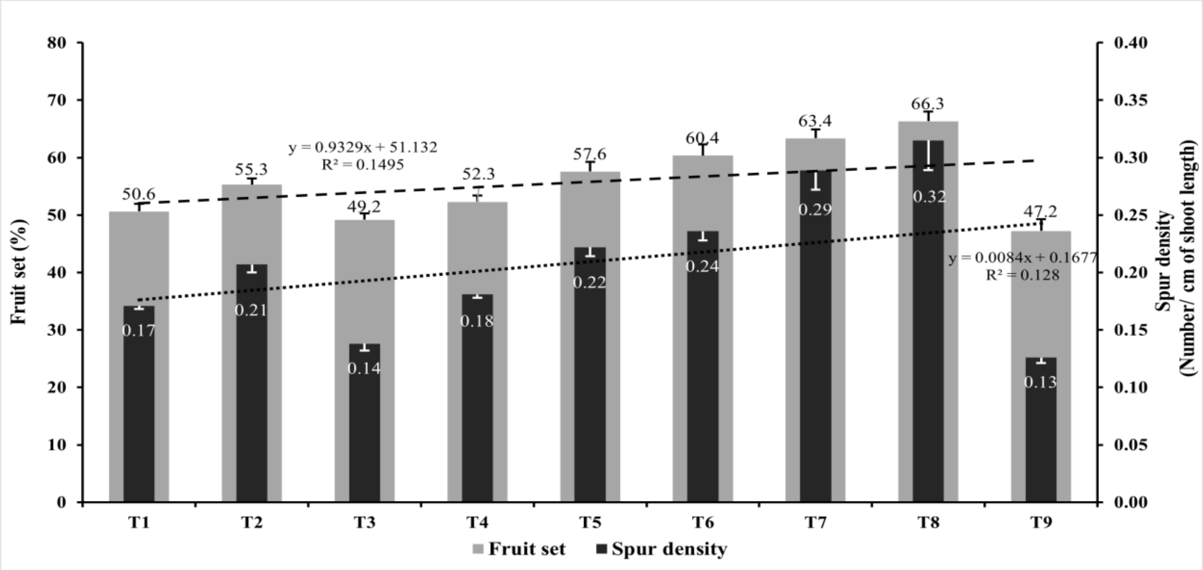


Fig. 20: Relationship between fruit set and spur density in apple under organic Zn and bio-organics nutrition

4.2.1.3.6 Spur density

Conjoint application of different organic Zn and bio-organics sources exerted a significant influence on spur density in apple trees (Fig. 19). The average spur density ranged between 0.13 and 0.32. The treatment of T_8 exhibited maximum spur density (0.32) which was statistically at par with T_7 (0.29). However, treatment of T_9 exhibited the minimum spur density (0.13) which was also statistically at par with T_3 (0.14). The order followed pertaining to spur density for various organic Zn and bio-organic treatments applied in the order of $T_8 > T_7 > T_6 > T_5 > T_2 > T_4$. In addition, treatment T_8 recorded an increase of 2.2 times followed by T_7 and T_6 (1.8 times each), T_5 (1.7 times), T_2 (1.6 times) and T_4 (1.4 times) over T_9 treatment.

The results inferred that the flowering indicators were significantly improved when nano Zn amino acid chelate and bio-organic nutrient sources in combinations were applied. The high solubility of nanoparticles and subsequent effect of Zn on plant reproductive growth might be responsible for improving flowering traits of the trees (Naderi and Danesh-Shahraki, 2013; Rahman et al., 2016). Furthermore, better flowering characteristics might have resulted from the targeted distribution of amino acid chelates and subsequent progressive release (Niu et al., 2020). In addition, amino acid chelates promote plant flowering by accelerating N transport and assimilation throughout plant tissues (Al-Said and Kamal, 2008; Marschner, 2012; Kolota et al., 2013; Calvo et al., 2014; Abbass et al., 2020). The function of zinc in photosynthesis and chlorophyll production further supports this (Alloway, 2008). Baligah et al. (2020) and Xu et al. (2022) have reported that different organic Zn complexes were more effective than zinc sulphate. According to Prasad et al. (2012), the early flowering initiation was due to the impact of nano amino acid chelate, which increases the penetration of Zn molecules through the leaf surface. Additionally, it could result from the enhanced accessibility of vital nutrients with the dual treatment application of vermicompost and AM fungi (Singh et al., 2015).

In addition, the earthworms, *Eisenia fetida* releases hormones into the vermicompost, including gibberellins and auxins (Suthar, 2010; Aremu et al., 2015; Kist Steffen et al., 2019). This might have caused an early emergence of pink buds and the beginning of flowering. Improvement in flowering with inoculation of PGPR is also ascribed to production of phytohormones (Sgroy et al., 2009; Shah et al., 2021). Our results are also corroborating with the findings of Moghadam and Shoor (2013), who concluded that PGPR inoculation enhanced the transport and mobilization of cytokinins from source to sink, which led to an early shift of vegetative to reproductive phase. Likewise, days from full bloom to harvest, the length of the flowering shoot and the spur density might have been attributed to a higher accumulation of carbohydrates through organic compounds (Jat, 2023). Amiri et al. (2016) reported the faster rate of carbohydrate buildup caused by nano Zn amino acid chelate application. Furthermore, it has been demonstrated that humic acid stimulates the synthesis of flower stimulus through altering the ratio of components that promote and inhibit flowering (Ngullie et al., 2014).

4.2.2 YIELD CONTRIBUTING PARAMETERS

The data on the conjoint effect of organic Zn and bio-organic supplements on the yield contributing parameters of apple trees under high density planting are presented in Table 20 and Fig. 20.

4.2.2.1 Number of flowers/shoot

Number of flowers per shoot was significantly influenced when combined application of organic Zn and bio-organics in apple trees was supplemented (Table 20; Plate 9). Treatment of T_8 recorded maximum number of flowers per shoot (39.27), whereas, it was least in T_9 (29.51). Statistically equivalent results were also observed among treatments T_7 (38.29) and T_6 (37.55), and T_4 (32.72) and T_1 (32.09). Furthermore, the results indicated 4.5, 13.5, 20.0 and 33.1 per cent increment in the superior treatment T_8 as compared to T_6, T_2, T_4 and T_9 treatments, respectively.

Table 20. Comparative assessment of organic Zn and bio-organic fertilizers on yield contributing traits of apple

Treatment	Number of flowers/shoot	Number of fruits/shoot	Fruit set (%)	Fruit yield (kg/tree)
T_1	32.09	20.66	50.63	5.15
T_2	34.59	21.95	55.27	5.46
T_3	31.58	19.11	49.16	4.97
T_4	32.72	21.23	52.28	5.31
T_5	36.22	22.87	57.58	5.63
T_6	37.55	23.50	60.36	5.82
T_7	38.29	24.30	63.35	5.98
T_8	39.27	24.98	66.34	6.13
T_9	29.51	17.20	47.21	4.81
$CD_{0.05}$	0.84	0.62	3.47	0.14

T_1: Zn-Gly$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$ + HA$_{100}$; T_2: Zn-Gly$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$ + HA$_{120}$; T_3: Zn-Glu$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$ + HA$_{100}$; T_4: Zn-Glu$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$ + HA$_{120}$; T_5: Zn-AAC$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$ + HA$_{100}$; T_6: Zn-AAC$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$ + HA$_{120}$; T_7: Nano Zn-AAC$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$; T_8: Nano Zn-AAC$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$; T_9: Inorganic N:P:K (105:53:105 g/plant) + $ZnSO_4$ @0.5%

4.2.2.2 Number of fruits/shoot

A cursory glance at the data highlighted the significant impact of soil organic Zn and bio-organic sources on number of fruits per shoot which varied from 17.20 to 24.98 (Table 20). Highest number of fruits per shoot (24.98) was recorded in treatment T_8, which was statistically superior to all other applied treatments (Plate 10). The lowest number of fruits per shoot (17.20) however, was noticed in T_9. Among other treatments, the treatments of T_4 and T_1 marked statistically significant results with the corresponding values of 21.23 and

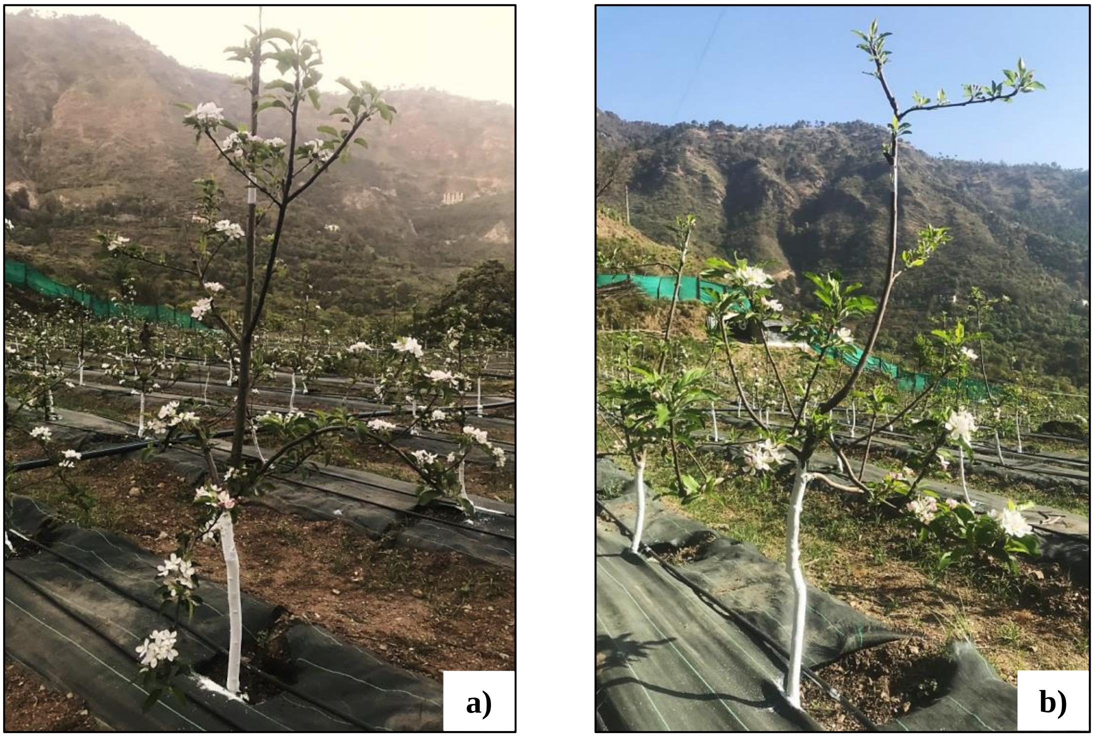


Plate 9: Flowering in apple trees a) Nano Zn-AAC$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$ b) Inorganic N:P:K (105:53:105 g/plant) + ZnSO$_4$ @0.5% (control)

Plate 10: Fruit development stage in apple trees a) Nano Zn-AAC$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$ b) Inorganic N:P:K (105:53:105 g/plant) + ZnSO$_4$ @0.5% (control)

20.66. Moreover, the order followed pertaining to number of fruits per shoot among various organic Zn and bio-organic nutrient sources as $T_8 > T_7 > T_6 > T_5 > T_2 > T_4$.

4.2.2.3 Fruit set

Conjoint application of organic Zn and bio-organics supplements resulted in a marked increase in fruit set of apple trees (Table 20). Fruit set was recorded maximum (66.34%) in T_8 which was statistically at par with T_7 (63.35%) treatment. The least values for fruit set however, were recorded in T_9 (47.21%), which also displayed uniform results with treatment T_3 (49.16%). Among all treatments, the treatment of T_8 exhibited maximum per cent increase of 40.5, followed by T_7 (34.1%), T_6 (27.8%), T_5 (21.9%), T_2 (17.1%) and T_4 (10.7%) as compared to T_9 treatment.

4.2.2.4 Fruit yield

In the context of different organic Zn and bio-organics application, the fruit yield of apple trees varied from 4.81 to 6.13 kg/tree. Highest fruit yield (6.13 kg/tree) was observed in treatment T_8 followed by T_7, T_6, T_5, T_2 and T_4 with the corresponding values of 5.98, 5.82, 5.63, 5.46 and 5.31 kg/tree. However, the lowest fruit yield of 4.81 kg/tree was attained by T_9 treatment. Treatment of T_8 exhibited statistically superior results to all other applied treatments. Among different conjoint application of organic Zn and bio-organics treatments, fruit yield was followed in the order of $T_8 > T_7 > T_6 > T_5 > T_2 > T_4$ (Table 20). Furthermore, the superior treatment of T_8 also exhibited an increase of 5.3, 12.3, 15.4 and 27.4 per cent compared to treatments T_6, T_2, T_4 and T_9, respectively.

4.2.2.5 Yield efficiency

Perusal of the data observed the variation in yield efficiency in terms of leaf area, TCSA, CV and CA of apple trees subjected to different organic Zn and bio-organic sources (Fig. 20). Yield efficiency as yield per leaf area (LA) varied recorded insignificant results among all the applied Zn treatments. However, treatments T_5 and T_6 observed the maximum yield per leaf area (0.16 kg/cm^2 of LA). Moreover, all the other applied treatments depicted similar values (0.15 kg/cm^2 of LA each). Furthermore, yield per TCSA of the trees ranged between 0.40 and 0.64 kg/cm^2 of TCSA and was also recorded statistically insignificant results. Treatment T_4 and T_9 exhibited maximum yield efficiency (0.64 kg/cm^2 of TCSA), however, it was least in treatment T_8 (0.40 kg/cm^2 of TCSA). Similarly, yield efficiency in terms of yield per CV varied from 2.11 to 5.02 kg/m^3. Maximum yield efficiency (5.02 kg/m^3 of CV) was recorded in treatment T_9. However, treatment T_8 reflected the lowest value (2.11 kg/m^3 of CV) in terms of yield per CV, which exhibited statistically equivalent results with treatment T_7 (2.32 kg/m^3 of CV). Data analysis in relation to yield efficiency in terms of CA showed that treatment T_9 exhibited the maximum yield efficiency (5.23 kg/m^2 of CA). The superior treatment was followed by treatments T_3, T_1, T_6, T_4 and T_5 with the corresponding values of 5.16, 5.07, 4.58, 4.46 and 4.44 kg/m^2. However, it was recorded minimum (3.76 kg/m^2 of CA) in treatment T_8.

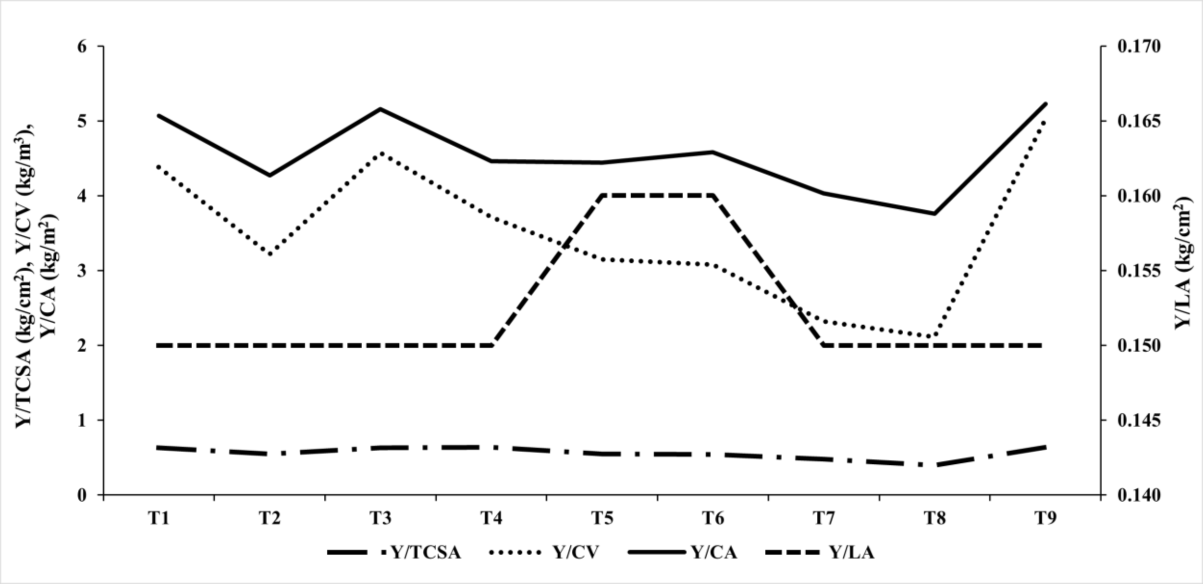


Fig. 21: Yield efficiency of apple as affected by conjoint application of organic Zn and bio-organic fertilizers

In contrast to Zn sulphate and inorganic NPK, the current investigation showed that Zn amino acid complexes in conjugation with bio-organics increased the yield contributing indicators. However, the most significant effect was demonstrated by the nano Zn amino acid chelate which ascribed to the increased absorption of nutrients brought accessible by zinc chelated with amino acids (Souri and Hatamian, 2019). Additionally, El-Sayed et al. (2017) suggested that the positive impacts of nanoparticles on targeted nutrient release and the avoidance of unexpected loss could also have contributed. The reason for the increase in number of flowers by nano Zn amino chelate might be due to the involvement of Zn in the synthesis of tryptophan (IAA precursor) or the combined effect of N and Zn on the metabolic pathways (Cakmak et al., 2010). Similarly, improved pollen tube growth and pollination with Zn foliar spray led to better flower bud formation (Najizadeh and Khoshgoftarmanesh, 2019). Amino acids are also essential for cell division and the assimilation of carbohydrates. According to Ahmed and Abd El-Hameed (2003) and Baqir et al. (2019), organic zinc chelates provide a higher translocation of assimilates to the sink, which possibly contributed to the increase in the production of fruits. An additional explanation for the increase in fruits and flowers could be the accelerated flower growth induced by addition of vermicompost (Singh et al., 2015).

Also, more fruits are produced as a result of the higher gibberellin concentration in vermicompost (Kist Steffen et al., 2019).

Bio-organic nutrient sources and nano Zn amino acid chelate also had an impact on fruit set and development. According to Kamiab and Zamanibahramabadi (2016), increased leaf area through improved absorption of amino acid-chelated Zn might have promoted photosynthetic activity and therefore increased fruit set. It could also be related to the fact that bio-organics stimulate photosynthetic activity through promotion of growth factors (Das et al., 2017). Earlier literature is well documented on increased in the quantity of flowers and fruit set as a consequence of gibberellic acid produced by PGPR (Karadeniz et al., 2006; Zalewska and Antkowiak, 2013; Kumar et al., 2014b; Adeleke et al., 2019).

It is well known that amino acid chelates in nano form improve nutritional availability, which increases fruit yield (Yeboah et al., 2021). Furthermore, higher yield indicated as increased biomass due to amino acids for stimulating physiological processes involved in the synthesis of carbohydrates (Kandil et al., 2016). The stimulatory effect of Zn amino complexes on vegetative growth and fruit set might have led to increased fruit production (Datir et al., 2010). Incorporation of organic matter to the soil through *Azolla* also has a beneficial effect on yield (Aref et al., 2009). Furthermore, the results also confirmed the findings of Taha and El-Shahat (2017), who claimed that the application of humic acid and *Azolla* enhanced leaf area by increased photosynthetic area within crop plants. Moreover, the application of AM fungi increased leaf chlorophyll and the assimilation of CO_2, which resulted in an improvement in fruit yield (Mikiciuk et al., 2019). Previous research indicates that the twin effects of phosphate solubilization and N fixation that might have contributed to the unique impacts of PGPR inoculation on fruit production (Garcia de Salamone et al., 2012; Salomon et al., 2014; Perez-Rodriguez et al., 2020). Additionally, dual inoculation of AM fungi with PGPR showed a 30 per cent decrease in fertilizer usage yet maintained high yields (Adesemoye et al., 2009).

4.2.3 FRUIT QUALITY

4.2.3.1 PHYSICAL

The data depicted the effect of different organic Zn and bio-organic sources on the physical parameters of apple fruit samples have been presented in Table 21.

4.2.3.1.1 Fruit length

Perusal of the data has shown a pronounced effect on length of fruit samples over the cropping period due to the application of organic Zn and bio-organic sources. The treatment of T_8 demonstrated maximum fruit length (57.99 cm) which was statistically similar to T_7 (57.28 cm). The lowest values (52.66 cm) however, were recorded in treatment T_9. Moreover, treatments of T_6, T_5, T_2 and T_4 exhibited statistically uniform effects with corresponding values of 56.15, 55.45, 55.11 and 54.61 cm. This treatment also experienced an increase of 3.3, 5.2, 6.1 and 10.1 per cent in fruit length over treatments T_6, T_2, T_4 and T_9, respectively.

4.2.3.1.2 Fruit breadth

Analysis of data pertaining to fruit breadth of apple fruit samples revealed a positive effect of conjoint application of organic Zn and bio-organics sources as compared to control (Table 21). Maximum fruit breadth (68.67 cm) was recorded in treatment T_8 which was statistically at par with T_7 (67.95 cm). The lowest value (62.23 cm), however, was observed in T_9. The treatment of T_8 also reported an increase of 3.9, 6.8, 7.1 and 10.3 per cent compared to T_6, T_2, T_4 and T_9 treatments, respectively.

4.2.3.1.3 Shape index

Shape index of fruit samples showed statistical insignificant difference among different Zn and bio-organic treatments applied and which was ranged between 0.84 and 0.86. Maximum value of shape index (0.86) was observed in treatment T_2, whereas, it was lowest (0.84) in treatments of T_1, T_7 and T_8. Moreover, treatments T_3, T_4, T_5, T_6 and T_9 also displayed the identical values (0.85 each).

Table 21. Physical parameters of apple fruit samples influenced by conjoint organic zinc and bio-organic fertilization

Treatment	Fruit length (mm)	Fruit breadth (mm)	Shape index	Fruit weight (g)	Fruit volume (cm^3)	Specific gravity	Fruit firmness (kg/cm^2)
T_1	53.85	63.98	0.84	110.68	121.67	0.90	5.86
T_2	55.11	64.27	0.86	115.25	128.34	0.90	5.53
T_3	53.42	62.95	0.85	107.41	120.00	0.89	5.97
T_4	54.61	64.13	0.85	113.55	124.17	0.91	5.76
T_5	55.45	65.00	0.85	118.49	129.17	0.91	5.51
T_6	56.15	66.08	0.85	124.74	142.50	0.87	5.35
T_7	57.28	67.95	0.84	132.92	151.67	0.87	5.11
T_8	57.99	68.67	0.84	152.04	170.00	0.90	4.94
T_9	52.66	62.23	0.85	104.32	117.45	0.89	6.27
$CD_{0.05}$	1.71	2.23	NS	5.99	5.43	NS	0.07

NS, non-significant; T_1: Zn-Gly$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$ + HA$_{100}$; T_2: Zn-Gly$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$ + HA$_{120}$; T_3: Zn-Glu$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$ + HA$_{100}$; T_4: Zn-Glu$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$ + HA$_{120}$; T_5: Zn-AAC$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$ + HA$_{100}$; T_6: Zn-AAC$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$ + HA$_{120}$; T_7: Nano Zn-AAC$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$; T_8: Nano Zn-AAC$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$; T_9: Inorganic N:P:K (105:53:105 g/plant) + $ZnSO_4$ @0.5%

4.2.3.1.4 Fruit weight

Application of organic Zn and bio-organic treatments exhibited significant impact on weight of apple fruit samples, which varied from 104.32 to 152.04 g. Treatment of T_8 recorded the highest fruit weight (152.04 g) which was statistically superior to all other applied treatments. However, lowest fruit weight (104.32 g) was observed in treatment T_9. The analogous results were also noticed with statistically equivalent treatments of T_2 (115.25 g), T_4 (113.55 g) and T_1 (110.68 g). Moreover, average fruit weight illustrated 21.8, 31.9, 33.8 and 45.7 per cent increase in T_8 compared to T_6, T_2, T_4 and T_9 treatments, respectively.

4.2.3.1.5 Fruit volume

The data on conjoint application of organic Zn and bio-organic treatments on the fruit volume is presented in Table 21. Average fruit volume varied between 117.45 to 170.00 cm^3. Maximum fruit volume (170.00 cm^3) was recorded in treatment T_8, which also exhibited statistical significance compared to all other treatments. However, it was minimum (117.45 cm^3) in treatment T_9. The results also reflected 1.2, 1.3, 1.4 and 1.5 times increase in fruit volume through superior treatment of T_8 over T_6, T_2, T_4 and T_9, respectively.

4.2.3.1.6 Specific gravity

Specific gravity of fruit samples observed statistically insignificant results among all the applied treatments. However, maximum specific gravity (0.91) was observed in treatments T_4 and T_5, whereas, minimum (0.87) values were registered in T_6 and T_7. Moreover, identical value of 0.90 each was recorded in treatments T_1, T_2 and T_8 and 0.89 each in T_3 and T_9 treatments.

4.2.3.1.7 Fruit firmness

The data presented in Table 21 indicated that fruit firmness varied from 4.94 to 6.27 kg/cm^2 among different applied treatments. Minimum fruit firmness (4.94 kg/cm^2) was recorded in treatment T_8, whereas, the treatment T_9 exhibited the maximum value (6.27 kg/cm^2). The treatment T_9 surpassed all other treatments significantly with respect to firmness of fruit samples. In addition, treatment T_9 also demonstrated a remarkable 26.9 per cent increase compared to T_8 treatment.

The findings suggested that different organic Zn complexes supplemented with bio-organics significantly affected the physical characteristics of fruit samples. The increased application of Zn (Alloway, 2008; Boettcher et al., 2010) and amino acids (Datir et al., 2010) is believed to have caused an increase in fruit dimension. According to Babu and Singh (2001), longer cells encourage the accumulation of metabolites, which might have improved the fruit's physical characteristics. Furthermore, improved fruit weight was due to increased metabolite translocation through zinc amino acid chelates from source to sink (Gourkhede et al., 2020). In addition, compared to its counterparts, Zn is more readily accessible due to its nano size and large surface area of nanoparticles (Prasad et al., 2012), which could have accelerated the translocation of photosynthates to sink (Lin and Xing, 2008). Laware and Raskar (2014) observed improved weight of fruit samples due to nano Zn ability to improve the volume of fruits. Also, *Azolla* application resulted in higher fruit weight and dimensions (Taha and El-Shahat, 2017; Taha et al., 2018; Ibrahim, 2022). This might be due to potential of *Azolla* to release nutrients into soil so that plants can receive the nutrition they need (Chaoudhury and Kennedy, 2004). Further, the addition of AM fungi into the rhizosphere improved nutrients that can be absorbed by the roots of the plant. This contributes to higher metabolic activity and subsequently better fruit weight (Jiang et al., 2023). Another possible explanation is the efficient distribution of photosynthates to the sink through the inoculation with AM fungi (Kumar et al., 2020). Also, the inoculation of PGPR improved the physical properties of fruits by stimulating the production of plant growth hormones while providing a balanced supply of nutrients (Lingua et al., 2013; Kurokura et al., 2017).

4.2.3.2 BIOCHEMICAL

The perusal of data pertaining to biochemical attributes of apple fruits on the significant influence conjoint application of Zn organic and bi-organic sources is presented in Table 22.

4.2.3.2.1 Total soluble solids

Conjoint application of organic Zn and bio-organic supplemented a notable impact on the total soluble solids (TSS) content of apple fruit samples. Application of treatment T_8 exhibited the maximum TSS content (14.02 °Brix) followed by T_7, T_6, T_5, T_2 and T_4 with corresponding values of 13.66, 13.31, 13.16, 12.95 and 12.56 °Brix. However, it was minimum (11.80 °Brix) in the treatment T_9. The treatments of T_6 and T_5 also depicted similar effects in relation to increment in TSS content in fruit samples. The superior treatment of T_8 also recorded a 1.0, 1.1, 1.1 and 1.2 times increase in TSS content compared to T_6, T_2, T_4 and T_9 treatments, respectively.

4.2.3.2.2 Titratable acidity

Different conjoint organic Zn and bio-organic sources combinations recorded non-significant results in terms of titratable acidity of fruits (Table 22). Minimum titratable acidity (0.36%) was recorded in treatment T_8, whereas, it was maximum (0.54%) in treatment T_9. The treatments combinations of T_3 and T_1, T_5 and T_6, T_7 and T_8 depicted analogous results with the corresponding values of 0.52 and 0.50, 0.43 and 0.41, 0.38 and 0.36 per cent. Moreover, a reduction of 13.8, 27.7, 36.1 and 50.0 per cent in titratable acidity was also observed in T_8 as compared to T_6, T_2, T_4 and T_9, respectively.

4.2.3.2.3 Reducing sugars

Data analysis in relation to reducing sugars depicted that reducing sugars content of apple fruits was positively influenced by the conjoint application of organic Zn sources and bio-organics in apple. Application of treatment T_8 registered maximum reducing sugars content (7.78%) demonstrating statistically significance with T_7 (7.61%). However, it was recorded minimum in treatment T_9 (6.21%). Treatments of T_5 (7.16%) and T_2 (7.00%) were statistically at par with each other, and the treatments of T_1 and T_3 also displayed uniform effects with corresponding values of 6.58 and 6.39 per cent. The results also reflected an increase of 5.5, 11.1, 16.4 and 25.2 per cent in reducing sugars content fruit samples through T_8 compared to T_6, T_2, T_4 and T_9, respectively.

4.2.3.2.5 Total sugars

The recorded data exhibited variation in total sugars content of apple fruits subjected to different organic Zn and bio-organics combinations. The values for total sugars content varied from 9.83 to 12.02 per cent. Treatment of T_8 recorded maximum total sugars content (12.02%) which was statistically at par with treatment T_7 (11.86%). However, the treatment T_9 recorded the minimum content (9.83%). Among other treatment combinations, the treatments of T_6 (11.37%) and T_5 (11.17%) and the treatments of T_4 and T_1 were statistically equivalent to each other with respective values of 10.65 and 10.40 per cent. The order followed pertaining to total sugars content among different organic Zn and bio-organic nutrient combinations as $T_8 > T_7 > T_6 > T_5 > T_2 > T_4$.

4.2.3.2.6 Ascorbic acid

A perusal of the data showed the positive impact of conjoint application of organic Zn and bio-organics combinations on ascorbic acid content of fruit samples. Average ascorbic acid content ranged between 2.65 and 4.65 mg/100g. The treatment combination of T_8 recorded maximum ascorbic acid content (4.65 mg/100g) which was statistically higher compared to all other applied treatments. Minimum (2.65 mg/100g) values however, were observed in treatment T_9. The findings also depicted that treatment combinations of T_6 (3.95 mg/100g) and T_5 (3.74 mg/100g), T_2 (3.65 mg/100g) and T_4 (3.30 mg/100g), T_1 (3.17 mg/100g) and T_3 (3.02 mg/100g) showed statistically similar effects during the investigations. Moreover, an increment of 17.7, 27.4, 40.9 and 75.4 per cent in ascorbic acid content was observed in treatment T_8 compared to T_6, T_2, T_4 and T_9, respectively.

Table 22. Bio-chemical parameters in apple fruit samples affected by organic Zn and bio-organic fertilizers

Treatment	TSS (°B)	Titratable acidity (%)	Reducing sugars (%)	Non-reducing sugars (%)	Total sugars (%)	Ascorbic acid (mg/100g)	Anthocyanins content (mg/100g)	Total phenols (mg/100g)
T_1	12.39	0.50	6.58	3.63	10.40	3.17	9.69	60.53
T_2	12.95	0.46	7.00	3.76	10.95	3.65	10.17	66.88
T_3	12.14	0.52	6.39	3.61	10.18	3.02	9.57	58.75
T_4	12.56	0.49	6.68	3.77	10.65	3.30	9.87	63.71
T_5	13.16	0.43	7.16	3.81	11.17	3.74	10.38	70.67
T_6	13.31	0.41	7.37	3.81	11.37	3.95	10.70	74.36
T_7	13.66	0.38	7.61	4.04	11.86	4.32	11.06	77.20
T_8	14.02	0.36	7.78	4.02	12.02	4.65	11.37	78.20
T_9	11.80	0.54	6.21	3.44	9.83	2.65	9.37	54.40
$CD_{0.05}$	0.32	NS	0.19	0.27	0.31	0.24	0.30	1.58

TSS, Total soluble solids; NS, non-significant; T_1: Zn-Gly$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$ + HA$_{100}$; T_2: Zn-Gly$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$ + HA$_{120}$; T_3: Zn-Glu$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$ + HA$_{100}$; T_4: Zn-Glu$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$ + HA$_{120}$; T_5: Zn-AAC$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$ + HA$_{100}$; T_6: Zn-AAC$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$ + HA$_{120}$; T_7: Nano Zn-AAC$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$; T_8: Nano Zn-AAC$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$; T_9: Inorganic N:P:K (105:53:105 g/plant) + $ZnSO_4$ @0.5%

4.2.3.2.7 Anthocyanins content

The data presented in Table 22 showed notable variations in anthocyanin content through application of different Zn nutrient sources and bio-organics combinations. The conferred data revealed that the treatment T_8 recorded maximum anthocyanin (11.37 mg/100g) content, which was statistically at par with treatment T_7 (11.06 mg/100g). However, minimum anthocyanin (9.37 mg/100g) content was recorded in the treatment T_9. Among other treatments, the treatments T_4, T_1 and T_3 were also statistically equivalent to each other with corresponding values of 9.87, 9.69 and 9.57 mg/100g, respectively. The superior treatment also demonstrated a 6.2, 11.7, 15.2 and 21.3 per cent increase compared to T_6, T_2, T_4 and T_9, respectively.

4.2.3.2.8 Total phenols

Total phenols of apple fruit samples ranged from 54.40 to 78.20 mg/100g. Maximum total phenols content (78.20 mg/100g) was registered in T_8 which was statistically similar to T_7 (77.20 mg/100g). The lowest values (54.40 mg/100g) however, were recorded in T_9 treatment. Moreover, statistically non-significant results were recorded among all the other treatments utilizing Zn and bio-organic nutrient sources. Among different organic Zn and bio-organics treatments combinations, total phenols content of fruit samples was followed in the order of $T_8 > T_7 > T_6 > T_5 > T_2 > T_4$.

In the present investigation, the biochemical traits of fruit samples were positively affected by Zn amino acid complexes and bio-organic amendments. This might be explained by the increased photosynthetic activity and starch metabolism ascribed to foliar application of Zn (Hassan et al., 2020; Fan et al., 2021; Kandil et al., 2022). According to Al-Ali (2006) and Shalan (2020), the translocation of sugars from leaves to fruits after starch metabolism led to increased total soluble solids, total sugars and anthocyanin content. Further, a decrease in titratable acidity content of fruit samples was also recorded. It might also be implicated to the role of nano Zn amino acid chelates in accelerating the functioning of metabolic processes owing to their greater surface area (Subramanian et al., 2015; Qureshi et al., 2018). In addition, by slowing down the rate of dissolution, nano fertilizers improve the interaction and ensure steady release of nutrients at different growth stages of plants (Naderi and Danesh-Shahraki, 2013; El-Ghamry et al., 2018; Kumari and Singh, 2019). Moreover, the organic amino acid chelates facilitated immediate chelation and breakdown of nutrients within plant system (Areche et al., 2023) which might have resulted in improved bio-chemical quality characteristics of fruit samples.

In the study, the improvement in ascorbic acid content of fruit samples might be due the increased auxin biosynthesis through application of Zn fertilization (Nawaz et al., 2008). It was further augmented by the role of amino acids on enhanced Zn assimilation (Santi et al., 2017). Earlier literature documented the positive influence of amino acid chelates on increased TSS and ascorbic acid content in fruits (Keutgen and Pawelzik, 2008; Fahimi et al., 2016). Besides, the inoculation of *Azolla* enhanced the availability of macro- and micronutrient to plants, and thereby improved TSS, total sugars and ascorbic acid content (Taha and El-Shahat, 2017). Moreover, higher utilization of carbohydrates during fruit ripening has drawn from reserves in the roots and stems through inoculation of PGPR (Kumar et al., 2020). In addition, the positive effects of inoculation of AM fungi on the total sugars and ascorbic acid content of fruits have also been reported by Bona et al. (2017). AM fungi influenced the plant secondary metabolism which in turn led to enhanced production of anthocyanin content (Basu et al., 2018). Besides, AM fungi activated the host-defense response to increase the anthocyanin content (Lingua et al., 2013; Rouphael et al., 2015). Song et al. (2015) revealed that photosynthetic activity was directly related to phenol metabolism. Furthermore, the accompanying amino acids with nano Zn increased the total fruit phenol content (Tavallali et al., 2018). Increased fruit quality attributes are also linked to the higher translocation of photosynthates towards the sink through stimulation of P and K uptake induced by the application of humic acid (Mikkelsen, 2005).

4.2.4 SOIL PROPERTIES

4.2.4.1 CHEMICAL

The data collected to investigate the influence of conjoint application of organic Zn sources and bio-organics combinations on chemical properties of soils of apple has been summarized in Table 23, 24 and Fig. 21.

4.2.4.1.1 pH

Application of different Zn nutrient sources and bio-organics combinations recorded statistically non-significant results for soil pH during the course of study. Minimum soil pH (7.00) however, was recorded in treatment T_8, which was statistically equivalent to treatment T_7 (7.03). The treatments combinations of T_6, T_5 and T_2 also depicted analogous results with corresponding values of 7.14, 7.15 and 7.15, respectively (Table 23).

4.2.4.1.2 Electrical conductivity

Non-significant effect of different organic Zn and bio-organics combinations on the electrical conductivity (EC) of soil. Minimum EC values (0.20 dS/m) were registered in treatment T_8 followed by T_7, T_6, T_5, T_2 and T_4 with corresponding values of 0.21, 0.23, 0.24, 0.26 and 0.27

dS/m. However, maximum EC (0.30 dS/m) values were observed in T_9 treatment. The treatment combination of T_8 was statistically similar to T_7 (0.21 dS/m) and T_6 (0.23 dS/m).

4.2.4.1.3 Organic carbon

It is evident from the data that soil organic carbon content was significantly influenced when conjoint application of organic Zn sources and bio-organics in apple trees was supplemented. Maximum organic carbon content (2.77%) was recorded in treatment T_8, which was statistically similar to T_7 (2.74%) and T_6 (2.73%). However, minimum organic carbon (2.58%) content was exhibited by treatment T_9. Moreover, statistically equivalent results were also observed with treatments combinations of T_5, T_2, T_4, T_1 and T_3 with corresponding values of 2.68, 2.67, 2.64, 2.63 and 2.62 per cent. The treatment of T_8 also recorded an increment of 1.4, 3.7, 4.9 and 7.3 per cent when compared to T_6, T_2, T_4 and T_9, respectively.

4.2.4.1.4 Available nitrogen

Application of different Zn nutrient sources and bio-organics combinations reported a significant increase in available N content of soil during the course of investigation (Table 23). Available N content was maximum (432.40 kg/ha) in treatment T_8, which was statistically superior to all other applied treatment combinations. However, the lowest values were observed in T_9 (370.41 kg/ha). Moreover, statistically non-significant results were observed among all other applied treatments. Furthermore, an increment of 3.8, 10.4, 14.3 and 16.7 per cent was observed in T_8 compared to T_6, T_2, T_4 and T_9, respectively.

Table 23. Effect of conjoint application of organic Zn and bio-organic fertilizers on chemical properties of soil of apple

Treatment	pH	EC (dS/m)	OC (%)	Available N (kg/ha)	Available P (kg/ha)	Available K (kg/ha)
T_1	7.33	0.28	2.63	383.74	161.51	254.95
T_2	7.15	0.26	2.67	391.32	174.89	269.37
T_3	7.41	0.28	2.62	378.04	156.36	247.18
T_4	7.27	0.27	2.64	387.99	167.83	262.63
T_5	7.15	0.24	2.68	406.69	178.40	283.59
T_6	7.14	0.23	2.73	416.56	183.37	293.12
T_7	7.03	0.21	2.74	420.44	187.77	313.34
T_8	7.00	0.20	2.77	432.40	189.83	314.92
T_9	7.45	0.30	2.58	370.41	149.86	232.82
$CD_{0.05}$	NS	NS	0.07	3.60	1.15	4.18

EC, Electrical conductivity; OC, organic carbon; NS, non-significant; T_1: Zn-Gly_{200} + VC_{80} + AZ_{300} + AMF_{25} + $PGPR_{200}$ + HA_{100}; T_2: Zn-Gly_{400} + VC_{75} + AZ_{600} + AMF_{20} + $PGPR_{250}$ + HA_{120}; T_3: Zn-Glu_{200} + VC_{80} + AZ_{300} + AMF_{25} + $PGPR_{200}$ + HA_{100}; T_4: Zn-Glu_{400} + VC_{75} + AZ_{600} + AMF_{20} + $PGPR_{250}$ + HA_{120}; T_5: Zn-AAC_{200} + VC_{80} + AZ_{300} + AMF_{25} + $PGPR_{200}$ + HA_{100}; T_6: Zn-AAC_{400} + VC_{75} + AZ_{600} + AMF_{20} + $PGPR_{250}$ + HA_{120}; T_7: Nano Zn-AAC_{200} + VC_{80} + AZ_{300} + AMF_{25} + $PGPR_{200}$; T_8: Nano Zn-AAC_{400} + VC_{75} + AZ_{600} + AMF_{20} + $PGPR_{250}$; T_9: Inorganic N:P:K (105:53:105 g/plant) + $ZnSO_4$ @0.5%

4.2.4.1.5 Available phosphorus

Available P content of soil showed a positive effect through the conjoint application of organic Zn and bio-organics in apple trees. Treatment combination of T_8 recorded maximum available P content (189.83 kg/ha) which was significantly superior to all the other applied treatments. However, treatment of T_9 recorded the minimum (149.86 kg/ha). The order followed pertaining to available P content for different Zn nutrient sources and bio-organics combinations as $T_8 > T_7 > T_6 > T_5 > T_2 > T_4$.

4.2.4.1.6 Available potassium

A perusal of the data indicated the significant influence of conjoint application of organic Zn and bio-organics combinations on available K content of soil (Table 23). The treatment T_8 recorded the maximum available K content (314.92 kg/ha) which was statistically similar to treatment T_7 (313.34 kg/ha). However, the lowest value (232.82 kg/ha) was found in treatment T_9. The per cent increase of 7.4, 16.9, 19.9 and 35.2 was reported in treatment T_8 as compared to T_6, T_2, T_4 and T_9 treatments, respectively. Moreover, the available K content in soil through the application of different organic Zn and bio-organics combinations was followed in the order $T_8 > T_7 > T_6 > T_5 > T_2 > T_4$.

4.2.4.1.7 Exchangeable calcium

Perusal of the data revealed that conjoint application of different organic Zn and bio-organics combinations exerted a significant influence on exchangeable Ca content of soil (Table 24). Average exchangeable Ca content ranged between 1517.07 and 1578.70 mg/kg. Treatment T_8 exhibited the maximum exchangeable Ca content (1578.70 mg/kg) which was statistically superior to all other applied treatments. However, treatment T_9 exhibited the minimum value (1517.07 mg/kg). Among different combinations, the treatment of T_8 displayed 1.0 times increase over T_9.

4.2.4.1.8 Exchangeable magnesium

Soil exchangeable Mg content was significantly influenced when supplemented with conjoint application of organic Zn and bio-organics combinations in apple. The treatment combination of T_8 recorded the maximum exchangeable Mg content (589.33 mg/kg) which was statistically superior compared to all other treatments, whereas, it was lowest in T_9 (554.05 mg/kg). Statistically equivalent results were also observed among treatments combinations of T_4 and T_1 with corresponding values of 568.98 and 566.65 mg/kg. Furthermore, the results indicated 1.4, 2.6, 3.6 and 6.3 per cent increment in the treatment combination of T_8 as compared to T_6, T_2, T_4 and T_9 treatments, respectively.

Table 24. Comparative assessment of organic zinc and bio-organic fertilizers on meso-nutrients and micronutrient cations at 0-15 cm depth of apple rhizosphere

Treatment	Meso-nutrients (mg/kg)		DTPA extractable micronutrient cations (mg/kg)			
	Exchangeable Ca	Exchangeable Mg	Zn	Fe	Cu	Mn
T_1	1529.56	566.65	27.22	72.20	11.75	32.07
T_2	1544.74	574.23	29.48	72.74	12.70	33.89
T_3	1522.37	561.52	26.89	70.97	10.93	31.19
T_4	1539.79	568.98	27.50	72.54	12.19	32.62
T_5	1551.76	577.45	31.36	73.70	13.16	36.13
T_6	1556.53	581.03	34.74	74.62	14.23	36.59
T_7	1571.89	583.87	35.14	76.37	15.17	37.20
T_8	1578.70	589.33	35.82	78.48	15.84	38.66
T_9	1517.07	554.05	26.16	68.07	9.85	29.35
$CD_{0.05}$	2.18	2.80	0.95	2.30	0.38	1.02

T_1: Zn-Gly$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$ + HA$_{100}$; T_2: Zn-Gly$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$ + HA$_{120}$; T_3: Zn-Glu$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$ + HA$_{100}$; T_4: Zn-Glu$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$ + HA$_{120}$; T_5: Zn-AAC$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$ + HA$_{100}$; T_6: Zn-AAC$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$ + HA$_{120}$; T_7: Nano Zn-AAC$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$; T_8: Nano Zn-AAC$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$; T_9: Inorganic N:P:K (105:53:105 g/plant) + $ZnSO_4$ @0.5%

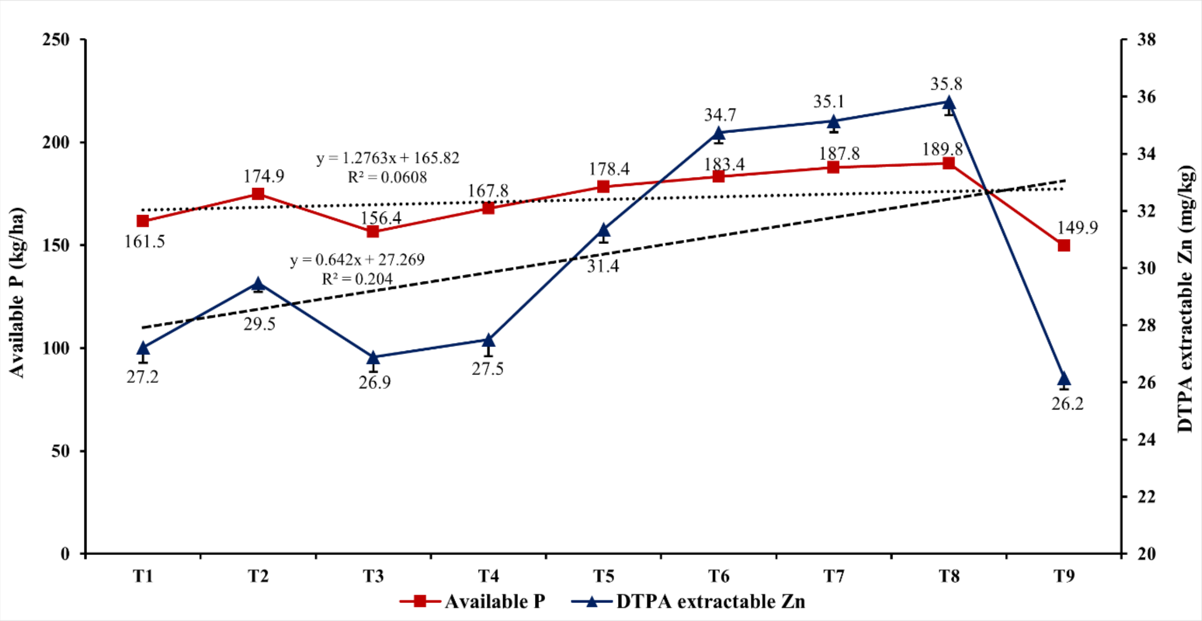


Fig. 22: Relationship between available P and DTPA extractable Zn in soils of apple under organic Zn and bio-organics nutrition

4.2.4.1.9 Zinc

A cursory glance at the data highlighted the significant impact of Zn organic sources and bio-organics on DTPA extractable Zn content which varied from 26.16 to 35.82 mg/kg (Table 24; Fig. 21). Highest DTPA extractable Zn content (35.82 mg/kg) was recorded in the treatment T_8, which was statistically at par with T_7 (35.14 mg/kg). The lowest (26.16 mg/kg) values however, were recorded in the treatment T_9. Among other treatments, the treatment combinations of T_4 and T_1 were observed statistically significant results with the corresponding values of 27.50 and 27.22 mg/kg, respectively. Moreover, the order followed on DTPA extractable Zn content of soil for different organic Zn and bio-organics combinations applied as $T_8 > T_7 > T_6 > T_5 > T_2 > T_4$.

4.2.4.1.10 Iron

Conjoint application of organic Zn and bio-organics resulted in a marked increase in the DTPA extractable Fe content of soil. Maximum DTPA extractable Fe content (78.48 mg/kg) was registered in treatment T_8 which was statistically at par with T_7 (76.37 mg/kg). The least values however, were recorded in T_9 (68.07 mg/kg). Among all treatments, the treatment combinations of T_8 exhibited the maximum per cent increase of 15.2 followed by T_7 (12.1%), T_6 (9.6%), T_5 (8.2%), T_2 (6.8%) and T_4 (6.5%) as compared to T_9.

4.2.4.1.11 Copper

DTPA extractable Cu content varied from 9.85 to 15.84 mg/kg. Highest DTPA extractable Cu content (15.84 mg/kg) was exhibited by T_8 followed by T_7, T_6, T_5, T_2 and T_4 with the corresponding values of 15.17, 14.23, 13.16, 12.70 and 12.19 mg/kg. However, the lowest value (9.85 mg/kg) was attained by treatment T_9. Treatment T_8 exhibited statistically superior results to all other applied treatments. DTPA extractable Cu content for conjoint organic Zn and bio-organics combinations was followed in the order $T_8 > T_7 > T_6 > T_5 > T_2 > T_4$.

4.2.4.1.12 Manganese

The data depicted the variation in DTPA extractable Mn content of soil subjected to different organic Zn and bio-organic sources (Table 24). DTPA extractable Mn content varied from 29.35 to 38.66 mg/kg. Treatment combination of T_8 observed the maximum DTPA extractable Mn content (38.66 mg/kg), however, T_9 recorded the minimum values (29.35 mg/kg). Moreover, the treatment combinations of T_4 and T_1 also observed statistically similar results with the corresponding values of 32.62 and 32.07 mg/kg. The order of DTPA extractable Mn content through conjoint organic Zn and bio-organic combinations as $T_8 > T_7 > T_6 > T_5 > T_2 > T_4$.

The use of Zn amino acid complexes and bio-organics significantly improved the chemical properties of soil. Areche et al. (2023) who suggested that application of Zn amino acid chelates improved chemical properties of soil due to increased microbial population in rhizosphere. The organic nature of amino acid complexes results in increased available nutrients along with reduced leaching losses (Dolev et al., 2020). Conversely, the chemical fertilizers are prone to fixation in soil and precipitation (Kumar et al., 2019) thus lowering availability of nutrients accessible to plants. Improvement of physico-chemical properties of soil through interaction between nanostructured chelates and organic minerals were also reported by Liu et al. (2006). Our results also confirmed the findings of Souri (2016) who reported increased available nutrient content through buffering effect of amino acid complexes. In another study, Wei-Hong et al. (2007) suggested improved macronutrient and micronutrient content of soil through higher dissolution and chelation of nutrients caused by the addition of amino acids complexes. Increased soil N content could also be due to slow-release mechanism and availability for longer period though nano-fertilization (Rajonee et al., 2016). These results further align with studies which indicated improved available N, P, K content in soil through Zn fertilization (Lonergan et al., 2009; Wu et al., 2011; Ghoneim, 2016). Using Zn amino acid chelates also significantly increased concentration of micronutrients in the soil (Habashy et al., 2006).

Furthermore, the integration of bio-organic nutrient sources enhanced the chemical properties of the soil. Due to microbial metabolism, the chemicals caused the production of organic acids, NH_4^+ ions and CO_2 which decreased pH of the soil (Sharma et al., 2011). El-Shahat (2007) found a correlation between the use of *Azolla* and a decrease in soil pH in rhizosphere. Vermicompost and *Azolla* are supplemental bio-organic sources that supply soil with organic matter (Sharifi et al., 2019; Abou El-Goud et al., 2021). Also, increased organic carbon content and accessibility to nutrients which resulted from the faster breakdown of organic matter due to quicker microbial activity due to organic Zn supplements (Babu and Sharma, 2005; Shashidhar et al., 2009). Taha and El-Shahat (2017) observed similar findings in apricot, and Taha et al. (2018) in apple using *Azolla* and humic acid application. According to Roy et al. (2016), *Azolla* is a rich source of N, P, K, Ca, Mg, Fe, Zn and Cu. By promoting a variety of chemical reactions and biological processes in soil. Besides, the inoculation with AM fungus improved P solubilization, which was reflected in the quantity of available P content in soil (Zhu et al., 2018). Furthermore, it aids in absorbing nutrients especially K, Ca, Mg, Zn, Cu, Fe and Mn (Garcia et al., 2016; Hashem et al., 2018). Likewise, the inoculation of PGPR indicates the capacity to accelerate the breakdown of organic matter, solubilize less-mobile nutrients and release nutrients in their available form (Kumar et al., 2020; Zhang et al., 2023). Additionally, production of organic acids in the plant rhizosphere by plants and bacteria led to increased nutrient acquisition in the soil (Sundara et al., 2002; Esitken et al., 2010).

4.2.4.2 MICROBIOLOGICAL

The data presented in Table 25, 26 and Fig. 22 depicted the influence of conjoint application of Zn nutrient and bio-organic sources on the microbiological properties of rhizosphere soils of apple.

4.2.4.2.1 Total bacterial count

The data illustrated a pronounced effect of conjoint application of organic Zn and bio-organic sources on total bacterial count in the rhizosphere of apple ($\log_{10}$ transformed). The treatment of T_8 demonstrated maximum total bacterial count (1.62×10^6 cfu/g) which was statistically higher compared to all other applied treatments. The lowest values (1.35×10^6 cfu/g) were, however, recorded in treatment T_9 (Plate 11). The superior treatment also experienced an increase of 2.5, 5.2, 7.3 and 20.0 per cent in total bacterial count in rhizosphere soils of apple over treatments T_6, T_2, T_4 and T_9, respectively.

Table 25. Effect of conjoint application of organic zinc and bio-organic fertilizers on soil microbial properties ($\log_{10}$ transformed) in apple rhizosphere at 0-15 cm depth

Treatment	Total bacterial count ($\times 10^6$ cfu/g)	Total soil fungi ($\times 10^3$ cfu/g)	Actinomycetes population ($\times 10^4$ cfu/g)
T_1	1.45 (28.37)	0.99 (9.69)	1.15 (14.06)
T_2	1.54 (34.41)	1.06 (11.38)	1.23 (16.89)
T_3	1.39 (24.70)	0.97 (9.33)	1.08 (11.94)
T_4	1.51 (32.31)	1.01 (10.35)	1.20 (16.00)
T_5	1.55 (35.85)	1.07 (11.74)	1.25 (17.83)
T_6	1.58 (38.13)	1.09 (12.22)	1.27 (18.83)
T_7	1.60 (39.50)	1.10 (12.55)	1.29 (19.68)
T_8	1.62 (41.80)	1.14 (13.70)	1.31 (20.32)
T_9	1.35 (22.23)	0.85 (7.10)	1.00 (10.00)

Figures in parentheses are actual values; cfu; colony forming units; T_1: Zn-Gly$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$ + HA$_{100}$; T_2: Zn-Gly$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$ + HA$_{120}$; T_3: Zn-Glu$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$ + HA$_{100}$; T_4: Zn-Glu$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$ + HA$_{120}$; T_5: Zn-AAC$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$ + HA$_{100}$; T_6: Zn-AAC$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$ + HA$_{120}$; T_7: Nano Zn-AAC$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$; T_8: Nano Zn-AAC$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$; T_9: Inorganic N:P:K (105:53:105 g/plant) + $ZnSO_4$ @0.5%

4.2.4.2.2 Soil fungi

Soil fungal population was positively affected by conjoint application of organic Zn and bio-organics supplements in rhizosphere soils of apple trees ($\log_{10}$ transformed). Maximum total soil fungal population (1.14×10^3 cfu/g) was recorded in T_8, which was statistically higher than all other applied treatments. The lowest value of 0.85×10^3 cfu/g, however, was observed in T_9 (Table 25; Plate 12). The treatment of T_8 also reported an increase of 4.6, 7.5, 12.8 and 34.1 per cent compared to T_6, T_2, T_4 and T_9 treatments, respectively.

4.2.4.2.3 Actinobacterial count

The results pertaining to actinobacterial count in rhizosphere of apple trees showed statistically significant difference among different organic Zn and bio-organic treatments and was

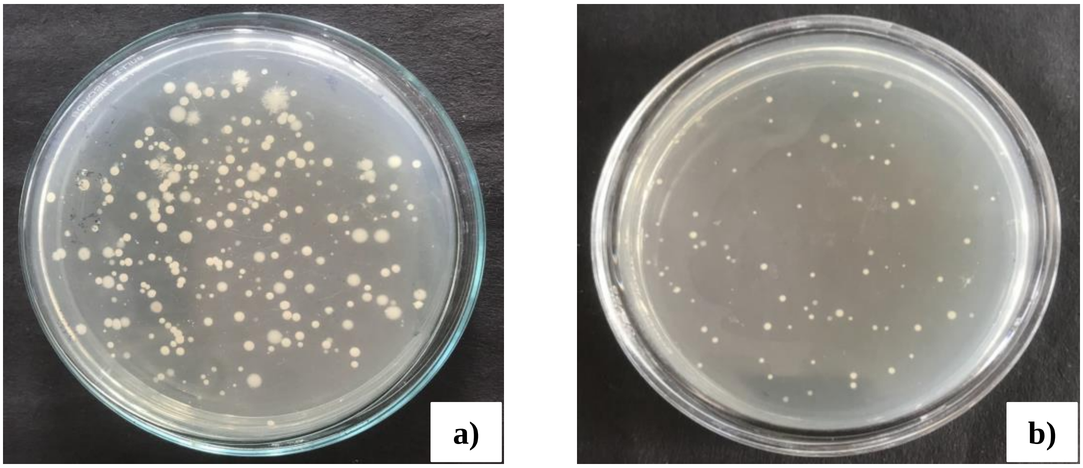


Plate 11: Total bacterial population (× 10^6 cfu/g) in rhizosphere of apple influenced by a) Nano Zn-AAC_{400} + VC_{75} + AZ_{600} + AMF_{20} + $PGPR_{250}$ b) Inorganic N:P:K (105:53:105 g/plant) + $ZnSO_4$ @0.5% (control)

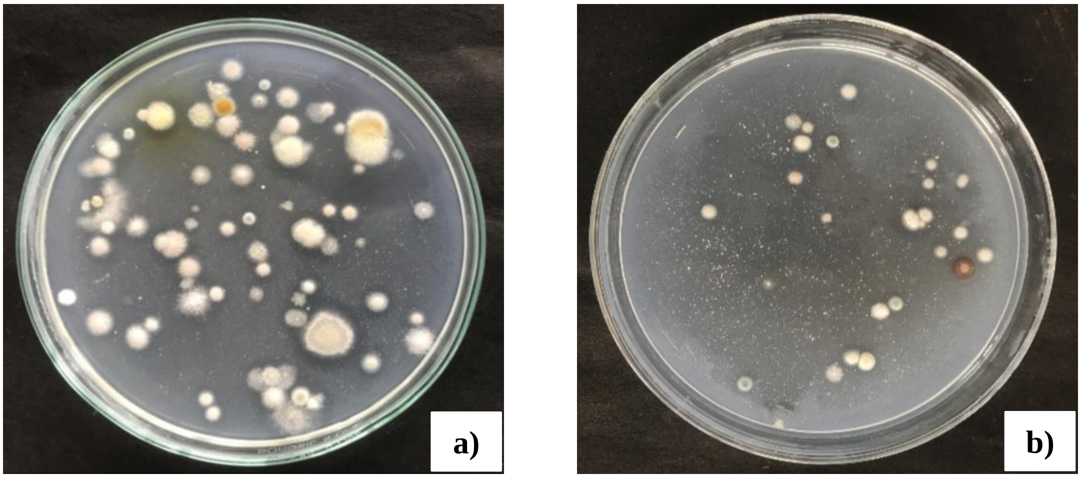


Plate 12: Soil fungal population (× 10^3 cfu/g) in rhizosphere of apple influenced by a) Nano Zn-AAC_{400} + VC_{75} + AZ_{600} + AMF_{20} + $PGPR_{250}$ b) Inorganic N:P:K (105:53:105 g/plant) + $ZnSO_4$ @0.5% (control)

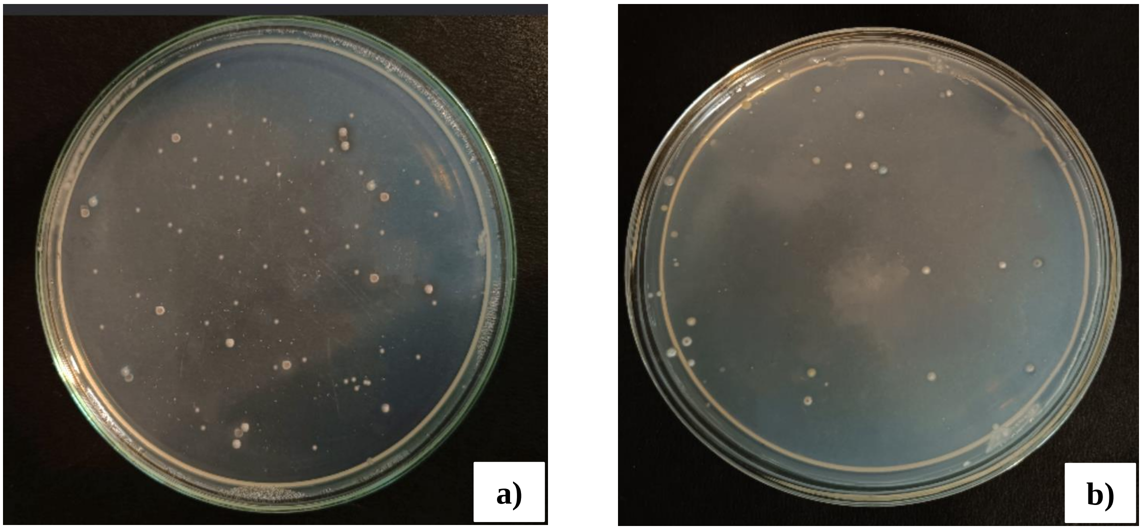


Plate 13: Actinobacterial count (× 10^4 cfu/g) in rhizosphere of apple influenced by a) Nano Zn-AAC_{400} + VC_{75} + AZ_{600} + AMF_{20} + $PGPR_{250}$ b) Inorganic N:P:K (105:53:105 g/plant) + $ZnSO_4$ @0.5% (control)

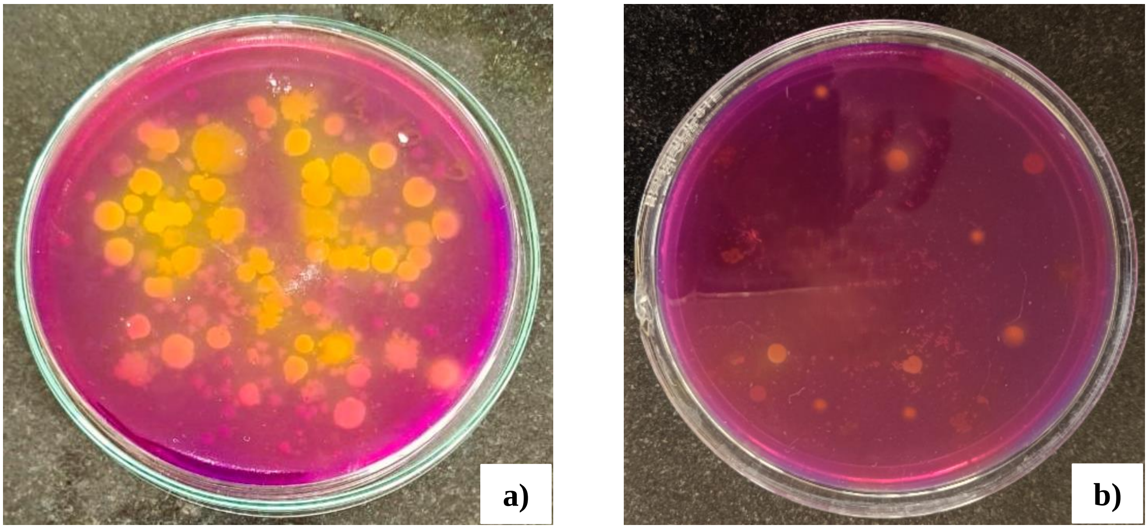


Plate 14: Phosphorus solubilizing bacteria (× 10^4 cfu/g) in rhizosphere of apple influenced by a) Nano Zn-AAC_{400} + VC_{75} + AZ_{600} + AMF_{20} + $PGPR_{250}$ b) Inorganic N:P:K (105:53:105 g/plant) + $ZnSO_4$ @0.5% (control)

ranged between 1.00 × 10^4 cfu/g and 1.31 × 10^4 cfu/g ($\log_{10}$ transformed). Actinobacterial count was recorded maximum (1.31 × 10^4 cfu/g) in treatment T_8. However, lowest value (1.00 × 10^4cfu/g) was exhibited by treatment T_9 (Plate 13). Furthermore, the results indicated 1.0, 1.1, 1.1 and 1.3 times increment in the superior treatment of T_8 as compared to T_6, T_2, T_4 and T_9 treatments, respectively.

4.2.4.2.4 Phosphorus solubilizing bacteria

Application of conjoint combinations of organic Zn and bio-organic supplements exhibited significant impact on population of phosphorus solubilizing bacteria (PSB) in rhizosphere soils of apple. PSB count varied from 0.84 × 10^4 cfu/g to 1.11 × 10^4 cfu/g ($\log_{10}$ transformed). Treatment T_8 recorded the highest PSB count (1.11 × 10^4 cfu/g) which was statistically superior to all other applied treatments. However, it was lowest (0.84 × 10^4 cfu/g) in treatment T_9 (Table 26; Plate 14). Moreover, the results also illustrated an increment of 3.7, 6.7, 11.0 and 32.1 per cent in T_8 compared to T_6, T_2, T_4 and T_9 treatments, respectively.

Table 26. Bacterial population in apple rhizosphere ($\log_{10}$ transformed) influenced by combined application of organic zinc and bio-organic fertilizers

Treatment	PSB (× 10^4 cfu/g)	KSB (× 10^5 cfu/g)	ZSB (× 10^5 cfu/g)	*Azotobacter* count (× 10^5 cfu/g)
T_1	0.99 (9.77)	0.92 (8.35)	0.92 (8.31)	1.25 (17.86)
T_2	1.04 (10.88)	0.96 (9.13)	0.95 (8.88)	1.28 (18.86)
T_3	0.96 (9.10)	0.91 (8.05)	0.90 (8.00)	1.21 (16.07)
T_4	1.00 (10.10)	0.94 (8.74)	0.93 (8.55)	1.26 (18.40)
T_5	1.05 (11.13)	0.98 (9.62)	0.99 (9.82)	1.33 (21.59)
T_6	1.07 (11.65)	1.00 (10.10)	1.02 (10.42)	1.35 (22.17)
T_7	1.09 (12.23)	1.02 (10.49)	1.06 (11.49)	1.38 (24.26)
T_8	1.11 (12.82)	1.04 (10.96)	1.10 (12.46)	1.42 (26.58)
T_9	0.84 (6.93)	0.85 (7.04)	0.78 (5.97)	1.12 (13.06)

Figures in parentheses are actual values; PSB, phosphorus solubilizing bacteria; KSB, potassium solubilizing bacteria; ZSB, zinc solubilizing bacteria; cfu; colony forming units; T_1: Zn-Gly_{200} + VC_{80} + AZ_{300} + AMF_{25} + $PGPR_{200}$ + HA_{100}; T_2: Zn-Gly_{400} + VC_{75} + AZ_{600} + AMF_{20} + $PGPR_{250}$ + HA_{120}; T_3: Zn-Glu_{200} + VC_{80} + AZ_{300} + AMF_{25} + $PGPR_{200}$ + HA_{100}; T_4: Zn-Glu_{400} + VC_{75} + AZ_{600} + AMF_{20} + $PGPR_{250}$ + HA_{120}; T_5: Zn-AAC_{200} + VC_{80} + AZ_{300} + AMF_{25} + $PGPR_{200}$ + HA_{100}; T_6: Zn-AAC_{400} + VC_{75} + AZ_{600} + AMF_{20} + $PGPR_{250}$ + HA_{120}; T_7: Nano Zn-AAC_{200} + VC_{80} + AZ_{300} + AMF_{25} + $PGPR_{200}$; T_8: Nano Zn-AAC_{400} + VC_{75} + AZ_{600} + AMF_{20} + $PGPR_{250}$; T_9: Inorganic N:P:K (105:53:105 g/plant) + $ZnSO_4$ @0.5%

4.2.4.2.5 Potassium solubilizing bacteria

The data on the influence of organic Zn and bio-organic treatments on the potassium solubilizing bacteria (KSB) count in apple rhizosphere soils is presented in Table 26 ($\log_{10}$ transformed). KSB count varied from 0.85 × 10^5 cfu/g to 1.04 × 10^5 cfu/g. Maximum KSB count (1.04 × 10^5 cfu/g) was recorded in treatment T_8, however, the minimum value 0.85 × 10^5 cfu/g was observed in treatment T_9. The results also reflected 1.0, 1.1, 1.1 and 1.2 times increase in KSB count through superior treatment of T_8 over T_6, T_2, T_4 and T_9 treatments, respectively.

4.2.4.2.6 Zinc solubilizing bacteria

Among different applied treatments, the population of zinc solubilizing bacteria (ZSB) varied from 0.78 × 10^5 cfu/g to 1.10× 10^5 cfu/g ($\log_{10}$ transformed). Minimum ZSB count (0.78 × 10^5 cfu/g) was recorded in treatment T_9, whereas, the treatment T_8 exhibited the maximum (1.10 × 10^5 cfu/g) count. Treatment T_8 also demonstrated a remarkable increase of 7.8, 15.8, 18.3 and 26.8 per cent compared to T_6, T_2, T_4 and T_9 treatments, respectively. The ZSB count for the various organic Zn and bio-organics combinations was followed in the order of $T_7 > T_8 > T_5 > T_6 > T_4 > T_2$ (Plate 15).

4.2.4.2.7 *Azotobacter* count

Conjoint application of organic Zn organic and bi-organic sources exerted a notable impact on the *Azotobacter* count of rhizosphere soils of apple trees (Table 26; Plate 16). Treatment T_8 exhibited the maximum *Azotobacter* count (1.42 × 10^5 cfu/g) followed by T_7, T_6, T_5, T_2 and T_4 with corresponding values of 1.38, 1.35, 1.33, 1.28 and 1.26 × 10^5 cfu/g, respectively. It was however, minimum (1.12 × 10^5 cfu/g) in the treatment T_9. Furthermore, the results further indicated an increase of 3.7, 6.7, 11.0 and 32.1 per cent in T_8 compared to T_6, T_2, T_4 and T_9 treatments, respectively.

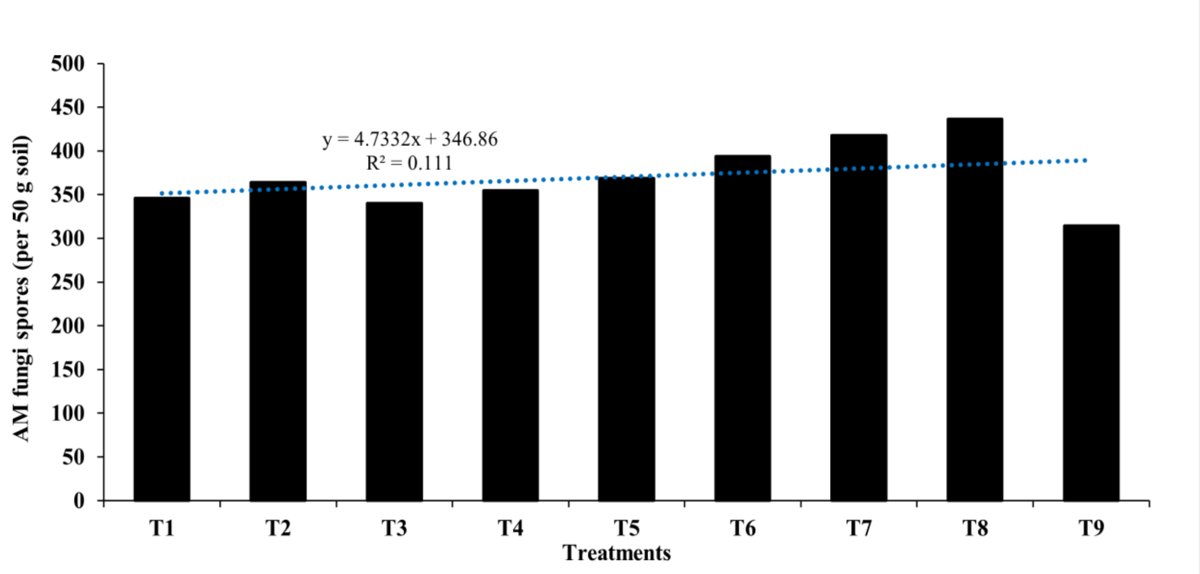


Fig. 23: AM fungi spore count in apple rhizosphere influenced by the application of organic Zn and bio-organic fertilizers

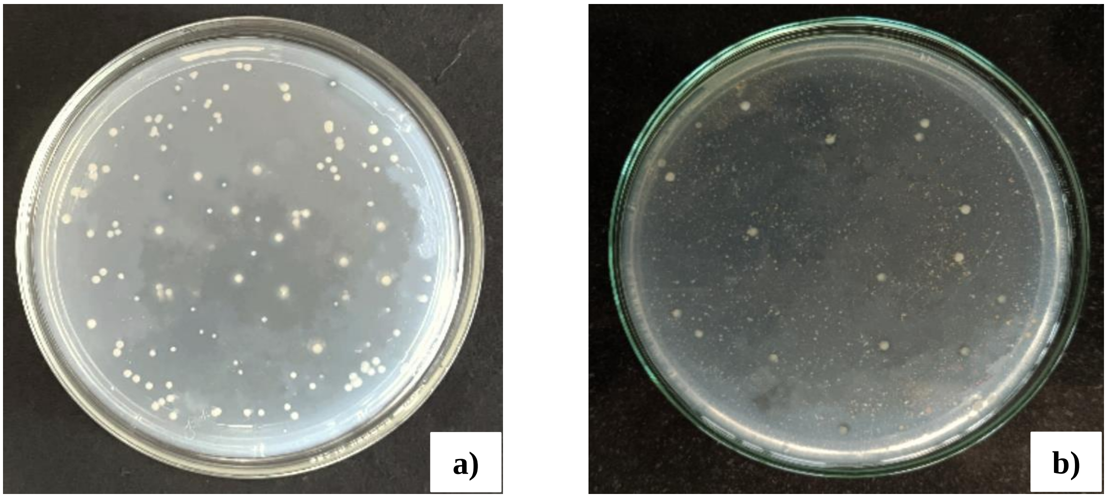


Plate 15: Zinc solubilizing bacteria (× 10^5 cfu/g) in rhizosphere of apple influenced by a) Nano Zn-AAC_{400} + VC_{75} + AZ_{600} + AMF_{20} + $PGPR_{250}$ b) Inorganic N:P:K (105:53:105 g/plant) + $ZnSO_4$ @0.5% (control)

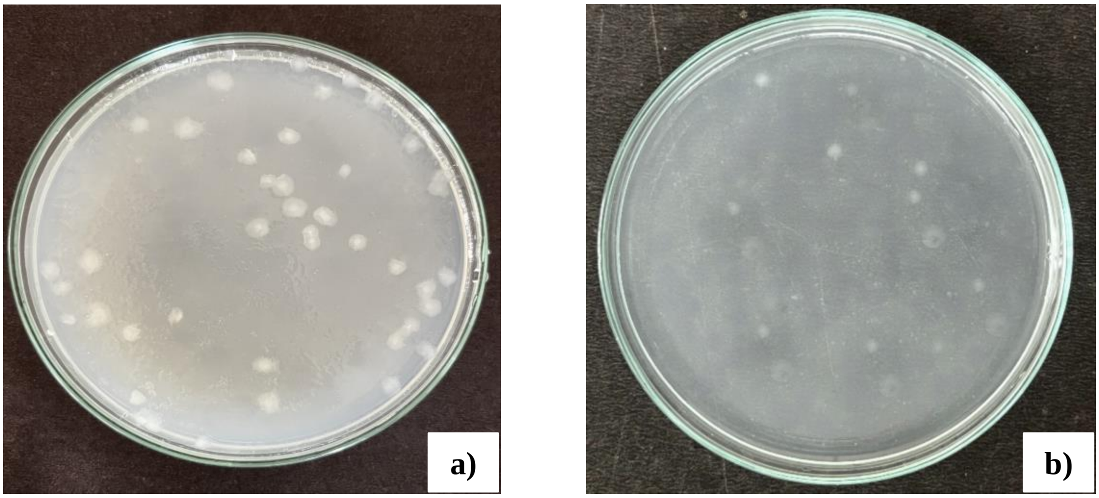
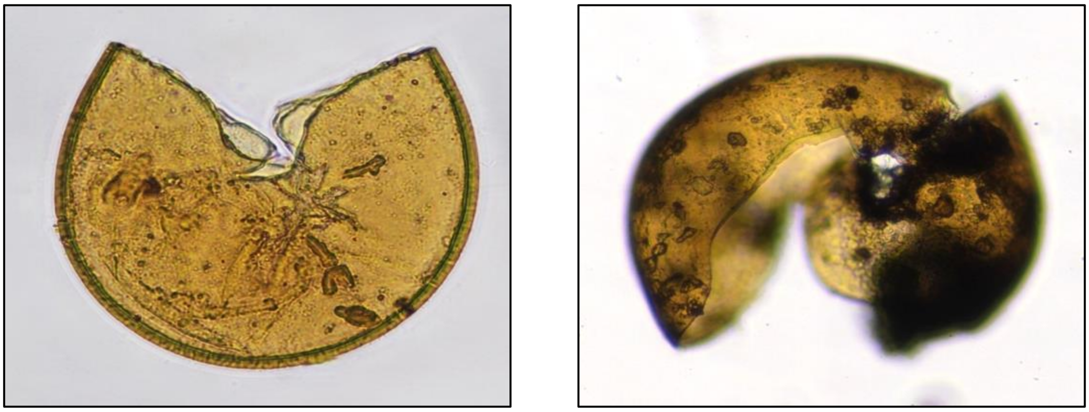
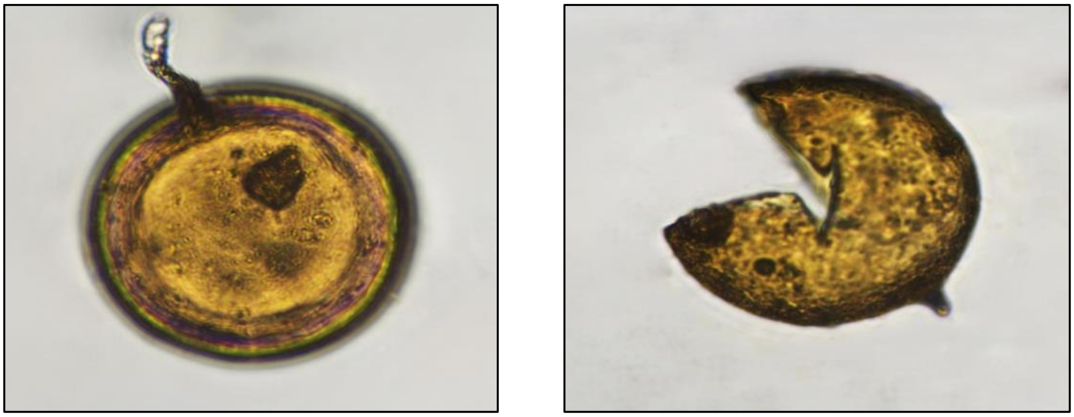


Plate 16: *Azotobacter* count (× 10^5 cfu/g) in rhizosphere of apple influenced by a) Nano Zn-AAC_{400} + VC_{75} + AZ_{600} + AMF_{20} + $PGPR_{250}$ b) Inorganic N:P:K (105:53:105 g/plant) + $ZnSO_4$ @0.5% (control)



Glomus caesaris **Sieverd. & Oehl** *Glomus fasciculatum* **Gerd. & Trappe**

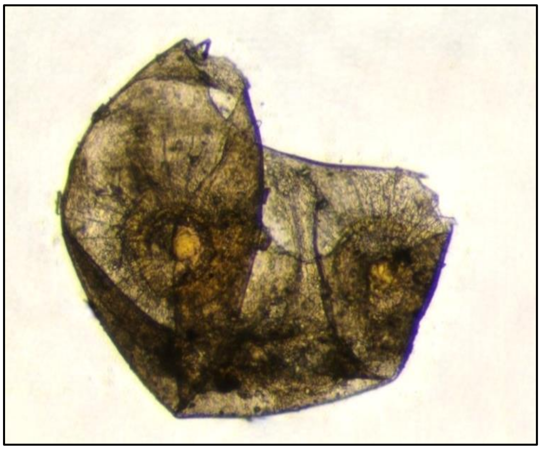


Glomus minutum **Tadych & Madej** *Glomus macrocarpum* **Tul. & C. Tul.**

Plate 17: Indigenous AMF species from rhizosphere soils of apple

Glomus versiforme **S.M. Berch**

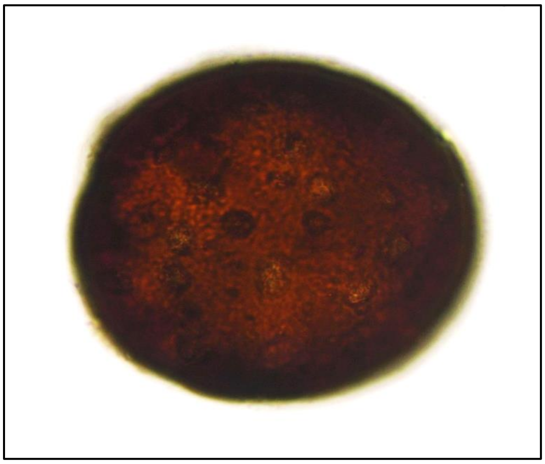
Glomus etunicatum **N. Becker & Gerd.**



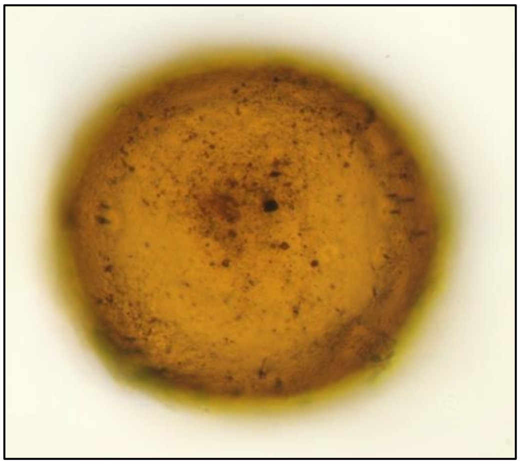
Glomus clarum **Nicol. & N.C. Schenck**

Gigaspora albida **N.C. Schenck & G.S. Sm.**

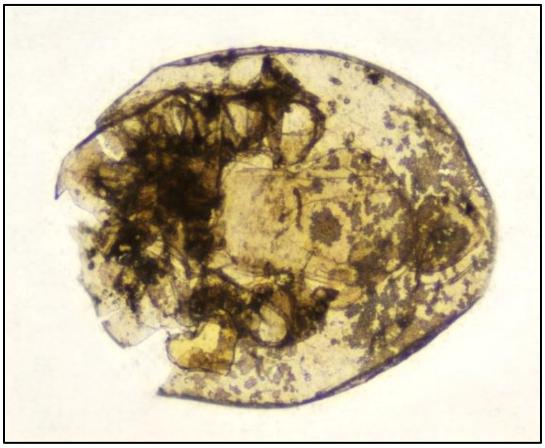
Plate 17: Indigenous AMF species from rhizosphere soils of apple



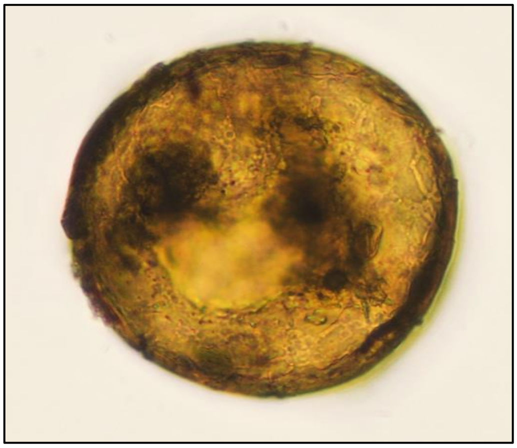
Gigaspora heterogamma **Nicol. & Gerd.**



Gigaspora margarita **W.N. Becker & I.R. Hall**

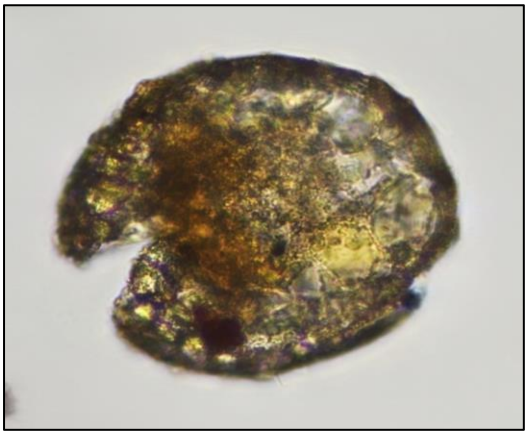


Gigaspora decipiens **I.R. Hall & L.K. Abbott**



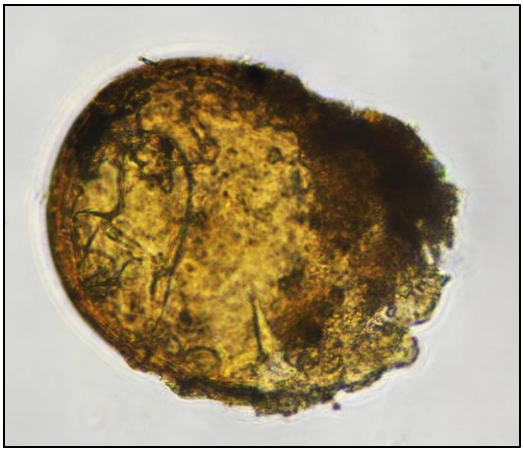
Acaulospora laevis **Gerd. & Trappe**

Plate 17: Indigenous AMF species from rhizosphere soils of apple



Acaulospora denticulate **Sieverd. & S. Toro**

Acaulospora foveata **Trappe & Janos**

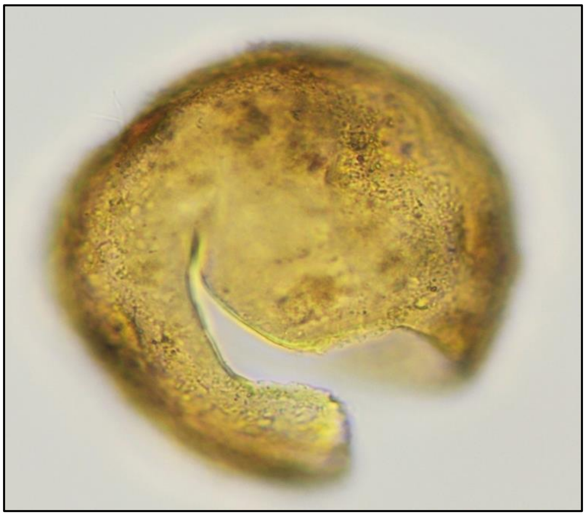


Acaulospora spinosa **C. Walker & Trappe**

Acaulospora brasiliensis
B.T. Goto, L.C. Maia & Oehl

Plate 17: Indigenous AMF species from rhizosphere soils of apple

Scutellospora sp. **C. Walker & F.E. Sanders**



Scutellospora pellucida **Nicol. & Schenck**

Plate 17: Indigenous AMF species from rhizosphere soils of apple

4.2.4.2.8 AM fungal population

Application of organic Zn and bio-organic sources recorded a significant increase in AM fungal (AMF) spore population in rhizosphere soils of apple (Fig. 22). Maximum AMF spore count (436.44 spores/50 g of soil) was recorded in treatment T_8, whereas, it was minimum (314.03 spores/50 g of soil) in treatment T_9. The order of organic Zn and bio-organic sources in stimulating AMF spore population was recorded in treatment combinations of $T_8 > T_7 > T_6 > T_5 > T_2 > T_4$. Moreover, an increment of 10.9, 19.9, 23.1 and 38.9 per cent on AMF spore population was also observed in T_8 as compared to T_6, T_2, T_4 and T_9, respectively.

During the course of study, fifteen AM fungi species were isolated from the experimental orchard and were identified (Plate 17). The isolated genera were comprised of *Glomus*, *Gigaspora*, *Acaulospora* and *Scutellospora*. Among the identified species, *Glomus* species was the most prevalent. *Glomus caesaris* (Sieverd. & Oehl), G. *fasciculatum* (Gerd. & Trappe), *G. minutum* (Tadych & Madej), *G. macrocarpum* (Tul. & C. Tul.), *G. etunicatum* (W.N. Becker & Gerd.) and *G. clarum* (Nicol. & N.C. Schenck) were the identified *Glomus* species. Apart from this, *Gigaspora* species namely, *Gigaspora albida* (N.C. Schenck & G.S. Sm.), *Gigaspora heterogamma* (Nicol. & Gerd.), *Gigaspora margarita* (W.N. Becker & I.R. Hall) and *Gigaspora decipiens* (I.R. Hall & L.K. Abbott) were also identified. The species of *Acaulospora* constituted *A. denticulate* (Sieverd. & S. Toro), *A. laevis* (Gerd. & Trappe), *A. foveata* (Trappe & Janos), *A. spinosa* (C. Walker & Trappe) and *A. brasiliensis* (B.T. Goto, L.C. Maia & Oehl). Besides, the lowest AM fungi spores identified belonged to *Scutellospora* sp. (C. Walker & F.E. Sanders) and *S. pellucida* (Nicol. & Schenck). Similar results have been reported by Kumar et al. (2006) in apple, Sharma et al. (2014a) in mango and Sharma et al. (2009) in citrus.

4.2.4.3 SOIL ENZYMES

4.2.4.3.1 Acid phosphatase

Acid phosphatase enzymatic activity was positively influenced by the application of organic Zn and bio-organics sources in rhizosphere soils of apple (Fig. 23). Treatment combination of T_8 registered the maximum acid phosphatase activity (175.76 µmole PNP/h/g soil) demonstrating statistically superior results over all other applied treatments. However, it was recorded minimum (124.49 µmole PNP/h/g soil) in treatment T_9. The results also reflected an increase of 7.1, 18.4, 22.6 and 41.1 per cent on acid phosphatase activity through superior treatment of T_8 compared to T_6, T_2, T_4 and T_9, respectively.

4.2.4.3.2 Alkaline phosphatase

Appraisal of the data unveiled that alkaline phosphatase activity in rhizosphere of apple ranged between 137.86 and 193.45 µmole PNP/h/g soil. Maximum alkaline phosphatase activity (193.45 µmole PNP/h/g soil) was recorded in treatment T_8, which was statistically superior compared to all other applied treatments. However, the treatment combination of T_9 reflected the lowest value (137.86 µmole PNP/h/g soil). The alkaline phosphatase activity for the various organic Zn and bio-organic combinations applied was followed in the order of $T_7 > T_8 > T_5 > T_6 > T_4 > T_2$.

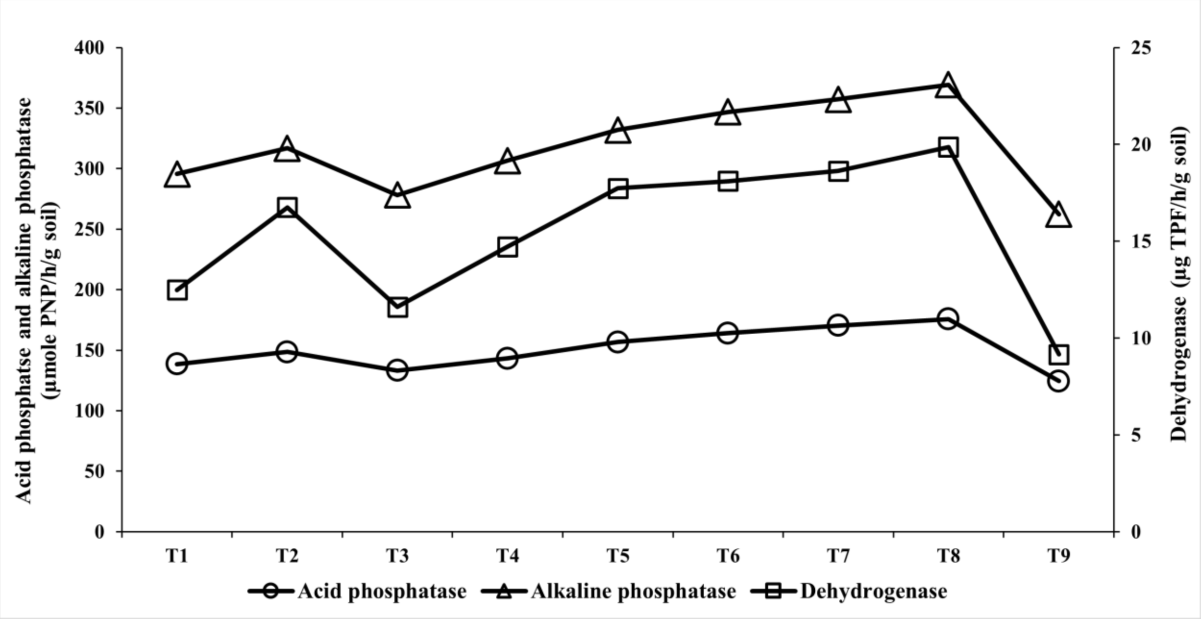


Fig. 24: Soil enzymatic activity in apple rhizosphere at depth of 0-15 cm in apple affected by organic Zn and bio-organic fertilizers

4.2.4.3.3 Dehydrogenase

There was significant variation in dehydrogenase activity in rhizosphere of apple subjected to different organic Zn sources and bio-organics combinations (Fig. 23). The values for soil dehydrogenase activity varied from 9.15 to 19.87 µg TPF/h/g soil. Treatment combination of T_8 recorded the maximum dehydrogenase (19.87 µg TPF/h/g soil) activity, however, the treatment combination of T_9 recorded the minimum (9.15 µg TPF/h/g soil). Among other treatment combinations, the treatments T_7, T_6 and T_5 were statistically equivalent to each other with corresponding values of 18.62, 18.10 and 17.73 µg TPF/h/g soil. The order followed pertaining to soil dehydrogenase activity for different conjoint combinations of organic Zn and bio-organic nutrient sources applied as $T_8 > T_7 > T_6 > T_5 > T_2 > T_4$.

Conjoint application of different organic and bio-organic Zn sources had a substantial impact on the microbiological and enzymatic properties of the soil when compared to the control. By providing soil microorganisms the ability to consume amino acids as a source of nutrition, amino acid chelates significantly increased microbial activity in rhizosphere (Souri, 2015). This finding is supported by previous studies, as compared to Zn sulphate, Zn amino acid chelates are primarily responsible for the improved nutrient availability due to increased microbial communities in soil (Raza et al., 2023). In addition, Zn amino acid complexes increased the diversity of fungi and bacteria by reducing the pH of the soil through the production of organic acids (Upadhayay et al., 2022). An explanation for this might be that increased zinc solubilization led to an increase in zinc availability for microbiological activity in soil (Srithaworn et al., 2023). In addition, the gradual release of nano Zn amino acid chelates is associated with the production of root exudates and humic acid, which increased the C: N ratio and might have boosted the soil microbial community (Nandy et al., 2023). Railya and Tarafdar (2013), Bala et al. (2019) and Khardia et al. (2022) previously described the positive effects of nano Zn complexes on microbial communities and enzymatic activity in soil. Application of nano Zn affected soil dehydrogenase activity through stimulating chemical processes in soil (Vijayalaxmi et al., 2013). Also, higher dehydrogenase activity has been associated to the catalytic and regulatory functions of nano Zn (Wyszkowska et al., 2013).

The results of the present study indicate an increased microbial population in rhizosphere through organic nutrient sources compared to chemical fertilizers, which aligns with the prior research of Zhan et al. (2018). This was explained by the fact that adding vermicompost improved pore density in soil resulting in more nutrients and water accessible to soil microbes (Manivannan et al., 2009). In addition, they further observed that using inorganic fertilisers reduced the porosity, bulk density and quantity of soil microbes. Also, vermicompost produces plant growth hormones as well as soil enzymes that promote microbial proliferation (Lim et al., 2015). Incorporation of *Azolla* and humic acid has been shown to increase the diversity of bacteria and soil dehydrogenase activity in apricot (Taha and El-Shahat, 2017) and apple (Taha et al., 2018). Microbial sources such as AM fungi and PGPR applied to the compost material stimulate the residential microbial activity through their multiplication (Boraste et al., 2009; Mahmud et al., 2021). In addition, the host plant relationship promotes the allocation of carbon (C) below soil when AM fungi occur, thereby boosting the number of soil microorganisms (Huang et al., 2020). Jabborova et al. (2021) reported that the inoculation of AM fungi led to a substantial increase in the quantity of AM fungal spores by 126 to 150 per cent. Furthermore, the inoculation of AM fungus was also reported to boost soil alkaline phosphatase (Peng et al.,

2020) and acid phosphatase (Ndoye et al., 2015). Kumar et al. (2020) also recorded due to enhanced degradation of metabolizable compounds in the plant rhizosphere. According to Kumar et al. (2020), the higher degradation of metabolizable substances is also linked to the increased acid, alkaline phosphatases and dehydrogenases enzyme activity in the rhizosphere (Fig. 24-26).

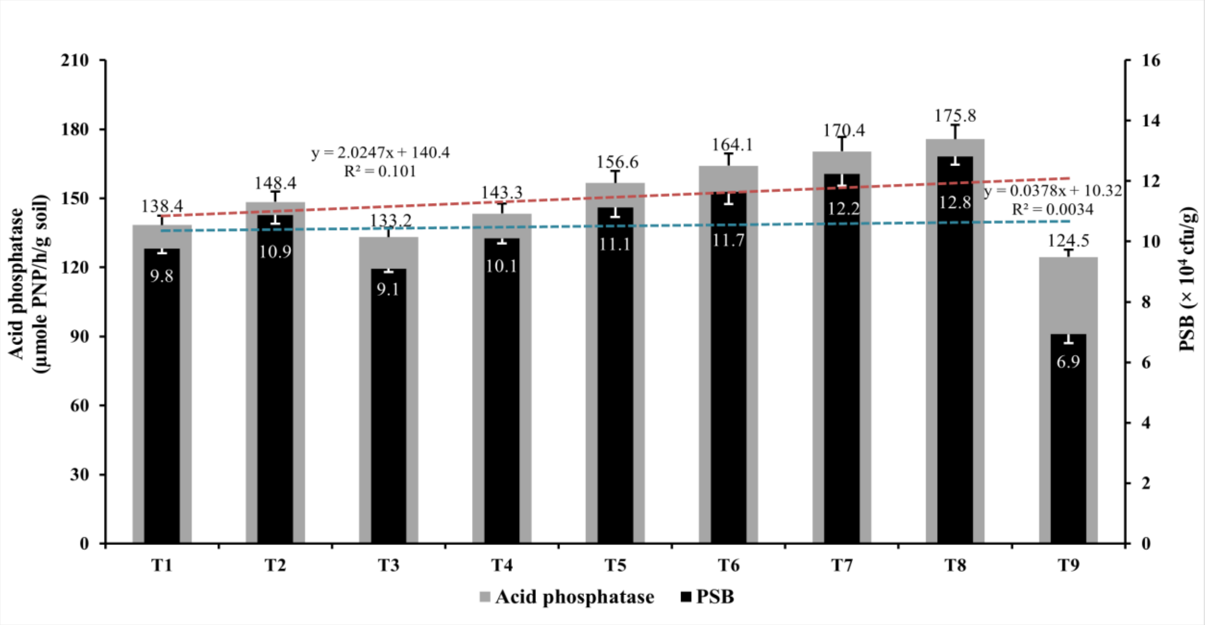


Fig. 25: Correlation between acid phosphatase and PSB in rhizosphere of apple under organic Zn and bio-organics nutrition

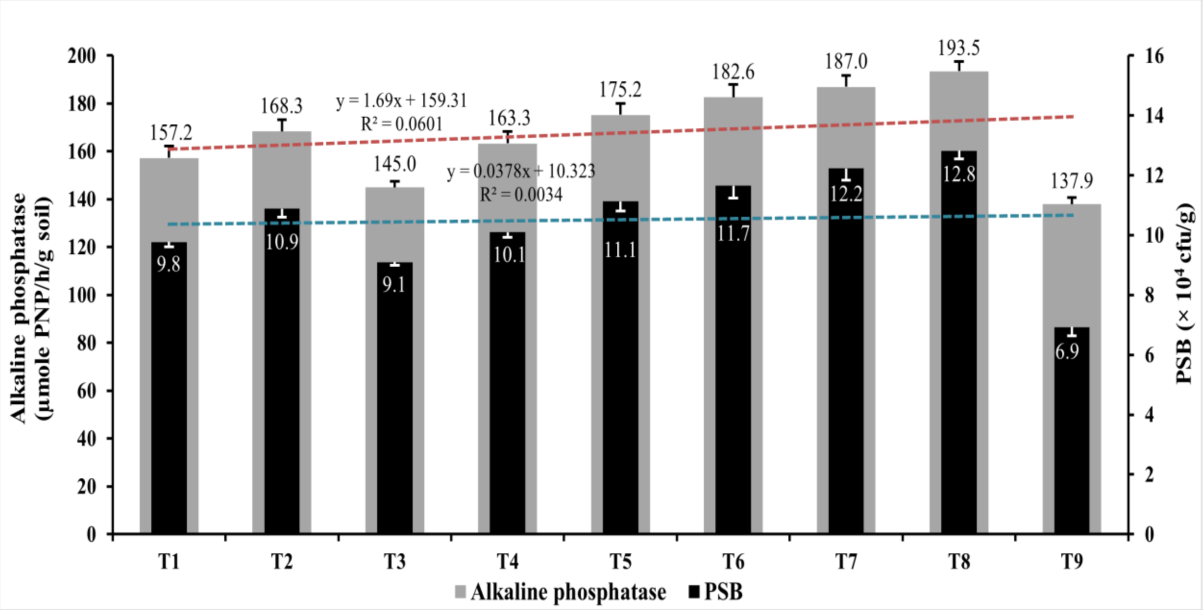


Fig. 26: Relationship studies on alkaline phosphatase and PSB in soils of apple under organic Zn and bio-organics fertilization

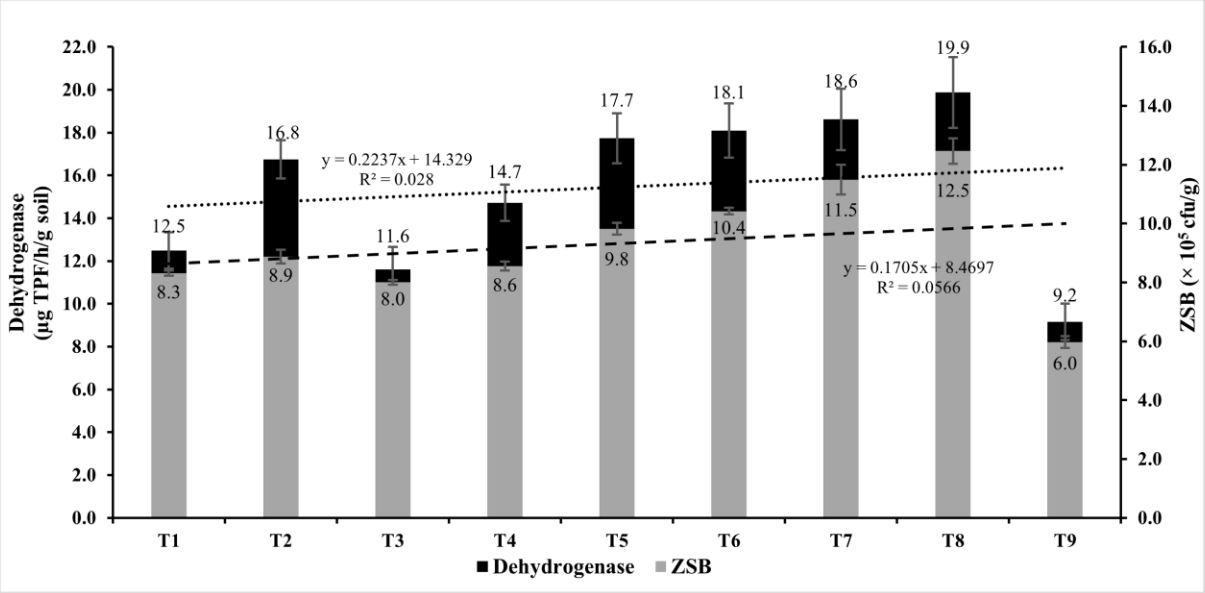


Fig. 27: Relationship between dehydrogenase and ZSB in rhizosphere of apple under organic Zn and bio-organics nutrition

4.2.5 PHYSIOLOGICAL PARAMETERS

The data on the impact of organic Zn and bio-organic sources on the physiological parameters of apple trees are illustrated in Table 27 and Fig. 27.

4.2.5.1 Total chlorophylls

A perusal of the data showed the positive impact of conjoint application of organic Zn and bio-organics sources on total leaf chlorophylls content of apple trees (Table 27). Average total leaf chlorophylls content ranged between 3.10 and 4.04 mg/g. The treatment combination of T_8 recorded maximum leaf chlorophylls content (4.04 mg/g) which was statistically higher compared to all other applied treatments. Minimum value of 3.10 mg/g however, was observed in treatment T_9. The result also depicted that treatment combinations of T_2 (3.42 mg/g) and T_4 (3.38 mg/g), T_1 (3.28 mg/g) and T_3 (3.22 mg/g) also showed statistically identical effects. During the cropping period, an increment of 11.2, 18.1, 19.5 and 30.3 per cent in leaf chlorophylls content was noticed in T_8 compared to T_6, T_2, T_4 and T_9 treatments, respectively.

4.2.5.2 Photosynthetic efficiency

The data presented in Table 27 showed notable variations in photosynthetic efficiency through conjoint application of different Zn nutrient and bio-organics sources in apple trees. The treatment combination of T_8 displayed the maximum photosynthetic efficiency (9.90 µmol CO_2 m^2/s), which was statistically superior to all other applied treatments. It was however, minimum (6.47 µmol CO_2 m^2/s) in the treatment T_9. Among other treatments, the treatments T_1 and T_3 were also statistically equivalent to each other with corresponding values of 7.18 and 7.01 µmol CO_2 m^2/s. The treatment combination of T_8 also demonstrated a 12.2, 25.6, 41.2 and 53.0 per cent increase compared to T_6, T_2, T_4 and T_9, respectively.

Table 27. Impact of conjoint application of organic zinc and bio-organic fertilizers on physiological parameters of apple

Treatment	Total chlorophylls (mg/g)	Photosynthetic efficiency (µmol CO_2 m^2/s)	Transpiration rate (mmol H_2O m^2/s)	Stomatal conductance (mmol H_2O m^2/s)
T_1	3.28	7.18	2.19	0.124
T_2	3.42	7.88	2.49	0.130
T_3	3.22	7.01	2.10	0.122
T_4	3.38	7.54	2.35	0.126
T_5	3.53	8.41	2.59	0.132
T_6	3.63	8.82	2.71	0.137
T_7	3.84	9.12	2.85	0.142
T_8	4.04	9.90	2.98	0.146
T_9	3.10	6.47	2.04	0.120
$CD_{0.05}$	0.09	0.18	0.07	NS

NS, Non-significant; T_1: Zn-Gly_{200} + VC_{80} + AZ_{300} + AMF_{25} + $PGPR_{200}$ + HA_{100}; T_2: Zn-Gly_{400} + VC_{75} + AZ_{600} + AMF_{20} + $PGPR_{250}$ + HA_{120}; T_3: Zn-Glu_{200} + VC_{80} + AZ_{300} + AMF_{25} + $PGPR_{200}$ + HA_{100}; T_4: Zn-Glu_{400} + VC_{75} + AZ_{600} + AMF_{20} + $PGPR_{250}$ + HA_{120}; T_5: Zn-AAC_{200} + VC_{80} + AZ_{300} + AMF_{25} + $PGPR_{200}$ + HA_{100}; T_6: Zn-AAC_{400} + VC_{75} + AZ_{600} + AMF_{20} + $PGPR_{250}$ + HA_{120}; T_7: Nano Zn-AAC_{200} + VC_{80} + AZ_{300} + AMF_{25} + $PGPR_{200}$; T_8: Nano Zn-AAC_{400} + VC_{75} + AZ_{600} + AMF_{20} + $PGPR_{250}$; T_9: Inorganic N:P:K (105:53:105 g/plant) + $ZnSO_4$ @0.5%

4.2.5.3 Transpiration rate

The data unveiled that transpiration rate of apple trees ranged from 2.04 to 2.98 mmol H_2O m^2/s. Maximum transpiration rate (2.98 mmol H_2O m^2/s) was registered in treatment T_8. The lowest values (2.04 mmol H_2O m^2/s) were, however, observed in the treatment T_9 which also exhibited statistically identical effects with T_3 (2.10 mmol H_2O m^2/s). Moreover, statistically non-significant results were also recorded among all the other treatments utilizing organic Zn and bio-organic nutrient sources. In apple trees, transpiration rate for various organic Zn nutrient and bio-organic treatments was followed in the order of $T_8 > T_7 > T_6 > T_5 > T_2 > T_4$.

4.2.5.4 Stomatal conductance

Application of organic Zn and bio-organic sources recorded non-significant results for stomatal conductance during the course of study. Maximum stomatal conductance (0.146 mmol H_2O m^2/s) was recorded in treatment T_8, however, it was observed minimum (0.120 mmol H_2O m^2/s) in treatment T_9. The treatment combinations of T_1 and T_3 also depicted analogous results with the corresponding values of 0.124 and 0.122 mmol H_2O m^2/s (Table 27). Moreover, an increment of 1.2 time of stomatal conductance in trees was also observed in T_8 as compared to T_9 treatment.

4.2.5.5 Total carbohydrates content

4.2.5.5.1 Fruiting shoots

Data analysis revealed a beneficial effect of Zn nutrient and bio-organics sources on total carbohydrate content of apple trees (Fig. 27). In fruiting shoots, maximum carbohydrate content (76.05 mg/g dry weight) was registered in treatment combination of T_8 followed by T_7, T_6, T_5, T_2 and T_4 with corresponding values of 74.27, 72.02, 69.18, 67.51 and 65.25 mg/g dry weight. However, minimum (61.19 mg/g dry weight) values were observed in treatment T_9. The treatment also showed statistically similar results with T_7 (74.27 mg/g dry weight). The order followed pertaining to total carbohydrate content of fruiting shoots of trees for the different organic Zn and bio-organic nutrient sources applied as $T_8 > T_7 > T_6 > T_5 > T_2 > T_4$.

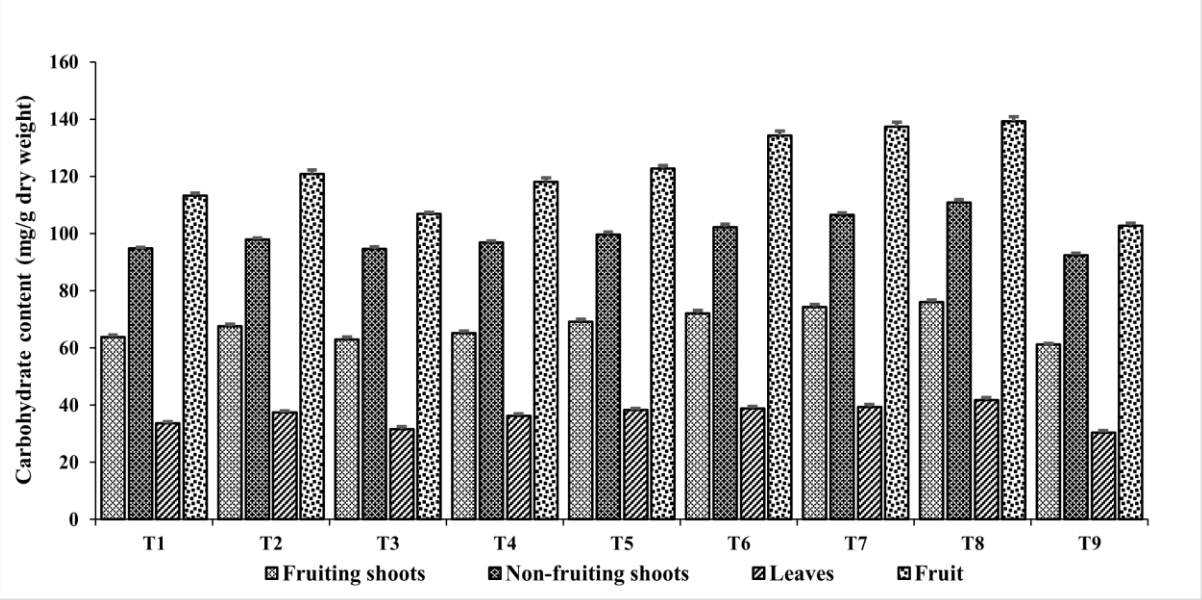


Fig. 28: Comparative assessment of organic Zn and bio-organic fertilizers application on total carbohydrate content of apple

4.2.5.5.2 Non-fruiting shoots

It is evident from the data that carbohydrate content of non-fruiting shoots was significantly influenced when conjoint application of organic Zn sources and bio-organics was supplemented in apple trees. Maximum carbohydrate content of non-fruiting shoots (110.78 mg/g dry weight) was recorded in the treatment T_8 which was statistically superior to all other applied treatment combinations. However, it was minimum (92.39 mg/g dry weight) in treatment T_9. Moreover, statistically equivalent results were also observed with treatment combinations of T_1 and T_3 with respective values of 94.78 and 94.56 mg/g dry weight. The treatment combination of T_8 also recorded an increment of 8.4, 13.0, 14.2 and 19.9 per cent compared to T_6, T_2, T_4 and T_9, respectively.

4.2.5.5.3 Leaves

Application of organic Zn and bio-organics supplements reported a significant increase on total carbohydrate content of leaves in apple (Fig. 27). Maximum total carbohydrate content of leaves (41.83 mg/g dry weight) was registered in treatment T_8 which was statistically superior to all other applied treatment combinations. However, the lowest values were observed in treatment T_9 (30.46 mg/g dry weight). Moreover, statistically uniform results were observed among treatment combination of T_6 and T_5 with the corresponding values of 38.80 and 38.24 mg/g dry weight. Furthermore, the per cent increment of 7.8, 11.8, 15.5 and 37.3 was observed in treatment T_8 compared to T_6, T_2, T_4 and T_9, respectively.

4.2.5.5.4 Fruit

The data pertaining to carbohydrate content of apple fruits showed a positive effect through the conjoint application of organic Zn and bio-organics sources (Fig. 27 and 28). The treatment combination of T_8 recorded maximum carbohydrate content of fruit samples (139.26 mg/g dry weight) which was statistically at par with treatment T_7 (137.29 mg/g dry weight). However, treatment of T_9 recorded the minimum (102.78 mg/g dry weight). Furthermore, the order followed pertaining to total carbohydrate content of fruits when supplemented with conjoint organic Zn and bio-organic nutrient sources as $T_8 > T_7 > T_6 > T_5 > T_2 > T_4$. The results also showed 1.0, 1.1, 1.2 and 1.4 times increase in total carbohydrate content of fruit samples through treatment combination of T_8 over T_6, T_2, T_4 and T_9, respectively.

The application of organic Zn complexes in combination with bio-organic fertilizers showed a substantial impact on physiological indicators of apple trees. This can be explained by the direct and quick uptake of amino acids chelates by the plants, which additionally facilitated the improvement of physiological processes (Nasholm et al., 2009). Also, higher leaf chlorophylls content was attained through increasing synthesis chlorophyll ascribed due to application of amino acid chelates and simultaneous reduction of chlorophyll degradation (Souri et al., 2017; Kaluzewicz et al., 2018; Noroozlo et al., 2019a; Souri and Hatamian, 2019). Zn also involved in the activation of chlorophyll pigments through protein synthesis (Mirbolook et al., 2020) and by catalyzing the carbonic anhydrase enzyme activity (Kamiab and Zamanibahramabadi, 2016).

In particular, the total chlorophyll content was increased (Yousuf and Abbass, 2023) due to the easier penetration of nanoparticles through the casparian strip into the cortical cells (Larue et al., 2012). Zeid (2009) and Amin et al. (2011) reported that the application of amino chelates stimulates photosynthetic activity through increased chlorophyll content of plants. In addition, the findings of this study are corroborated by the regulatory action of Zn in photosynthetic activity and carbohydrate metabolism (Rehman et al., 2018). According to Yeboah et al. (2017), the canopy area was positively affected by nano Zn amino acid chelate, which in turn improved the tree microclimate and photosynthetic activity. Simultaneously, the positive impact on anthocyanin content of fruit samples also improved photosynthetic efficiency through the protection of photosynthetic system against reactive oxygen (Xu and Rothstein, 2018; Chattha et al., 2022). Furthermore, the improvement in photosynthetic activity, transpiration rate and stomatal conductance attributed to Zn amino acid chelate application aligned with the earlier conducted research by Pradhan et al. (2014). Zn also promoted translocation of assimilates through increased leaf area and CO_2 metabolism (Dawood, 2000). This translocation further resulted in the deposition of assimilates in the sink (leaves, fruits and shoots), thereby increasing total carbohydrate content. The competition for sink, however, depicted a major impact on the further distribution of carbohydrates (Litton et al., 2007). Moreover, the process was facilitated by the easier transfer of assimilates from phloem to sink mediated by amino acid chelates (Ge et al., 2009; Fahimi et al., 2016).

Lakhdar et al. (2009) proposed that vermicompost might improve the photosynthetic system's resilience. This was due to vermicompost’s high porosity and water-holding capacity which enhanced the CO_2 supply required for photosynthesis and inhibition stomatal closure (Arancon et al., 2004). Furthermore, researchers found a correlation between the higher water availability and an increase in transpiration rate (Oliva et al., 2008). *Azolla* and humic acid application also showed higher total chlorophylls content because of higher absorption of nutrients (Taha and El-Shahat, 2017). This is in line with previous studies of Abd El-Razek et al. (2012) who found a positive association between humic acid and the total chlorophyll content of peaches. However, the chlorophyll content and photosynthetic efficiency were also enhanced by the symbiotic relationship between AM fungi and plant roots (Hajiboland et al., 2010; Abdel Latef and Chaoxing, 2014; Elhindi et al., 2017). The symbiotic interaction led to an increase in water status which in turn enhanced photosynthesis in plants (Chen et al., 2017). According to Kapoor et al. (2008) and Chandrasekaran et al. (2019), the extensive hyphal network of AM fungi strengthened the hydraulic conductivity which in turn improved transpiration and stomatal conductance. Likewise, the inoculation of PGPR led to a higher amount of chlorophyll content due to the activation of enzymes that helped to maintain photosynthetic pigments and improved performance of electron transporters associated with photosynthetic pathways (Pinnola et al., 2016; Enebe and Babalola, 2018).

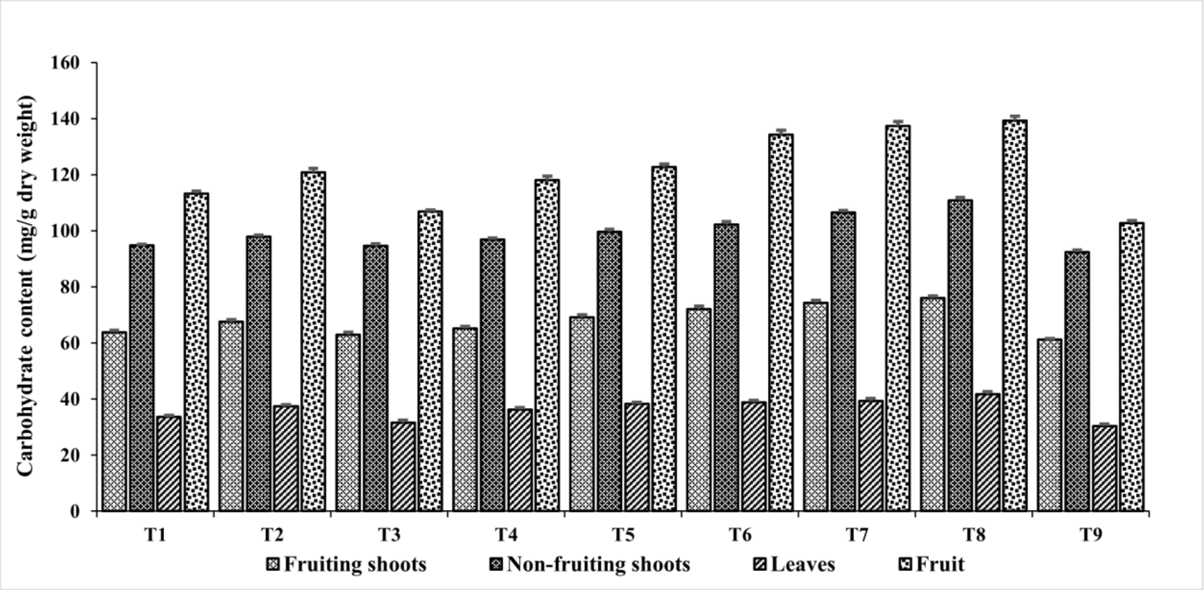


Fig. 29: Relationship between fruit carbohydrate and fruit Zn content in apple under organic Zn and bio-organics nutrition

4.2.6 LEAF NUTRIENTS

4.2.6.1 Nitrogen (N)

A perusal of the data indicated the positive influence of conjoint application of soil organic Zn and bio-organics supplements on leaf N content of apple trees (Table 28). The treatment combination of T_8 observed maximum leaf N content (2.42%) which was statistically similar to T_7 (2.40%) and T_6 (2.35%). However, the minimum leaf N (2.08%) content was found in treatment T_9. The per cent increase of 2.9, 7.1, 9.0 and 16.3 was also recorded T_8 as compared to T_6, T_2, T_4 and T_9 treatments, respectively. Moreover, the leaf N content for various organic Zn and bio-organic combinations was followed in the order of $T_8 > T_7 > T_6 > T_5 > T_2 > T_4$.

4.2.6.2 Phosphorus (P)

Analysis of data of Table 28 revealed that conjoint application of organic Zn and bio-organics exerted a non-significant influence on leaf P content of apple. The average values for leaf P content however, ranged between 0.17 and 0.28 per cent. Treatment T_8 exhibited maximum leaf P (0.28%) content and was statistically uniform with treatment T_7 (0.27%). However, treatment T_9 exhibited minimum leaf P (0.17%) content. In addition, treatment T_8 displayed significant increase over T_6 (1.1), T_2 and T_4 (1.3 each) and T_9 (1.6) times.

4.2.6.3 Potassium (K)

Leaf K content was positively influenced when supplemented with conjoint application of soil organic Zn and bio-organics supplements. Treatment T_8 recorded the highest leaf K content (1.78%) which was statistically similar to T_7 (1.75%) and T_6 (1.73%). However, it was lowest in T_9 (1.50%), which was also statistically at par with treatment T_3 (1.54%). Furthermore, the results indicated 2.8, 6.5, 10.5 and 18.6 per cent increment in the treatment combination of T_8 as compared to T_6, T_2, T_4 and T_9 treatments, respectively.

Table 28. Effect of conjoint application of organic zinc and bio-organic fertilizers on leaf nutrients content of apple

Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Zn (mg/kg)
T_1	2.16	0.20	1.57	1.68	0.30	60.27
T_2	2.26	0.22	1.67	1.75	0.33	68.80
T_3	2.14	0.18	1.54	1.64	0.28	54.55
T_4	2.22	0.22	1.61	1.72	0.31	64.71
T_5	2.31	0.23	1.69	1.80	0.33	75.12
T_6	2.35	0.25	1.73	1.84	0.34	80.91
T_7	2.40	0.27	1.75	1.87	0.36	86.03
T_8	2.42	0.28	1.78	1.90	0.37	91.38
T_9	2.08	0.17	1.50	1.62	0.27	47.67
$CD_{0.05}$	NS	NS	NS	NS	NS	1.58

NS, Non-significant; T_1: Zn-Gly_{200} + VC_{80} + AZ_{300} + AMF_{25} + $PGPR_{200}$ + HA_{100}; T_2: Zn-Gly_{400} + VC_{75} + AZ_{600} + AMF_{20} + $PGPR_{250}$ + HA_{120}; T_3: Zn-Glu_{200} + VC_{80} + AZ_{300} + AMF_{25} + $PGPR_{200}$ + HA_{100}; T_4: Zn-Glu_{400} + VC_{75} + AZ_{600} + AMF_{20} + $PGPR_{250}$ + HA_{120}; T_5: Zn-AAC_{200} + VC_{80} + AZ_{300} + AMF_{25} + $PGPR_{200}$ + HA_{100}; T_6: Zn-AAC_{400} + VC_{75} + AZ_{600} + AMF_{20} + $PGPR_{250}$ + HA_{120}; T_7: Nano Zn-AAC_{200} + VC_{80} + AZ_{300} + AMF_{25} + $PGPR_{200}$; T_8: Nano Zn-AAC_{400} + VC_{75} + AZ_{600} + AMF_{20} + $PGPR_{250}$; T_9: Inorganic N:P:K (105:53:105 g/plant) + $ZnSO_4$ @0.5%

4.2.6.4 Calcium (Ca)

A cursory glance at the data highlighted the insignificant impact of Zn organic and bio-organics sources on leaf Ca content which varied from 1.62 to 1.90 per cent. Highest leaf Ca content (1.90%) was recorded in treatment T_8, which was statistically similar to T_7 (1.87%). The lowest value of 1.62 per cent however, was observed in the treatment T_9. Moreover, the order followed related to leaf Ca content for the conjoint application of organic Zn and bio-organics supplements as $T_8 > T_7 > T_6 > T_5 > T_2 > T_4$.

4.2.6.5 Magnesium (Mg)

Combined application of organic Zn and bio-organics combinations resulted in a marked increase in leaf Mg content of apple (Table 28). Maximum leaf Mg content (0.37%) was exhibited in the treatment T_8 which was statistically at par with treatment T_7 (0.36%). The lowest value however, was recorded in T_9 (0.27%). Among all treatments, T_8 exhibited maximum per cent increase of 37.0 followed by T_7 (33.3%), T_6 (25.9%), T_5, T_2 (22.2% each) and T_4 (14.8%) as compared to T_9.

4.2.6.6 Zinc (Zn)

Leaf Zn content varied from 47.67 to 91.38 mg/kg. Highest leaf Zn content (91.38 mg/kg) was registered by treatment combination of T_8 followed by T_7, T_6, T_5, T_2 and T_4 with the corresponding values of 86.03, 80.91, 75.12, 68.80 and 64.71 mg/kg. However, it was lowest (47.67 mg/kg) in treatment T_9. Moreover, treatment T_8 observed statistically superior results to all other applied treatments. Leaf Zn content for different conjoint application of organic Zn and bio-organic supplements was followed in the order of $T_8 > T_7 > T_6 > T_5 > T_2 > T_4$.

The findings demonstrated the positive impacts of application of bio-organics and organic Zn complexes on leaf nutritional content of apple. This increase was attributed to the synergistic effects of zinc and amino acid chelates on increased absorption of nutrients and their subsequent translocation (Zhou et al., 2007; Souri, 2016). Meanwhile, the results were validated by increased penetration of Zn into the cells, which can be attributed to the bonding between nanoparticles and protein carriers (Gonzalez-Melendi et al., 2008; Alzreejawi and Al-Juthery, 2021). This is also in line with further studies that nano Zn is more efficient than Zn sulphate (Prasad et al., 2012). In addition, the foliar application of amino acids promoted N2-fixation, which in turn increased the surface area available for nutrient intake and root development (Weiland et al., 2016, Khan et al., 2019; Souri and Hatamian, 2019). Also, with response to increased amino acid nutrient intake, higher leaf N, P and K content received (Radkowski et al., 2018). A further explanation for an increase in leaf N content was due to the involvement of amino acids to control signaling pathways associated with biological processes (Gioseffi et al., 2012; Santi et al., 2017). Earlier literature has also demonstrated that application of Zn in conjugation with organic chemicals improved the macro- and micronutrients content of plants (Soliman et al., 2023). Moreover, Zn amino acid complexes had a significant impact on soil enzymatic activities which in turn affected nutrient cycling within rhizosphere zone (Alzreejawi and Al-Juthery, 2021; Neemisha and Sharma, 2022).

The simulative effect of vermicompost on leaf nutrient content is assigned to the availability of nutrients in readily accessible form such as phosphates ($H_2PO_4^-$ and HPO_4^{2-}), soluble K, exchangeable Ca and Mg (Theunissen et al., 2010). Besides, the incorporation of *Azolla* improved the soil fertility, increased nutrient availability and ultimately improved leaf nutrient content (Awodun, 2008; Taha and El-Shahat, 2017). AM fungi have higher capability for the absorption of immobile nutrient elements attributed to the extensive mycelial growth which provide greater surface area for absorption compared to roots alone (Jakobsen et al., 1992). According to Cavender et al. (2003), the increase in leaf N and P content might have resulted from N_2-fixation and P solubilization by PGPR and further improved P uptake by AM fungi.

Moreover, the enhancement in uptake of P, K, Ca and Mg is directly associated with the increased leaf N content (Pirlak et al., 2007). Similar results with PGPR inoculation were recorded for increased leaf macro and micronutrient content in blueberry (De Silva et al., 2000), apricot (Esitken et al., 2003) and apple (Karakurt and Aslantas, 2010). Furthermore, the improved cell permeability through humic acid application resulted in higher nutrient passage into the cells which thereby promoted leaf nutrient content (Abourayya et al., 2020).

4.2.7 FRUIT NUTRIENTS

4.2.7.1 Flesh

4.2.7.1.1 Phosphorus

The recorded data exhibited variation in P content of apple flesh subjected to different organic Zn and bio-organic sources, which varied from 0.02 to 0.04 per cent (Table 29). Treatment T_8 observed the maximum P content (0.04%) in fruit flesh, however, the treatment combinations of T_1, T_3, T_4 and T_9 recorded the minimum (0.02%) values. Moreover, treatments T_2, T_5, T_6 and T_7 also depicted statistically similar results with the corresponding values of 0.03 per cent each.

4.2.7.1.2 Potassium

Data in Table 29 illustrated a pronounced effect on K content of apple flesh due to the application of organic Zn and bio-organic sources. The treatment combination of T_8 demonstrated the highest K content of fruit flesh (0.56%) which was statistically equivalent to T_7 (0.53%). The lowest values (0.40%) were, however, recorded in treatment T_9. The superior treatment also experienced an increase of 9.8, 19.1, 21.7 and 40.0 per cent in K content in apple flesh over T_6, T_2, T_4 and T_9, respectively.

4.2.7.1.3 Calcium

Data analysis pertaining to Ca content of apple flesh depicted positive affect of conjoint application of organic Zn and bio-organics as compared to control (Table 29). Highest Ca content (0.30%) of apple flesh was recorded in treatment combinations of T_7 and T_8, however, it was minimum (0.20%) in T_9.

4.2.7.1.4 Magnesium

The results pertaining to Mg content of apple flesh showed statistically insignificant difference among different Zn and bio-organic treatments, which ranged between 0.06 and 0.09 per cent. Mg content of fruit flesh was observed maximum (0.09%) in treatment T_8. However, minimum values (0.06%) were exhibited by treatment T_9. Furthermore, the results indicated 50 per cent increment in the superior treatment T_8 as compared to T_9 treatment.

Table 29. Fruit nutrients of apple affected by conjoint organic zinc and bio-organic fertilization

Treatment	P (%)		K (%)		Ca (%)		Mg (%)		Zn (%)	
	Flesh	**Peel**	**Flesh**	**Peel**	**Flesh**	**Peel**	**Flesh**	**Peel**	**Flesh**	**Peel**
T_1	0.02	0.05	0.44	0.58	0.24	0.49	0.07	0.14	0.02	0.02
T_2	0.03	0.06	0.47	0.62	0.26	0.54	0.07	0.15	0.02	0.03
T_3	0.02	0.05	0.43	0.55	0.22	0.47	0.07	0.13	0.01	0.02
T_4	0.02	0.06	0.46	0.61	0.25	0.52	0.07	0.14	0.02	0.02
T_5	0.03	0.07	0.49	0.65	0.27	0.55	0.08	0.16	0.02	0.03
T_6	0.03	0.07	0.51	0.74	0.28	0.56	0.08	0.16	0.02	0.03
T_7	0.03	0.08	0.53	0.77	0.30	0.60	0.08	0.17	0.02	0.04
T_8	0.04	0.09	0.56	0.88	0.30	0.63	0.09	0.17	0.03	0.04
T_9	0.02	0.04	0.40	0.53	0.20	0.44	0.06	0.13	0.01	0.02
$CD_{0.05}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS, Non-significant; T_1: Zn-Gly$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$ + HA$_{100}$; T_2: Zn-Gly$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$ + HA$_{120}$; T_3: Zn-Glu$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$ + HA$_{100}$; T_4: Zn-Glu$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$ + HA$_{120}$; T_5: Zn-AAC$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$ + HA$_{100}$; T_6: Zn-AAC$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$ + HA$_{120}$; T_7: Nano Zn-AAC$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$; T_8: Nano Zn-AAC$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$; T_9: Inorganic N:P:K (105:53:105 g/plant) + $ZnSO_4$ @0.5%

4.2.7.1.5 Zinc

In the present investigation, conjoint organic Zn and bio-organic treatments exhibited non-significant impact on Zn content of apple flesh, which varied from 0.01 to 0.03 per cent. Treatment T_8 recorded highest Zn content (0.03%), however, it was least (0.01% each) in T_3 and T_9. The results also illustrated 20.0 per cent increase in Zn content of apple flesh through treatment combination of T_8 over T_9.

4.2.7.2 Peel

4.2.7.2.1 Phosphorus

The influence of Zn and bio-organic treatments on P content of apple peel is presented in Table 29. It is clear from the data that P content of peel varied from 0.04 to 0.09 per cent. Maximum P content of fruit peel (0.09%) was recorded in treatment T_8. However, the minimum P content of fruit peel (0.04%) was observed in T_9. The results also reflected 2.3 times increase in P content of fruit peel through treatment combination of T_8 over T_9.

4.2.7.2.2 Potassium

Among different applied treatments, fruit peel K content varied from 0.53 to 0.88 per cent. Minimum K content of fruit peel (0.53%) was recorded in T_9, whereas, the treatment T_8 exhibited the maximum value (0.88%). The treatment of T_8 also demonstrated a remarkable increase of 18.9, 41.9, 44.2 and 66.0 per cent compared to T_6, T_2, T_4 and T_9 treatments, respectively.

4.2.7.2.3 Calcium

Conjoint application of Zn organic and bi-organic sources exerted a notable impact on the Ca content of peel of apple fruit samples. Treatment T_8 exhibited the maximum Ca content of fruit peel (0.63%) followed by T_7, T_6, T_5, T_2 and T_4 with corresponding values of 0.60, 0.56, 0.55, 0.54 and 0.52 per cent. However, minimum fruit peel Ca content (0.44%) was reported in T_9. The fruit peel Ca content for the various organic Zn and bio-organic sources was followed in the order of $T_7 > T_8 > T_5 > T_6 > T_4 > T_2$.

4.2.7.2.4 Magnesium

Application of organic Zn and bio-organic sources recorded significant increase in Mg content of apple peel during the course of study. Maximum Mg content of fruit peel (0.17% each) was recorded in treatments of T_8 and T_7, whereas, it was minimum (0.13%) in T_9 and also recorded an increment of 30.7 per cent compared to T_9.

4.2.7.2.5 Zinc

Data analysis in relation to Zn content of apple peel depicted that Zn content of apple peel was positively influenced by the application of organic Zn and bio-organics sources (Table 29). Treatment combinations of T_8 and T_7 registered the maximum fruit peel Zn content (0.04%), however, it was recorded minimum (0.02%) in T_3, T_4 and T_9. The results also reflected an increase of 100.0 per cent on Zn content of apple peel through T_8 and T_7 treatments compared to T_3, T_4 and T_9 treatments, respectively.

4.2.8 FRUIT NUTRIENTS RATIO

4.2.8.1 Flesh

4.2.8.1.1 K/Ca

Appraisal of the data unveiled that among different applied treatments, K/Ca ratio of apple flesh depicted statistically non-significant results which ranged between 1.75 and 1.94 (Fig. 29). Maximum K/Ca (1.95) was recorded in T_3 which was statistically similar to treatment T_9 (1.94). However, treatment T_2 reflected the lowest value (1.75). Furthermore, K/Ca ratio of fruit flesh for different organic Zn and bio-organics combinations was followed in the order of $T_3 > T_9 > T_1 > T_8 = T_4 > T_5$.

4.2.8.1.2 (K+Mg)/Ca

The recorded data exhibited non-significant effects on (K+Mg)/Ca in flesh subjected to different organic Zn and bio-organics sources. The values for (K+Mg)/Ca of fruit flesh varied from 2.04 to 2.25. Treatment T_3 recorded maximum (K+Mg)/Ca ratio of fruit flesh (2.25), however, the treatment combinations of T_2 and T_7 recorded the minimum (2.04 each). The order followed pertaining to (K+Mg)/Ca of fruit flesh for different conjoint organic Zn and bio-organic nutrient sources as $T_8 > T_7 > T_6 > T_5 > T_2 > T_4$.

4.2.8.2 Peel

4.2.8.2.1 K/Ca

A perusal of the data showed a non-significant impact of conjoint application of organic Zn sources and bio-organics on K/Ca ratio of apple peel. Average K/Ca ratio of fruit peel ranged between 1.15 and 1.38. The treatment of T_8 recorded maximum K/Ca ratio of fruit peel (1.38) which was statistically similar with treatment T_6 (1.31). Minimum value of 1.15 however, was observed in T_2. Moreover, the K/Ca ratio of fruit peel for the various organic Zn and bio-organic sources was followed as $T_8 > T_6 > T_7 > T_9 > T_1 > T_5$.

4.2.8.2.2 (K+Mg)/Ca

The data presented in Fig. 29 showed insignificant effects on (K+Mg)/Ca ratio of apple peel through the application of different organic Zn nutrient and bio-organics sources. The conferred data revealed that the treatment T_8 recorded the maximum (K+Mg)/Ca ratio of fruit peel (1.66) which was statistically at par with treatment T_6 (1.61). However, the minimum of (K+Mg)/Ca (1.43) were exhibited by T_2. Furthermore, the treatment combinations of T_1 and T_5 depicted similar values of 1.47. The order followed related to (K+Mg)/Ca ratio of fruit peel for the various applied Zn nutrient and bio-organic sources exhibited as $T_8 > T_6 > T_7 > T_9 > T_1 = T_5$.

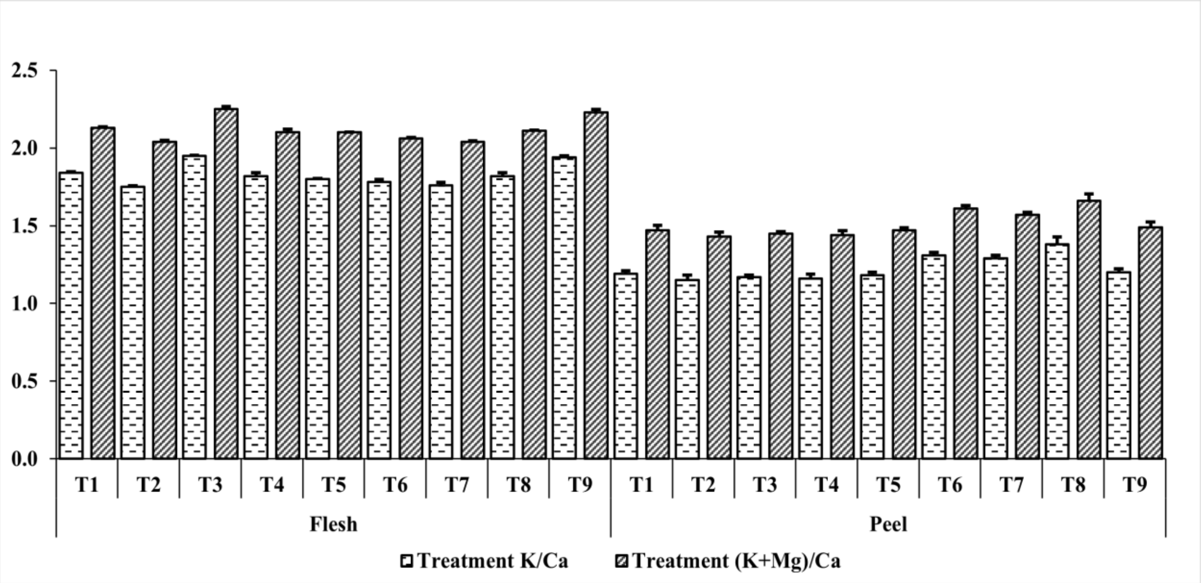


Fig. 30: Impact of foliar organic Zn and bio-organic sources on K/Ca and (K+Mg)/Ca content in flesh and peel of apple

Furthermore, the synergistic effect of Zn amino acid complexes and bio-organic sources on fruit nutrient content has been well documented. The application of Zn amino acid chelate led to a higher translocation of zinc from leaves to fruits, which in turn enhanced the nutrient content of the fruit samples (Anitha, 2020; Tabesh et al., 2020). It might also be attributed to the rapid absorption of nutrients administered through foliar application (Al-Juthery et al., 2019). Furthermore, it has been demonstrated by Garcia et al. (2011) and Ghoname et al. (2012) that chelation of nutrients via amino acids resulted in improved nutrient absorption and subsequent distribution throughout the plant system. The present study is further supported by the critical role of nano Zn in accelerating sugar accumulation and improved fruit nutrition (Sharma et al., 2014b). Besides, the low molecular weight, higher availability and easier penetration of Zn amino acid chelates compared to Zn sulphate led to improved fruit macro and micronutrient status (Colle et al., 2009; Wei et al., 2012; Ghasemi et al., 2013; Chen et al., 2022). Our results are in harmony with the findings of Wei et al. (2012) who advocated reduced phytic acid (PA) content and enhanced Zn availability through application of Zn amino acid complexes, which probably led to increased fruit nutrient content. Foliar application of nano Zn amino acid chelate exhibited an increase in Zn movement from leaves to fruits through phloem subsequently resulting in higher Zn assimilation during fruit development stages (Garcia-Lopez et al., 2019). Analogous research reports on fruit nutrient content were reported by Ning et al. (2021).

The incorporation of biofertilizers influences the nutrient availability (Awodun, 2008) which might be the reason for increased nutrient content of fruits (Taha and El-Shahat, 2017). Similar results with PGPR inoculation were observed in apple (Yildiz et al., 2022). Treder et al. (2022) also depicted significant improvement in macro and micronutrient content in fruits through PGPR inoculation. However, fruit Ca and Mg was not affected in the present investigation. The positive effects of *Azolla* and humic acid on fruit nutrient content were also reported by Taha and El-Shahat (2017) and Taha et al. (2018). Moreover, accumulation of more nutrients in fruit peel compared to fruit flesh has also been observed (Manzoor et al., 2012; Michailidis et al., 2021).

4.2.9 ANTIOXIDANTS ACTIVITY

4.2.9.1 Phytic acid

The examination of data unveiled that fruit phytic acid content ranged from 1.05 to 1.56 per cent (Table 30; Fig. 30). Minimum phytic acid (1.05%) content was registered in treatment T_8, which was statistically uniform with treatment T_7 (1.07%). However, it was recorded maximum (1.56%) in treatment T_9, which also exhibited statistically equivalent results with treatment T_3 (1.53%) and T_1 (1.50%). Furthermore, the per cent decrease of 10.4, 33.3, 34.2 and 48.6 was observed in T_8 compared to treatments T_6, T_2, T_4 and T_9, respectively.

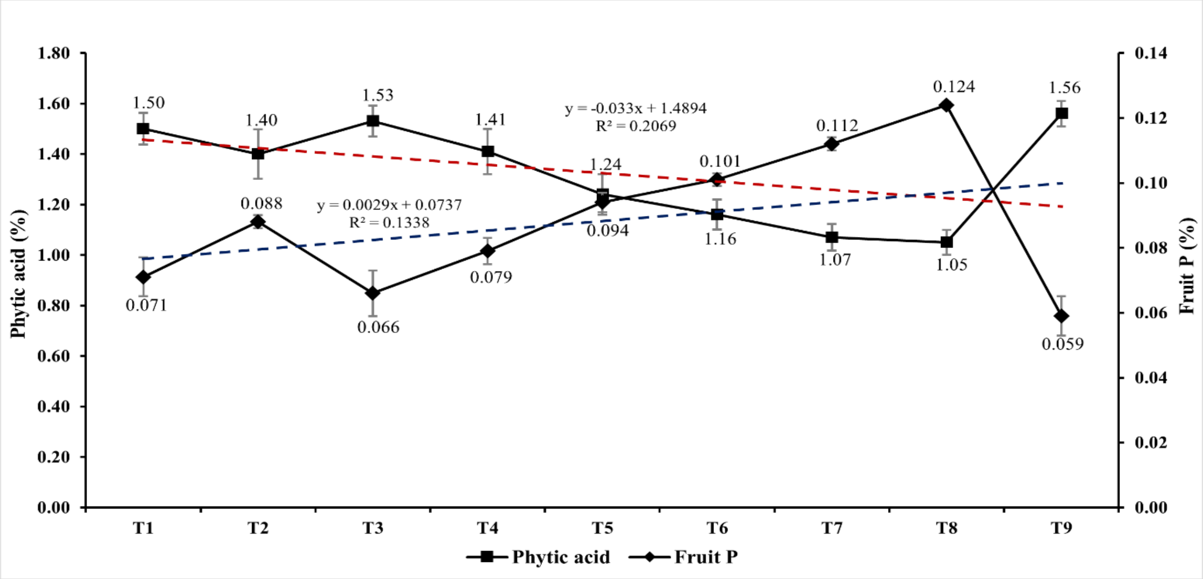


Fig. 31: Relationship studies on phytic acid and fruit P content in apple under organic Zn and bio-organics fertilization

4.2.9.2 Phytic acid/ Fruit nutrients

4.2.9.2.1 PA: Ca

Data analysis revealed that fruit PA: Ca ratio ranged between 1.13 and 2.51. Maximum fruit PA: Ca (2.51) was registered in treatment T_9, followed by T_3, T_1, T_4, T_2 and T_5 with corresponding values of 2.29, 2.11, 1.87, 1.79 and 1.56. However, minimum (1.13) values were observed in T_8. The treatment combinations of T_3 and T_1 also depicted analogous results with the corresponding values 2.29 and 2.11. Moreover, a decrease of 1.2, 1.6, 1.7 and 2.2 times of fruit PA: Ca was also observed in T_8 as compared to T_6, T_2, T_4 and T_9, respectively.

Table 30. Phytic acid and Ursolic acid content of apple influenced by conjoint application of organic Zn and bio-organic fertilizers

Treatment	Phytic acid (%)	PA: Ca	PA: Mg	PA: Zn	Ursolic acid (µg/mL)
T_1	1.50	2.11	7.30	45.12	207.80
T_2	1.40	1.79	6.25	33.76	214.55
T_3	1.53	2.29	7.76	50.31	182.26
T_4	1.41	1.87	6.72	36.87	208.66
T_5	1.24	1.56	5.38	26.67	215.90
T_6	1.16	1.39	4.80	23.22	216.65
T_7	1.07	1.21	4.25	19.55	247.84
T_8	1.05	1.13	4.07	16.92	297.27
T_9	1.56	2.51	8.63	62.16	180.98
$CD_{0.05}$	0.07	0.18	0.43	4.20	1.35

T_1: Zn-Gly$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$ + HA$_{100}$; T_2: Zn-Gly$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$ + HA$_{120}$; T_3: Zn-Glu$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$ + HA$_{100}$; T_4: Zn-Glu$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$ + HA$_{120}$; T_5: Zn-AAC$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$ + HA$_{100}$; T_6: Zn-AAC$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$ + HA$_{120}$; T_7: Nano Zn-AAC$_{200}$ + VC$_{80}$ + AZ$_{300}$ + AMF$_{25}$ + PGPR$_{200}$; T_8: Nano Zn-AAC$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$; T_9: Inorganic N:P:K (105:53:105 g/plant) + $ZnSO_4$ @0.5%

4.2.9.2.2 PA: Mg

Fruit PA: Mg varied from 4.07 to 8.63. Maximum PA: Mg (8.63) was recorded in T_9, which was statistically higher than all other applied treatment combinations (Table 30). However, minimum PA: Mg (4.07) was observed in T_8. Moreover, statistically equivalent results were observed in T_7 and T_8 with corresponding values of 4.25 and 4.07. The treatment combination of T_8 also recorded a per cent decrease of 17.9, 53.5, 65.1 and 112.1 compared to T_6, T_2, T_4 and T_9, respectively.

4.2.9.2.3 PA: Zn

Application of organic Zn and bio-organics reported significant increase on PA: Zn in fruit samples (Table 30; Fig. 31). Fruit PA: Zn was exhibited minimum (16.92) in T_8, which was statistically at par with T_7 (19.55). However, the maximum PA: Zn was observed in T_9 (62.16). Furthermore, the decrease of 37.2, 99.5, 117.9 and 267.3 per cent was observed in T_8 compared to

T_6, T_2, T_4 and T_9, respectively. The order followed pertaining to PA: Zn for the various conjoint organic Zn and bio-organic nutrient sources as $T_9 > T_3 > T_1 > T_4 > T_2 > T_5$.

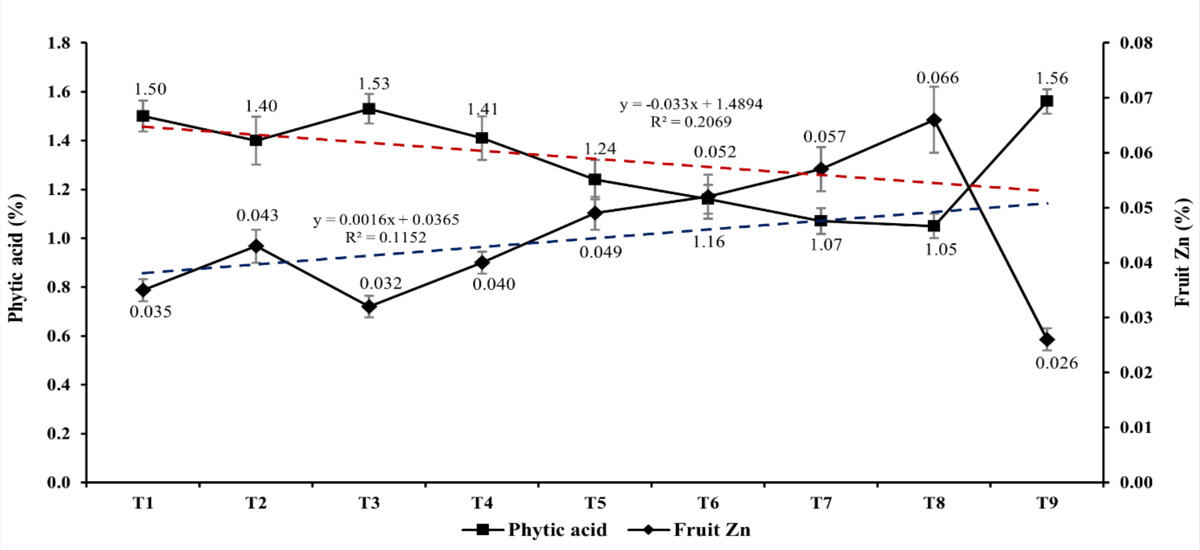


Fig. 32: Relationship between phytic acid and fruit Zn content in apple under organic Zn and bio-organics nutrition

4.2.9.2.4 Ursolic acid

The conjoint application of organic Zn and bio-organics showed a positive effect on ursolic acid content in apple fruit samples (Table 30; Fig. 32). Treatment T_8 recorded the maximum ursolic acid content (297.27 µg/mL) which was statistically superior to all other applied treatments. However, treatment T_9 recorded the minimum (180.98 µg/mL). Furthermore, the order followed pertaining to ursolic acid content through conjoint Zn nutrient and bio-organic sources as $T_8 > T_7 > T_6 > T_5 > T_2 > T_4$. The results also reflected 1.3, 1.4, 1.4 and 1.6 times increase in ursolic acid content through superior treatment over T_6, T_2, T_4 and T_9 treatments, respectively.

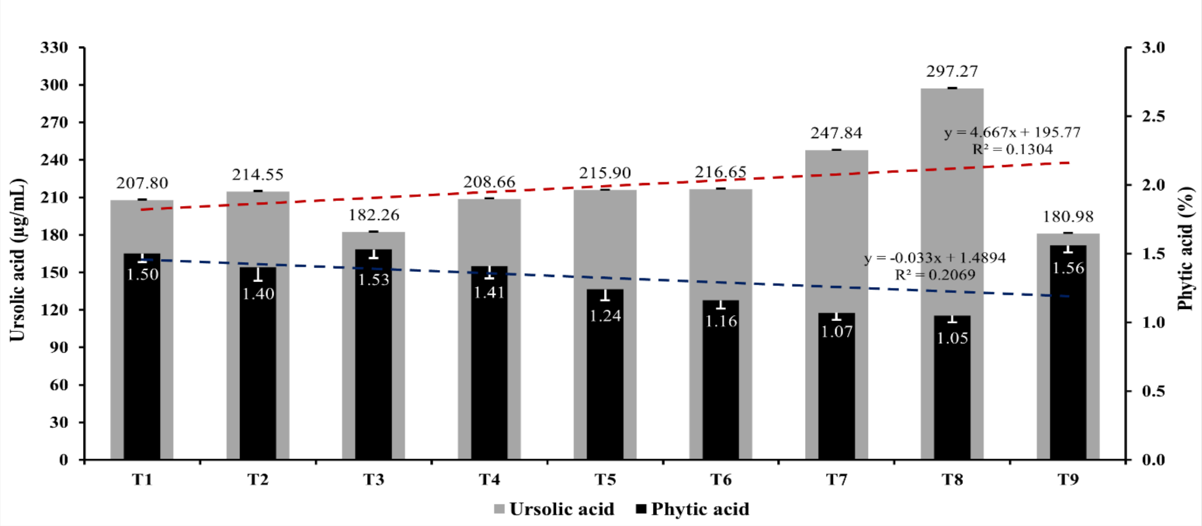


Fig. 33: Relationship studies on ursolic acid and phytic acid in apple under organic Zn and bio-organics fertilization

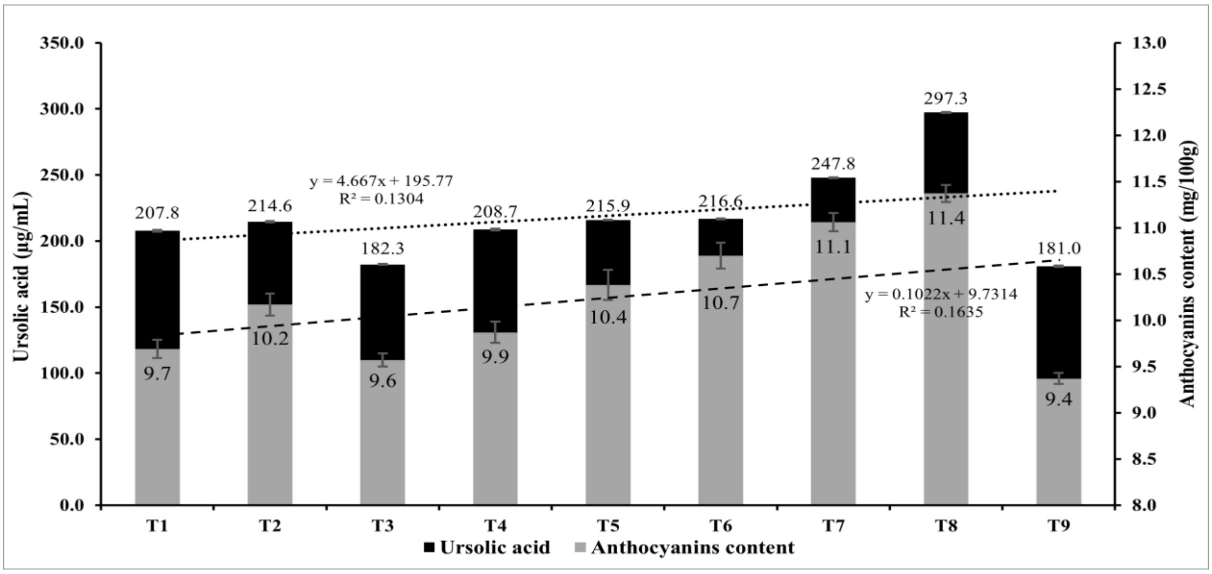


Fig. 34: Relationship between ursolic acid and anthocyanins content in apple under organic Zn and bio-organics nutrition

In the present investigation, the reduction of PA: Zn in fruit samples and increase of nutrient bioavailability determined efficacy of Zn (Raza et al., 2023). Similarly, the current investigation found that Zn amino complexes significantly reduced phytic acid when compared to Zn sulphate. Additionally, the fruit antioxidant activity was enhanced by the nano Zn amino acid chelate, which exhibited the lowest phytic acid content. According to Gupta et al. (2015), 60 to 80 per cent of total P is stored by plants as phytic acid, which drastically lowers the bioavailability of Zn for humans (Saha et al., 2017). Mirbolook et al. (2020) confirmed the beneficial effect of Zn amino acid chelates to reduce PA: Zn in fruits and thereby improved Zn bio-accessibility to humans. In contrast, Zn sulphate was observed to lower Zn availability (Schlegel et al., 2013). Other researchers also showed higher efficacy of Zn amino acid chelates over Zn sulphate (Ghasemi et al., 2013; Seddigh et al., 2016). It can also be ascribed to the complexation between amino acids and PA which thereby improved Zn uptake (Sun et al., 2018). Also, reduction in PA: Zn molar ratio was recorded with the application of bio fertilizers (Mohammad et al., 2015). PGPR and acid phosphatase enzyme accelerated the conversion of PA into soluble P forms (Irshad and Yergeau, 2018), which thereby increased Zn availability. Besides, humic acid application increased the enzymatic hydrolysis of PA via formation of humic acid-PA complex (Ge et al., 2022).

Ursolic acid (UA) is a major organic acid present in apple peel known for its anti-tumor, anti-inflammatory and antibacterial properties (Cargnin and Gnoatto, 2017). Nano Zn amino chelate exerted a positive influence on UA content of apple peel, ascribed to the increased antioxidant activity via Zn application (Yaish, 2015). Moreover, easier penetration of nano sized molecules (Tarafdar et al., 2012) might have elevated the UA content. Butkeviciute et al. (2018) showed that major part of UA is present in apple peel. In particular, according to Farag and Palta (1992) and Li et al. (2018) anthocyanin content was highly correlated with UA content (Fig. 33). Moreover, the accumulation of greater anthocyanins pigment through conjoint application of Zn amino acid chelate and bio-organics resulted in higher UA content in apple peel (Shalan, 2020). In spite of this, higher UA content was also recorded in organically grown crops compared to chemical fertilization (Itankar et al., 2015). Similarly, Bhoon (2011) reported increase in UA content through inoculation of AM fungi.

4.2.10 PRINCIPAL COMPONENT ANALYSIS

4.2.10.1 PCA analysis for growth, yield contributing and fruit quality parameters in apple under organic Zn and bio-organics fertilization

PCA was executed to visualize the similarities and dissimilarities among the growth, yield contributing and fruit quality parameters under the influence of conjoint application of Zn and bio-organic nutrient sources. The plot depicted the association between the factor and the illustrated axis of growth traits (leaf area, TCSA, CV, CA), yield contributing parameters (fruit set, fruit yield), and fruit quality parameters (fruit length, fruit breadth, fruit weight, fruit firmness, TSS, titratable acidity, reducing sugars, non-reducing sugars, total sugars, anthocyanins, phytic acid, ursolic acid). Based on *Eigen* value (>1), PCA further identified the primary components that explained 96.97 per cent (PC1), 98.45 per cent (PC2). 99.12 per cent (PC3), 99.49 per cent (PC4) and 99.70 per cent (PC5) of the total variance (Table 31). Variables with values equal to or more than two thirds of the maximum variable value in each PC were considered in the PCA investigations. In terms of growth, yield contributing and fruit quality parameters of apple, PC5 showed the maximum cumulative variance. The results further revealed that PC1 was significantly correlated with all the growth, yield contributing and fruit quality parameters except firmness, titratable acidity and phytic acid. The main influence on PC2, which accounted for 99.74 per cent of the variance were depicted by the parameters viz., leaf area, TCSA, CV, CA, fruit breadth, fruit weight, firmness, titratable acidity and phytic acid. Furthermore, PC3 loaded heavily for leaf area, CV, CA, fruit length, titratable acidity, non-reducing sugars, total sugars, phytic acid and ursolic acid. Furthermore, the parameters including leaf area, TCSA, CA, fruit set, fruit yield, TSS, titratable acidity, reducing sugars, phytic acid and ursolic acid loaded heavily for PC4 and the parameters TCSA,

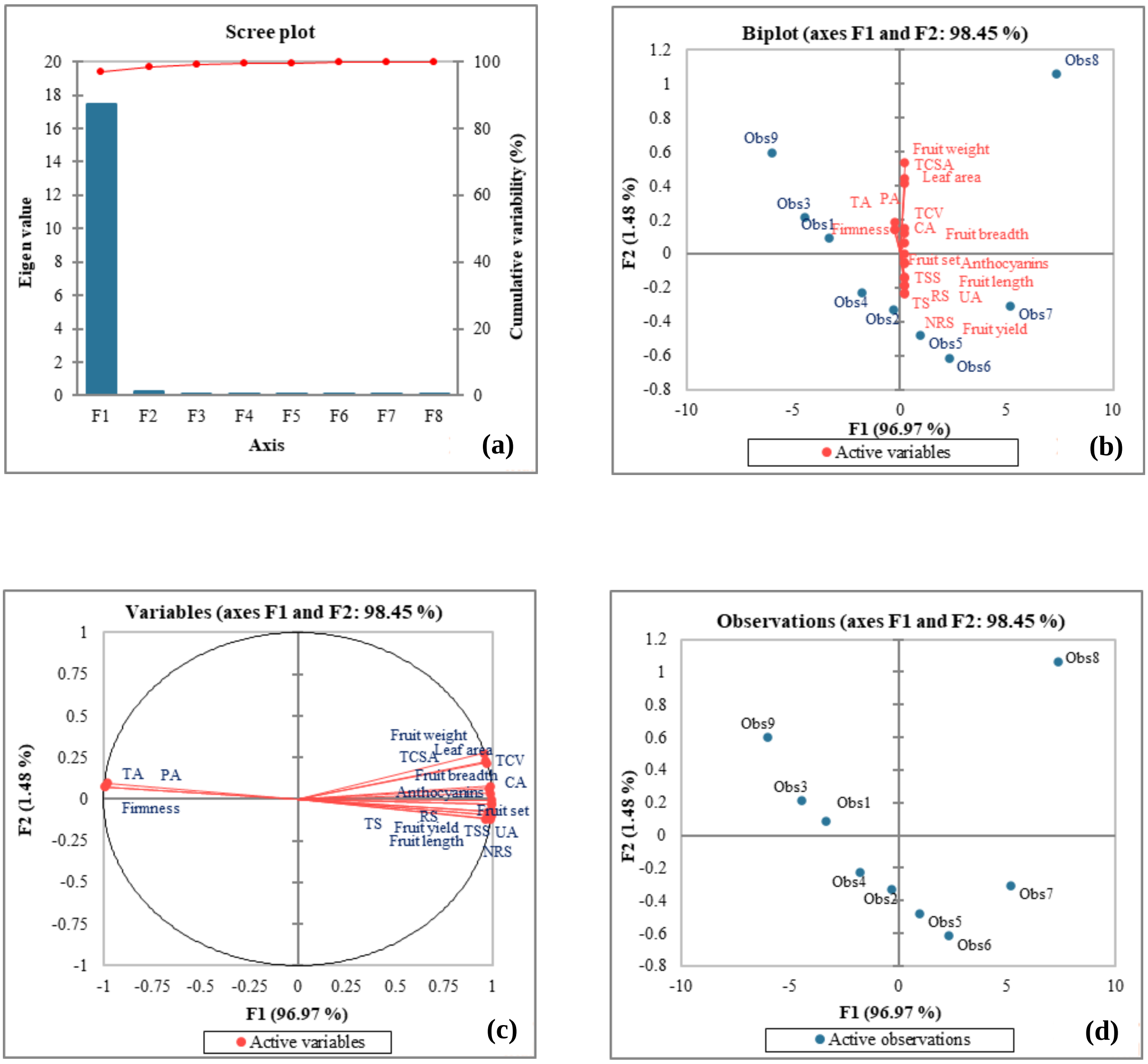


Fig. 35: PCA Scree plot (a) and Correlation biplot (b-d) for effect of organic Zn and bio-organic supplementation on growth, yield and quality parameters of apple

Table 31: PCA of growth, yield contributing and quality parameters of apple influenced by organic Zn and bio-organics fertilization

Parameter	Principal Component				
	PC1	PC2	PC3	PC4	PC5
Eigen value	17.45	0.27	0.12	0.07	0.04
Variability (%)	96.97	1.48	0.67	0.38	0.21
Cumulative variance (%)	96.97	98.45	99.12	99.49	99.70

Growth traits and fruit quality parameters	Factor loadings					Eigen vectors				
	F1	F2	F3	F4	F5	F1	F2	F3	F4	F5
Leaf area	0.969	0.216	0.096	0.003	-0.017	0.232	0.418	0.278	0.013	-0.090
TCSA	0.968	0.229	-0.068	0.045	0.028	0.232	0.444	-0.197	0.173	0.143
CV	0.991	0.079	0.028	-0.028	-0.036	0.237	0.153	0.080	-0.107	-0.187
CA	0.991	0.034	0.059	0.053	-0.039	0.237	0.067	0.170	0.204	-0.201
Fruit set	0.996	-0.028	-0.078	0.008	0.004	0.238	-0.054	-0.226	0.031	0.018
Fruit yield	0.991	-0.119	-0.038	0.024	0.003	0.237	-0.230	-0.110	0.092	0.017
Fruit length	0.999	-0.030	0.027	-0.007	0.002	0.239	-0.058	0.078	-0.026	0.013
Fruit breadth	0.985	0.062	-0.006	-0.145	0.017	0.236	0.120	-0.017	-0.555	0.088
Fruit weight	0.958	0.276	-0.012	-0.003	0.006	0.229	0.535	-0.035	-0.010	0.031
Firmness	-0.991	0.073	-0.046	-0.019	-0.100	-0.237	0.141	-0.131	-0.072	-0.515
TSS	0.994	-0.070	-0.005	0.053	0.056	0.238	-0.136	-0.014	0.203	0.286
TA	-0.993	0.075	0.087	0.006	-0.016	-0.238	0.145	0.251	0.022	-0.081
RS	0.992	-0.095	-0.067	0.026	0.034	0.237	-0.184	-0.194	0.101	0.174
NRS	0.962	-0.121	0.217	-0.101	-0.004	0.230	-0.234	0.627	-0.387	-0.020
TS	0.995	-0.098	0.011	-0.008	0.021	0.238	-0.190	0.033	-0.029	0.109
Anthocyanins	0.997	-0.002	-0.075	-0.009	-0.002	0.239	-0.003	-0.215	-0.036	-0.012
PA	-0.977	0.096	0.134	0.073	0.109	-0.234	0.186	0.385	0.282	0.564
UA	0.976	-0.076	0.092	0.145	-0.080	0.234	-0.147	0.267	0.557	-0.414

PC1, Principal component 1; PC2, Principal component 2; PC3, Principal component 3; PC4 Principal component 4; PC5, Principal component 5; F1, factor-1; F2, factor-2; F3, factor-3, F4, factor-4; F5, factor-5; TCSA, trunk cross sectional area; CV, canopy volume; CA, canopy area; TSS, total soluble solids; TA, titratable acidity; RS, reducing sugars; NRS, non-reducing sugars; TS, total sugars; PA, phytic acid; UA, ursolic acid

Factor scores					
Treatment (T)	**F1**	**F2**	**F3**	**F4**	**F5**
T_1	-3.321	0.089	0.029	-0.218	0.316
T_2	-0.289	-0.331	0.259	0.490	0.194
T_3	-4.437	0.213	0.084	-0.207	0.176
T_4	-1.772	-0.229	0.650	0.037	-0.287
T_5	0.980	-0.478	-0.153	0.128	-0.079
T_6	2.311	-0.615	-0.621	0.060	0.001
T_7	5.181	-0.306	0.120	-0.485	-0.080
T_8	7.348	1.061	0.034	0.148	0.032
T_9	-6.001	0.597	-0.401	0.046	-0.273

fruit set, fruit length, fruit breadth, fruit weight, TSS, reducing sugars, total sugars and phytic acid highly loaded for PC5. Among different PCs, variables with factor loadings greater than 0.40 were deemed significant (Wander and Bolero, 1999). In addition, the *Scree* plot and correlation biplot were illustrated graphically through generation of PCA biplots by integrating original factors as *Eigen* vectors (Fig. 34). The findings are in line with the studies of Kumar et al. (2017) and Kumar et al. (2020) for data reduction of different variables using PCA.

4.2.10.2 PCA analysis for soil chemical and microbiological properties in apple under organic Zn and bio-organics fertilization

Similarly, PCA was carried out to understand the effect of Zn and bio-organic nutrient sources on the soil chemical and microbiological properties. PCA studies highlighted that the first four components, characterized by *Eigen* values (>1) represented 97.40 per cent, 98.64 per cent, 99.38 per cent and 99.65 per cent of the variance distributed across PC1 to PC4 (Table 32). PCA biplots were utilized to represent *Scree* plot and correlation biplots, employing original factors as *Eigen* vectors to sum up the correlations among soil chemical (organic carbon, available N, P, K, exchangeable Ca, Mg, DTPA extractable Zn, Fe, Cu, Mn) and microbiological (bacteria, fungi, actinomycetes, PSB, KSB, ZSB, *Azotobacter*, AM fungi, acid phosphatase, alkaline phosphatase, dehydrogenase) properties across the illustrated axis (Fig. 35). Among different applied treatments, PC4 exhibited the highest cumulative variance for the studied soil chemical and microbiological properties. All the parameters loaded heavily for PC1 which showed 97.40 per cent of variance, followed by PC2 for most of the parameters except available P, exchangeable Ca, DTPA extractable Mn, bacteria, fungi, actinomycetes, PSB, KSB, alkaline phosphatase and dehydrogenase. On the other hand, exchangeable Mg, DTPA extractable Fe, Cu, fungi, PSB, KSB, ZSB, *Azotobacter* and AM fungi contributed highly to PC3. Similarly, PC4 was strongly correlated with fruit yield, organic carbon, available P, K exchangeable Ca, DTPA extractable Fe, Cu, bacteria, actinomycetes, *Azotobacter*, AM fungi, and alkaline phosphatase. Similar results in earlier research have been noted in apple (Kumar et al., 2017) and strawberry (Kumar et al., 2020) for summarizing the data between different factors.

Table 32: PCA of chemical and microbiological properties of soils of apple influenced by organic Zn and bio-organics fertilization

Parameter	Principal Component			
	PC1	PC2	PC3	PC4
Eigen value	21.43	0.27	0.16	0.06
Variability (%)	97.40	1.23	0.75	0.26
Cumulative variance (%)	97.40	98.64	99.38	99.65

Soil chemical and microbiological properties	Factor loadings				Eigen vectors			
	F1	F2	F3	F4	F1	F2	F3	F4
Fruit yield	0.997	0.021	-0.063	0.032	0.215	0.041	-0.155	0.134
OC	0.990	0.096	-0.011	-0.055	0.214	0.185	-0.028	-0.230
N	0.985	0.138	-0.052	-0.030	0.213	0.265	-0.128	-0.124
P	0.991	-0.083	-0.095	0.013	0.214	-0.159	-0.234	0.054
K	0.991	0.088	-0.021	0.042	0.214	0.169	-0.052	0.176
Ca	0.989	0.059	-0.026	0.118	0.214	0.114	-0.064	0.489
Mg	0.997	-0.061	0.010	-0.018	0.215	-0.118	0.024	-0.073
Zn	0.957	0.213	-0.173	-0.080	0.207	0.409	-0.427	-0.332
Fe	0.977	0.045	0.202	0.032	0.211	0.086	0.498	0.132
Cu	0.996	0.057	0.028	0.043	0.215	0.109	0.069	0.179
Mn	0.992	-0.001	-0.050	-0.073	0.214	-0.001	-0.122	-0.303
Bacteria	0.986	-0.131	-0.074	0.061	0.213	-0.253	-0.181	0.255
Fungi	0.982	-0.142	0.078	-0.080	0.212	-0.274	0.192	-0.331
Actinomycetes	0.980	-0.180	-0.047	0.049	0.212	-0.345	-0.116	0.205
PSB	0.977	-0.154	0.119	-0.054	0.211	-0.296	0.294	-0.226
KSB	0.999	-0.018	0.020	-0.032	0.216	-0.034	0.049	-0.134
ZSB	0.986	0.069	0.139	-0.034	0.213	0.133	0.344	-0.141
Azotobacter	0.992	0.041	0.087	0.007	0.214	0.079	0.214	0.030
AM Fungi	0.980	0.154	0.093	0.044	0.212	0.296	0.228	0.185
Acid Ph	0.998	0.050	-0.040	-0.002	0.211	-0.380	-0.188	-0.119
Alkaline Ph	0.994	-0.062	-0.049	0.041	0.216	0.096	-0.098	-0.008
Dehydrogenase	0.975	-0.198	-0.076	-0.029	0.215	-0.118	-0.120	0.172

PC1, Principal component 1; PC2, Principal component 2; PC3, Principal component 3; PC4 Principal component 4; F1, factor-1; F2, factor-2; F3, factor-3, F4, factor-4; OC, organic carbon; PSB, phosphorus solubilizing bacteria; KSB, potassium solubilizing bacteria; Acid Ph, acid phosphatase; Alkaline Ph, alkaline phosphatase

Factor scores				
Treatment (T)	**F1**	**F2**	**F3**	**F4**
T_1	-3.158	-0.096	0.504	0.018
T_2	-0.120	-0.859	-0.145	-0.002
T_3	-4.812	0.274	0.540	-0.386
T_4	-1.721	-0.601	0.180	0.373
T_5	1.669	-0.503	-0.311	-0.175
T_6	3.546	0.097	-0.549	-0.320
T_7	5.284	0.438	-0.076	0.250
T_8	7.181	0.524	0.414	0.057
T_9	-7.868	0.725	-0.556	0.185

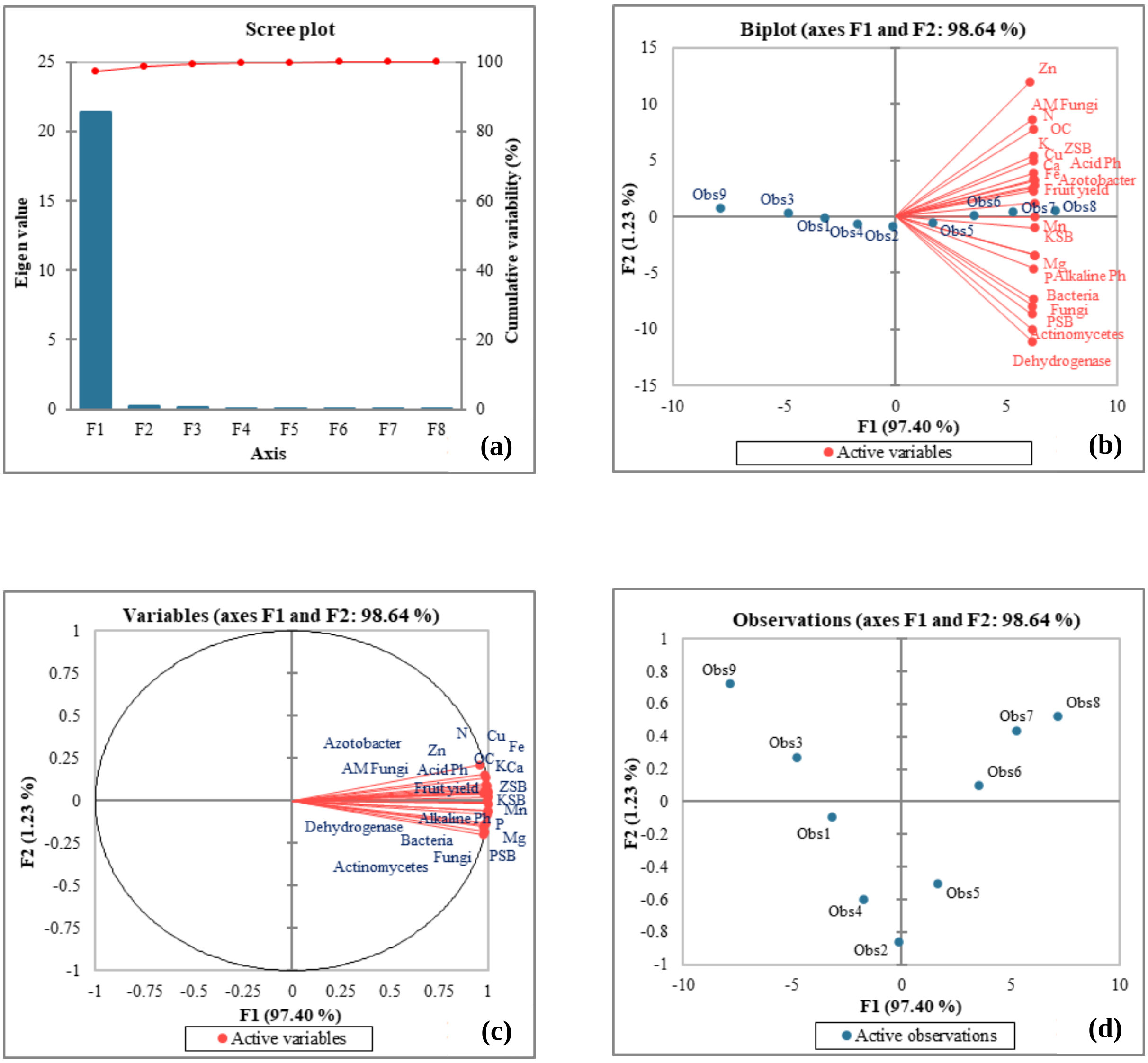


Fig. 36: PCA Scree plot (a) and Correlation biplot (b-d) for effect of organic Zn and bio-organic supplementation on chemical and microbiological properties of soils of apple

Chapter - 5

SUMMARY AND CONCLUSION

Zinc is a vital micronutrient for both plants and humans, but its deficiency is widespread. Zn biofortification as a means of foliar application is essential to fight malnutrition and ensure food security. Zn amino acid complexes offer enhanced agronomic biofortification by increasing nutritional content and crop yield due to improved utilization rate and complex stability. Besides, phytic acid has more tendencies to bind with Zn which can hamper the absorption rate. To overcome this, Zn amino acid chelates application has reduced fruit phytic acid content and increased Zn bioavailability for plants as well as humans. Furthermore, the supplementation of organic amendments elevated the crop biofortification via enhanced nutrient supply.

The salient findings of the present study entitled **"Zinc biofortification through organic Zn and bio-organic supplements in high density plantation of apple (*Malus* × *domestica* Borkh.)"** carried out during two consecutive cropping cycles between 2022 and 2023 are summarized as under:

EXPERIMENT 1:
EFFECT OF FOLIAR FEEDING OF ORGANIC Zn FERTILIZERS IN APPLE

5.1.1 Foliar application of nano Zn-AAC at 400 mg/L (NZn-AAC$_{400}$) compared to all other applied Zn sources showed positive influence on vegetative growth characteristics namely, plant height, trunk girth, canopy diameter, shoot growth and leaf area of apple.

5.1.2 Apple trees treated with NZn-AAC$_{400}$ exhibited maximum trunk cross-sectional area (TCSA), canopy volume (CV) and canopy area (CA) which increased by 2.0, 2.3 and 1.9 times compared to $ZnSO_4$ (control).

5.1.3 The treatment application of NZn-AAC$_{400}$ also recorded earliest date of pink bud emergence, initiation of flowering and date of full bloom. Furthermore, maximum increase in duration of flowering (34.4%), days from full bloom to harvest (6.6%), length of flowering shoot (28.2%) and spur density (92.3%) was also observed in NZn-AAC$_{400}$ compared to control.

5.1.4 Among different Zn treatments, foliar application of NZn-AAC$_{400}$ registered the most significant effect on numbers of flowers/shoot, number of fruits/shoot, fruit set and fruit yield with 1.4, 1.7, 1.5 and 1.8 times increase over the control.

5.1.5 Foliar application of NZn-AAC$_{400}$ registered the increment of 15.3, 15.7 and 41.5 per cent in terms of fruit length, fruit breadth and fruit weight compared to control. However, the maximum fruit firmness (6.19 kg/cm^2) was noted in control.

5.1.6 Significant increase in biochemical attributes of fruit samples *viz*., TSS, total sugars, ascorbic acid and anthocyanins content was also observed with NZn-AAC$_{400}$. Moreover, the treatment exhibited 1.2, 1.1, 1.5 and 1.2 times increase in TSS, total sugars, ascorbic acid and anthocyanins content, respectively, over control.

5.1.7 Post harvest chemical properties of soil of apple were also improved significantly by NZn-AAC$_{400}$ application compared to control. Organic carbon (8.8%), available N (12.6%), available P (28.2%) and available K (12.6%) content were considerably increased through NZn-AAC$_{400}$ treatment in comparison to control.

5.1.8 Among different Zn treatments, maximum exchangeable Ca and Mg content were recorded in NZn-AAC$_{400}$, which increased by 1.0 and 1.1 times compared to control. DTPA extractable Zn, Fe, Cu and Mn also depicted increase of 18.4, 13.7, 33.9 and 41.6 per cent, respectively over control.

5.1.9 Microbial biomass in terms of total bacterial count (1.50×10^6 cfu/g), soil fungi (1.03×10^3 cfu/g) and actinobacterial count (1.09×10^4 cfu/g) was highest in treatment NZn-AAC$_{400}$. Similarly, phosphorus solubilizing bacteria, potassium solubilizing bacteria, zinc solubilizing bacteria and *Azotobacter* were increased by 1.6, 1.3, 1.4 and 1.2 times, respectively compared to control. AM fungi spore count exhibited an increment of 30.7 per cent through NZn-AAC$_{400}$ treatment in comparison to control.

5.1.10 Soil enzymatic activity of acid phosphatase, alkaline phosphatase and dehydrogenase in rhizosphere of apple trees were markedly influenced by NZn-AAC$_{400}$. Furthermore, an increment of 29.7, 26.5 and 50.6 per cent was exhibited by NZn-AAC$_{400}$ over control.

5.1.11 Application of NZn-AAC$_{400}$ registered significant influence on total chlorophylls, photosynthetic efficiency and transpiration rate, which improved by 1.6, 1.4 and 1.5 times in comparison to control. Stomatal conductance was increased by 17.9 per cent over control.

5.1.12 Total carbohydrate content *viz.*, fruiting shoots, non-fruiting shoots, leaves and fruits also exhibited an increase of 20.8, 13.2, 39.7 and 27.8 per cent through NZn-AAC_{400} in comparison to control.

5.1.13 Among different Zn treatments, foliar application of NZn-AAC_{400} also noted positive influence on leaf N (2.38%), P (0.27%), K (1.71%), Ca (1.82%) and Mg (0.35%) content. Highest leaf Zn content was also exhibited by NZn-AAC_{400} with 1.2 per cent increase over control.

5.1.14 Fruit nutrient content in terms of fruit flesh and peel was also increased by the foliar application of NZn-AAC_{400}.

5.1.15 K/Ca and (K+Mg)/Ca ratio in apple flesh was recorded highest in control, whereas, the highest values for K/Ca and (K+Mg)/Ca ratio in apple peel was registered in NZn-AAC_{400} treatment.

5.1.16 Application of NZn-AAC_{400} exhibited the lowest value of phytic acid with a decrease of 46.7 per cent over control. In addition, PA: Ca, PA: Mg and PA: Zn ratios registered 2.1, 2.0 and 3.4 times decrease with $NZnAAC_{400}$ treatment compared to control. However, the ursolic acid content was recorded maximum with $NZnAAC_{400}$, which increased by 37.5 per cent in comparison to control.

5.1.17 Principal component analysis revealed that PC1 (95.70%) recorded the highest variability for vegetative growth, yield contributing and quality parameters of apple. Subsequently, PC1 also depicted maximum variability of 97.87 per cent on the basis of *Eigen* value (>1) for chemical and microbiological properties of soil.

EXPERIMENT 2:

CONJOINT EFFECT OF ORGANIC Zn AND BIO-ORGANICS FERTILIZATION

5.2.1 Among different applied treatments, conjoint application of treatment nano Zn-AAC_{400} + VC_{75} + AZ_{600} + AMF_{20} + $PGPR_{250}$ (T_8) registered the most significant influence on vegetative growth characteristics of apple *viz.*, plant height, trunk girth, canopy diameter, shoot growth and leaf area with 1.5, 1.4, 1.5, 1.3 and 1.3 times increase compared to the treatment Inorganic N: P: K @105:53:105 g/plant + $ZnSO_4$ @0.5% (T_9; control).

5.2.2 Foliar application of treatment T_8 compared to all other applied treatments showed positive influence on generative traits of apple namely, trunk cross-sectional area (TCSA), canopy volume (CV) and canopy area (CA) compared to control.

5.2.3 Earliest date of pink bud emergence, initiation of flowering and date of full bloom was recorded in the treatment combination T_8. Moreover, duration of flowering (31.6%), days from full bloom to harvest (6.9%), length of flowering shoot (44.9%) and spur density (146.1%) were considerably increased through superior treatment T_8 in comparison to control.

5.2.4 The superior treatment T_8 registered the increment of 33.1, 45.2, 40.5 and 27.4 per cent in terms of numbers of flowers/shoot, number of fruits/shoot, fruit set and fruit yield compared to control.

5.2.5 Fruit physical parameters in terms of fruit length, fruit breadth, and fruit weight were markedly influenced by treatment T_8 with an increment of 10.1, 10.3 and 45.7 per cent over control. However, fruit firmness was recorded highest (6.27 kg/cm^2) in control.

5.2.6 Among different treatments, fruit biochemical attributes namely, TSS, total sugars, ascorbic acid and anthocyanins content were recorded maximum in treatment combination T_8, which increased by 1.2, 1.2, 2.0 and 1.2 times compared to control.

5.2.7 Significant improvement in soil chemical properties was recorded with the treatment T_8. Furthermore, the superior treatment of T_8 registered the increase of 7.4, 16.7, 26.7 and 35.3 per cent in terms of soil organic carbon, available N, P and K content compared to control.

5.2.8 Application of treatment T_8 registered significant influence on exchangeable Ca and Mg content, which improved by and 1.0 and 1.1 times in comparison to control. Similarly, DTPA extractable Zn, Fe, Cu and Mn content was increased by 36.9, 15.3, 60.8 and 31.7 per cent over control.

5.2.9 Soil microbial properties in terms of total bacterial count, soil fungi and actinobacterial count were increased by 20.0, 34.1 and 31.0 per cent through treatment combination T_8 as compared to control. Moreover, the superior treatment exhibited 1.3, 1.2, 1.4 and 1.3 times increase in phosphorus solubilizing bacteria, potassium solubilizing bacteria, zinc solubilizing bacteria and *Azotobacter* over control. AM fungi spore count (38.9%) was significantly increased through treatment T_8 in comparison to control.

5.2.10 Soil enzymatic activity *viz.*, acid phosphatase, alkaline phosphatase and dehydrogenase were significantly increased when supplemented with treatment combination of T_8. Furthermore, acid phosphatase, alkaline phosphatase and dehydrogenase exhibited an increment of 41.1, 40.3 and 117.1 per cent through treatment of T_8 over control.

5.2.11 Physiological parameters in terms of total chlorophylls, photosynthetic efficiency and transpiration rate were markedly influenced through treatment T_8 with an increment of 30.3, 53.0 and 46.1 per cent over control. Similarly, stomatal conductance was increased by 1.2 times compared to control.

5.2.12 Application of treatment T_8 registered significant influence on total carbohydrate content *viz*., fruiting shoots, non-fruiting shoots, leaves and fruits, which were improved by 1.3, 1.2, 1.4 and 1.3 times in comparison to control.

5.2.13 Leaf nutrient content in terms of N, P, K, Ca and Mg also exhibited an increase of 16.3, 64.7, 18.6, 17.3 and 37.0 per cent through superior treatment T_8 over control. Similarly, maximum leaf Zn content was registered with treatment T_8 with 1.9 times increase over control.

5.2.14 Different Zn treatments also observed maximum P, K, Ca, Mg and Zn content both in fruit flesh and peel in treatment combination of T_8.

5.2.15 Treatment combination of Zn-Gly_{400} + VC_{75} + AZ_{600} + AMF_{20} + $PGPR_{250}$ + HA_{120} (T_2) exhibited the lowest K/Ca ratio in terms of both peel and flesh. Moreover, treatment T_2 also registered the lowest (K+Mg)/Ca ratio for fruit peel. However, (K+Mg)/Ca ratio for fruit flesh was recorded minimum in treatments T_2 and nano Zn-AAC_{200} + VC_{80} + AZ_{300} + AMF_{25} + $PGPR_{200}$ (T_7).

5.2.16 Phytic acid content, PA: Ca, PA: Mg and PA: Zn were registered lowest in treatment T_8, which were decreased by 1.5, 2.1, 2.0 and 3.5 times, respectively compared to control. However, ursolic acid content exhibited an increment of 64.2 per cent through treatment combination T_8 in comparison to control.

5.2.17 PCA analysis identified that PC1 recorded the maximum variability (96.97%) based on *Eigen* value (>1). Similarly, PC1 exhibited the highest variability of 97.40 per cent for chemical and microbiological properties of soil.

CONCLUSION

The results of the present study conclude that the foliar application of Zn amino acid complexes namely, nano Zn-AAC, Zn-AAC, Zn-Gly and Zn-Glu offers a significant impact on fruit yield and quality attributes of apple by improving the vegetative growth, physiological parameters and nutrient content in leaves and fruits. Among all the Zn amino acid chelates, nano Zn-AAC was the best in improving the overall growth, quality and yield parameters by improving nutrients bioavailability and translocation within plants. Besides, the results demonstrated that the reduction in phytic acid content and increase in ursolic acid content resulted in agronomic Zn biofortification through enrichment of fruit nutrients. Based on the results of current study, use of nano Zn-AAC followed by other amino acid complexes emerged as a potent alternative to commonly used $ZnSO_4$ in apple fertilization. Moreover, considering the high consumption of apple, use of environment friendly Zn amino acid complexes are effective in improving the Zn nutritional status in humans.

Moreover, organic Zn sources supplemented with bio-organics represents a promising eco-friendly alternative to conventional fertilizers to enhance the growth, yield and fruit quality of apple under high density plantation. Besides, the rhizosphere microbiome supplemented with Zn-AAC improved the soil nutrient status and nutrient availability through biotransformation of the soil nutrients into available forms. Among all the applied treatments, $NZn\text{-}AAC_{400}$ + VC_{75} + AZ_{600} + AMF_{20} + $PGPR_{250}$ showed the best results followed by other bio-organic treatment combinations. These findings further highlight that the Zn amino acid chelates and bio-organic sources emerged as environmentally and economically beneficial alternatives to conventional $ZnSO_4$ and NPK in apple fertilization and further provides a significant contribution to the scientific community.

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APPENDIX-I

Agro-meteorological data (2021-2023) of the Experimental site at Department of Fruit Science, Dr YS Parmar University of Horticulture and Forestry, Solan, HP

Month	**Air temperature (ºC)**		**Relative humidity (%)**	**Rainfall (mm)**
	Maximum	**Minimum**		
October, 2021	27.1	11.8	66	52.6
November, 2021	23.9	4.8	54	NIL
December, 2021	18.6	2.3	61	14.3
January, 2022	16.1	3.2	58	258.8
February, 2022	18.7	2.7	55	82.7
March, 2022	28.6	9.4	44	NIL
April, 2022	33.2	13.3	37	1.0
May, 2022	32.0	16.1	52	84.8
June, 2022	32.1	17.7	49	85.6
July, 2022	28.5	20.5	51	195.4
August, 2022	29.0	19.9	78	219.5
September, 2022	27.8	17.8	77	233.5
October, 2022	26.3	11.4	64	79.7
November, 2022	24.1	6.4	55	2.4
December, 2022	21.7	2.5	51	NIL
January, 2023	18.8	2.9	61	39.2
February, 2023	23.8	5.6	52	7.2
March, 2023	23.6	8.1	64	130.6
April, 2023	26.6	10.5	51	114.3
May, 2023	27.9	13.2	58	152.3
June, 2023	29.3	17.4	65	154.0
July, 2023	27.5	20.1	83	660.5
August, 2023	29.0	20.1	78	283.4

Source: Department of Environmental Science, Dr Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (HP)

APPENDIX - II

Analysis of variance (ANOVA) for the effect of bio-organic Zn sources on plant height of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	48.845			
Treatment	8	648.237	81.03	161.369	0
Error	40	20.086	0.502		
Total	53	717.168			

ANOVA for the effect of bio-organic Zn sources on trunk girth of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	27.081			
Treatment	8	82.062	10.258	21.493	0
Error	40	19.091	0.477		
Total	53	128.234			

ANOVA for the effect of bio-organic Zn sources on canopy diameter of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	1,415.90			
Treatment	8	8,244.64	1,030.58	161.909	0
Error	40	254.607	6.365		
Total	53	9,915.15			

ANOVA for the effect of bio-organic Zn sources on shoot growth of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	55.842			
Treatment	8	782.338	97.792	74.581	0
Error	40	52.449	1.311		
Total	53	890.629			

ANOVA for the effect of bio-organic Zn sources on leaf area of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	4.227			
Treatment	8	939.373	117.422	140.926	0
Error	40	33.329	0.833		
Total	53	976.929			

ANOVA for the effect of bio-organic Zn sources on leaf area index of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	8.644			
Treatment	8	12.546	1.568	174.34	0
Error	40	0.36	0.009		
Total	53	21.549			

ANOVA for the effect of bio-organic Zn sources on TCSA of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	79.723			
Treatment	8	231.633	28.954	16.957	0
Error	40	68.302	1.708		
Total	53	379.659			

ANOVA for the effect of bio-organic Zn sources on CV of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	3.097			
Treatment	8	10.615	1.327	84.093	0
Error	40	0.631	0.016		
Total	53	14.343			

ANOVA for the effect of bio-organic Zn sources on CA of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.543			
Treatment	8	2.983	0.373	116.968	0
Error	40	0.128	0.003		
Total	53	3.653			

ANOVA for the effect of bio-organic Zn sources on duration of flowering of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	14.37			
Treatment	8	56.926	7.116	9.293	0
Error	40	30.63	0.766		
Total	53	101.926			

ANOVA for the effect of bio-organic Zn sources on days from full bloom to harvest of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	128.759			
Treatment	8	345.037	43.13	34	0
Error	40	50.741	1.269		
Total	53	524.537			

ANOVA for the effect of bio-organic Zn sources on length of flowering shoot of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	31.328			
Treatment	8	731.899	91.487	113.65	0
Error	40	32.2	0.805		
Total	53	795.427			

ANOVA for the effect of bio-organic Zn sources on spur density of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.001			
Treatment	8	0.062	0.008	41.934	0
Error	40	0.007	0		
Total	53	0.07			

ANOVA for the effect of bio-organic Zn sources on number of flowers/shoot of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	19.297			
Treatment	8	661.777	82.722	138.056	0
Error	40	23.968	0.599		
Total	53	705.042			

ANOVA for the effect of bio-organic Zn sources on number of fruits/shoot of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	22.516			
Treatment	8	459.788	57.474	342.981	0
Error	40	6.703	0.168		
Total	53	489.007			

ANOVA for the effect of bio-organic Zn sources on fruit set of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	92.143			
Treatment	8	2,410.24	301.28	46.149	0
Error	40	261.136	6.528		
Total	53	2,763.52			

ANOVA for the effect of bio-organic Zn sources on fruit yield of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.858			
Treatment	8	7.658	0.957	48.38	0
Error	40	0.791	0.02		
Total	53	9.307			

ANOVA for the effect of bio-organic Zn sources on Y/LA of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.001			
Treatment	8	0.005	0.001	13.983	0
Error	40	0.002	0		
Total	53	0.007			

ANOVA for the effect of bio-organic Zn sources on Y/TCSA of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.22			
Treatment	8	0.523	0.065	14.45	0
Error	40	0.181	0.005		
Total	53	0.925			

ANOVA for the effect of bio-organic Zn sources on Y/CV of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	5.9			
Treatment	8	17.66	2.208	114.479	0
Error	40	0.771	0.019		
Total	53	24.331			

ANOVA for the effect of bio-organic Zn sources on Y/CA of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	3.574			
Treatment	8	19.263	2.408	64.152	0
Error	40	1.501	0.038		
Total	53	24.338			

ANOVA for the effect of bio-organic Zn sources on fruit length of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	1,449.38			
Treatment	8	298.591	37.324	10.402	0
Error	40	143.522	3.588		
Total	53	1,891.50			

ANOVA for the effect of bio-organic Zn sources on fruit breadth of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	502.11			
Treatment	8	364.717	45.59	7.208	0.00001
Error	40	253.011	6.325		
Total	53	1,119.84			

ANOVA for the effect of bio-organic Zn sources on shape index of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.113			
Treatment	8	0.009	0.001	0.782	0.62106
Error	40	0.056	0.001		
Total	53	0.178			

ANOVA for the effect of bio-organic Zn sources on fruit weight of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	13,413.42			
Treatment	8	6,962.00	870.25	33.2	0
Error	40	1,048.49	26.212		
Total	53	21,423.90			

ANOVA for the effect of bio-organic Zn sources on fruit volume of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	16,864.64			
Treatment	8	10,027.41	1,253.43	18.757	0
Error	40	2,673.02	66.825		
Total	53	29,565.07			

ANOVA for the effect of bio-organic Zn sources on specific gravity of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.001			
Treatment	8	0.036	0.005	4.779	0.00036
Error	40	0.038	0.001		
Total	53	0.075			

ANOVA for the effect of bio-organic Zn sources on fruit firmness of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	16.797			
Treatment	8	10.547	1.318	78.303	0
Error	40	0.673	0.017		
Total	53	28.017			

ANOVA for the effect of bio-organic Zn sources on TSS content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	7.915			
Treatment	8	33.696	4.212	63.635	0
Error	40	2.648	0.066		
Total	53	44.258			

ANOVA for the effect of bio-organic Zn sources on titratable acidity of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.018			
Treatment	8	0.238	0.03	137.791	0
Error	40	0.009	0		
Total	53	0.264			

ANOVA for the effect of bio-organic Zn sources on reducing sugars content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	2.696			
Treatment	8	5.926	0.741	26.053	0
Error	40	1.137	0.028		
Total	53	9.759			

ANOVA for the effect of bio-organic Zn sources on non-reducing sugars content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	1,415.90			
Treatment	8	8,244.64	1,030.58	161.909	0
Error	40	254.607	6.365		
Total	53	9,915.15			

ANOVA for the effect of bio-organic Zn sources on total sugars content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	12.71			
Treatment	8	17.035	2.129	30.867	0
Error	40	2.759	0.069		
Total	53	32.504			

ANOVA for the effect of bio-organic Zn sources on ascorbic acid content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	3.246			
Treatment	8	3.021	0.378	8.748	0
Error	40	1.727	0.043		
Total	53	7.994			

ANOVA for the effect of bio-organic Zn sources on anthocyanins content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	1.225			
Treatment	8	10.941	1.368	51.904	0
Error	40	1.054	0.026		
Total	53	13.22			

ANOVA for the effect of bio-organic Zn sources on total phenols of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	1.202			
Treatment	8	11.899	1.487	37.685	0
Error	40	1.579	0.039		
Total	53	14.679			

ANOVA for the effect of bio-organic Zn sources on pH in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	68.662			
Treatment	8	761.183	95.148	26.401	0
Error	40	144.159	3.604		
Total	53	974.004			

ANOVA for the effect of bio-organic Zn sources on electrical conductivity in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.97			
Treatment	8	0.618	0.077	1.393	0.22906
Error	40	2.216	0.055		
Total	53	3.803			

ANOVA for the effect of bio-organic Zn sources on organic carbon content in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.053			
Treatment	8	0.033	0.004	1.885	0.0896
Error	40	0.088	0.002		
Total	53	0.175			

ANOVA for the effect of bio-organic Zn sources on available N content in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.051			
Treatment	8	0.244	0.031	8.754	0
Error	40	0.14	0.003		
Total	53	0.435			

ANOVA for the effect of bio-organic Zn sources on available P content in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	1,776.17			
Treatment	8	8,709.41	1,088.68	308.792	0
Error	40	141.024	3.526		
Total	53	10,626.60			

ANOVA for the effect of bio-organic Zn sources on available K content in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	722.038			
Treatment	8	6,084.46	760.557	598.428	0
Error	40	50.837	1.271		
Total	53	6,857.33			

ANOVA for the effect of bio-organic Zn sources on exchangeable Ca content in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	1,500.74			
Treatment	8	6,195.99	774.498	1,114.50	0
Error	40	27.797	0.695		
Total	53	7,724.53			

ANOVA for the effect of bio-organic Zn sources on exchangeable Mg content in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	1,500.74			
Treatment	8	6,195.99	774.498	1,114.50	0
Error	40	27.797	0.695		
Total	53	7,724.53			

ANOVA for the effect of bio-organic Zn sources on DTPA extractable Zn content in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	726.008			
Treatment	8	13,952.77	1,744.10	289.785	0
Error	40	240.743	6.019		
Total	53	14,919.52			

ANOVA for the effect of bio-organic Zn sources on DTPA extractable Fe content in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	759.553			
Treatment	8	7,953.00	994.125	194.53	0
Error	40	204.416	5.11		
Total	53	8,916.97			

ANOVA for the effect of bio-organic Zn sources on DTPA extractable Cu content in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	114.589			
Treatment	8	86.957	10.87	26.631	0
Error	40	16.327	0.408		
Total	53	217.873			

ANOVA for the effect of bio-organic Zn sources on DTPA extractable Mn content in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	114.825			
Treatment	8	337.585	42.198	50.634	0
Error	40	33.336	0.833		
Total	53	485.745			

ANOVA for the effect bio-organic Zn sources on bacterial count in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	717.033			
Treatment	8	5,410.43	676.304	148.716	0
Error	40	181.905	4.548		
Total	53	6,309.37			

ANOVA for the effect of bio-organic Zn sources on fungal count in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	4,134.95			
Treatment	8	6,465.80	808.225	78.575	0
Error	40	411.441	10.286		
Total	53	11,012.19			

ANOVA for the effect of bio-organic Zn sources on actinobacterial count in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	76.994			
Treatment	8	1,316.55	164.568	335.108	0
Error	40	19.644	0.491		
Total	53	1,413.19			

ANOVA for the effect of bio-organic Zn sources on PSB count in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	12.663			
Treatment	8	136.291	17.036	149.923	0
Error	40	4.545	0.114		
Total	53	153.5			

ANOVA for the effect of bio-organic Zn sources on KSB count in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	10.721			
Treatment	8	119.243	14.905	292.898	0
Error	40	2.036	0.051		
Total	53	131.999			

ANOVA for the effect of bio-organic Zn sources on ZSB count in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	37.745			
Treatment	8	261.024	32.628	110.897	0
Error	40	11.769	0.294		
Total	53	310.538			

ANOVA for the effect of bio-organic Zn sources on *Azotobacter* count in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	12.491			
Treatment	8	108.177	13.522	220.801	0
Error	40	2.45	0.061		
Total	53	123.118			

ANOVA for the effect of bio-organic Zn sources on AM Fungi count in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	14.53			
Treatment	8	109.529	13.691	278.43	0
Error	40	1.967	0.049		
Total	53	126.026			

ANOVA for the effect of bio-organic Zn sources on acid phosphatase activity in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	89.446			
Treatment	8	672.621	84.078	117.247	0
Error	40	28.684	0.717		
Total	53	790.751			

ANOVA for the effect of bio-organic Zn sources on alkaline phosphatase activity in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	13.202			
Treatment	8	148.213	18.527	17.128	0
Error	40	43.267	1.082		
Total	53	204.682			

ANOVA for the effect of bio-organic Zn sources on dehydrogenase activity in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	7.794			
Treatment	8	67.779	8.472	161.007	0
Error	40	2.105	0.053		
Total	53	77.678			

ANOVA for the effect of bio-organic Zn sources on total chlorophylls in leaves of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.321			
Treatment	8	0.494	0.062	4.197	0.00102
Error	40	0.588	0.015		
Total	53	1.403			

ANOVA for the effect of bio-organic Zn sources on photosynthetic efficiency of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.021			
Treatment	8	0.485	0.061	47.315	0
Error	40	0.051	0.001		
Total	53	0.557			

ANOVA for the effect of bio-organic Zn sources on transpiration rate of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0			
Treatment	8	0.002	0	29.092	0
Error	40	0	0		
Total	53	0.003			

ANOVA for the effect of bio-organic Zn sources on stomatal conductance of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.308			
Treatment	8	43.83	5.479	131.719	0
Error	40	1.664	0.042		
Total	53	45.802			

ANOVA for the effect of bio-organic Zn sources on total carbohydrate content of fruiting shoots of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	172.348			
Treatment	8	3,233.91	404.238	175.945	0
Error	40	91.901	2.298		
Total	53	3,498.16			

ANOVA for the effect of bio-organic Zn sources on total carbohydrate content of non-fruiting shoots of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	44.282			
Treatment	8	753.837	94.23	29.633	0
Error	40	127.195	3.18		
Total	53	925.314			

ANOVA for the effect of bio-organic Zn sources on total carbohydrate content of leaves of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.172			
Treatment	8	5.29	0.661	161.488	0
Error	40	0.164	0.004		
Total	53	5.626			

ANOVA for the effect of bio-organic Zn sources on total carbohydrate content of fruits of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	139.168			
Treatment	8	737.304	92.163	95.768	0
Error	40	38.494	0.962		
Total	53	914.967			

ANOVA for the effect of bio-organic Zn sources on leaf N content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	15.173			
Treatment	8	47.103	5.888	200.145	0
Error	40	1.177	0.029		
Total	53	63.453			

ANOVA for the effect of bio-organic Zn sources on leaf P content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	2,023.42			
Treatment	8	5,419.19	677.399	34.286	0
Error	40	790.294	19.757		
Total	53	8,232.91			

ANOVA for the effect of bio-organic Zn sources on leaf K content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.03			
Treatment	8	1.915	0.239	128.854	0
Error	40	0.074	0.002		
Total	53	2.02			

ANOVA for the effect of bio-organic Zn sources on leaf Ca content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.004			
Treatment	8	0.062	0.008	58	0
Error	40	0.005	0		
Total	53	0.071			

ANOVA for the effect of bio-organic Zn sources on leaf Mg content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.045			
Treatment	8	0.515	0.064	79.653	0
Error	40	0.032	0.001		
Total	53	0.593			

ANOVA for the effect of bio-organic Zn sources on leaf Zn content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.023			
Treatment	8	0.365	0.046	24.261	0
Error	40	0.075	0.002		
Total	53	0.463			

ANOVA for the effect of bio-organic Zn sources on flesh P content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.003			
Treatment	8	0.045	0.006	57.918	0
Error	40	0.004	0		
Total	53	0.051			

ANOVA for the effect of bio-organic Zn sources on peel P content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	474.57			
Treatment	8	10,902.12	1,362.77	263.717	0
Error	40	206.701	5.168		
Total	53	11,583.39			

ANOVA for the effect of bio-organic Zn sources on flesh K content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.002			
Treatment	8	0.001	0	72.486	0
Error	40	0	0		
Total	53	0.003			

ANOVA for the effect of bio-organic Zn sources on peel K content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.019			
Treatment	8	0.002	0	31.636	0
Error	40	0	0		
Total	53	0.021			

ANOVA for the effect of bio-organic Zn sources on flesh Ca content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.484			
Treatment	8	0.127	0.016	42.335	0
Error	40	0.015	0		
Total	53	0.626			

ANOVA for the effect of bio-organic Zn sources on peel Ca content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.507			
Treatment	8	0.557	0.07	58.777	0
Error	40	0.047	0.001		
Total	53	1.112			

ANOVA for the effect of bio-organic Zn sources on flesh Mg content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.012			
Treatment	8	0.067	0.008	127.332	0
Error	40	0.003	0		
Total	53	0.081			

ANOVA for the effect of bio-organic Zn sources on peel Mg content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.097			
Treatment	8	0.15	0.019	53.25	0
Error	40	0.014	0		
Total	53	0.261			

ANOVA for the effect of bio-organic Zn sources on flesh Zn content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.002			
Treatment	8	0.004	0.001	91.237	0
Error	40	0	0		
Total	53	0.006			

ANOVA for the effect of bio-organic Zn sources on peel Zn content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0			
Treatment	8	0.012	0.001	87.439	0
Error	40	0.001	0		
Total	53	0.013			

ANOVA for the effect of bio-organic Zn sources on flesh K/Ca ratio of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.438			
Treatment	8	0.401	0.05	8.348	0
Error	40	0.24	0.006		
Total	53	1.079			

ANOVA for the effect of bio-organic Zn sources on flesh (K+Mg)/Ca ratio of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.275			
Treatment	8	0.396	0.05	7.793	0
Error	40	0.254	0.006		
Total	53	0.925			

ANOVA for the effect of bio-organic Zn sources on peel K/Ca ratio of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	31.546			
Treatment	8	134.662	16.833	107.626	0
Error	40	6.256	0.156		
Total	53	172.464			

ANOVA for the effect of bio-organic Zn sources on peel (K+Mg)/Ca ratio of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	3,114.90			
Treatment	8	12,544.67	1,568.08	157.458	0
Error	40	398.35	9.959		
Total	53	16,057.92			

ANOVA for the effect of bio-organic Zn sources on phytic acid content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	31.04			
Treatment	8	2,058.83	257.353	76.32	0
Error	40	134.882	3.372		
Total	53	2,224.75			

ANOVA for the effect of bio-organic Zn sources on fruit PA: Ca ratio of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0			
Treatment	8	0.002	0	108.129	0
Error	40	0	0		
Total	53	0.002			

ANOVA for the effect of bio-organic Zn sources on fruit PA: Mg ratio of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	2	0.738			
Treatment	8	15,195.88	1,899.49	649.749	0
Error	16	46.775	2.923		
Total	26	15,243.39			

ANOVA for the effect of bio-organic Zn sources on fruit PA: Zn ratio of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	4.588			
Treatment	8	11.529	1.441	117.588	0
Error	40	0.49	0.012		
Total	53	16.606			

ANOVA for the effect of bio-organic Zn sources on ursolic acid content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0			
Treatment	8	0.001	0	77.236	0
Error	40	0	0		
Total	53	0.001			

ANOVA for the effect of organic Zn and bio-organic sources on plant height of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	70.413			
Treatment	8	1,321.38	165.172	262.468	0
Error	40	25.172	0.629		
Total	53	1,416.96			

ANOVA for the effect of organic Zn and bio-organic sources on trunk girth of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	10.593			
Treatment	8	88.535	11.067	173.601	0
Error	40	2.55	0.064		
Total	53	101.678			

ANOVA for the effect of organic Zn and bio-organic sources on canopy diameter of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	586.05			
Treatment	8	12,025.67	1,503.21	177.242	0
Error	40	339.245	8.481		
Total	53	12,950.96			

ANOVA for the effect of organic Zn and bio-organic sources on shoot growth of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	62.608			
Treatment	8	716.366	89.546	105.785	0
Error	40	33.86	0.846		
Total	53	812.834			

ANOVA for the effect of organic Zn and bio-organic sources on leaf area of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	9.973			
Treatment	8	452.246	56.531	97.816	0
Error	40	23.117	0.578		
Total	53	485.336			

ANOVA for the effect of organic Zn and bio-organic sources on leaf area index of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.035			
Treatment	8	1.081	0.135	62.393	0
Error	40	0.087	0.002		
Total	53	1.203			

ANOVA for the effect of organic Zn and bio-organic sources on TCSA of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	34.57			
Treatment	8	310.122	38.765	157.136	0
Error	40	9.868	0.247		
Total	53	354.56			

ANOVA for the effect of organic Zn and bio-organic sources on CV of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	2.515			
Treatment	8	22.397	2.8	120.926	0
Error	40	0.926	0.023		
Total	53	25.837			

ANOVA for the effect of organic Zn and bio-organic sources on CA of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.242			
Treatment	8	4.542	0.568	145.178	0
Error	40	0.156	0.004		
Total	53	4.94			

ANOVA for the effect of organic Zn and bio-organic sources on duration of flowering of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	13.481			
Treatment	8	42.037	5.255	10.413	0
Error	40	20.185	0.505		
Total	53	75.704			

ANOVA for the effect of organic Zn and bio-organic sources on days from full bloom to harvest of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	332.833			
Treatment	8	317.333	39.667	37.188	0
Error	40	42.667	1.067		
Total	53	692.833			

ANOVA for the effect of organic Zn and bio-organic sources on length of flowering shoot of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	73.203			
Treatment	8	978.1	122.262	91.867	0
Error	40	53.234	1.331		
Total	53	1,104.54			

ANOVA for the effect of organic Zn and bio-organic sources on spur density of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.017			
Treatment	8	0.195	0.024	47.902	0
Error	40	0.02	0.001		
Total	53	0.232			

ANOVA for the effect of organic Zn and bio-organic sources on number of flowers/shoot of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	45.461			
Treatment	8	549.276	68.659	133.058	0
Error	40	20.64	0.516		
Total	53	615.377			

ANOVA for the effect of organic Zn and bio-organic sources on number of fruits/shoot of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	15.992			
Treatment	8	302.471	37.809	134.039	0
Error	40	11.283	0.282		
Total	53	329.746			

ANOVA for the effect of organic Zn and bio-organic sources on fruit set of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	290.357			
Treatment	8	2,096.50	262.063	29.861	0
Error	40	351.043	8.776		
Total	53	2,737.90			

ANOVA for the effect of organic Zn and bio-organic sources on fruit yield of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.476			
Treatment	8	9.975	1.247	91.02	0
Error	40	0.548	0.014		
Total	53	10.999			

ANOVA for the effect of organic Zn and bio-organic sources on Y/LA of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0			
Treatment	8	0.001	0	3.264	0.0059
Error	40	0.001	0		
Total	53	0.002			

ANOVA for the effect of organic Zn and bio-organic sources on Y/TCSA of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.071			
Treatment	8	0.324	0.04	54.189	0
Error	40	0.03	0.001		
Total	53	0.425			

ANOVA for the effect of organic Zn and bio-organic sources on Y/CV of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	5.32			
Treatment	8	47.9	5.988	180.489	0
Error	40	1.327	0.033		
Total	53	54.547			

ANOVA for the effect of organic Zn and bio-organic sources on Y/CA of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	1.153			
Treatment	8	12.583	1.573	5.339	0.00014
Error	40	11.784	0.295		
Total	53	25.521			

ANOVA for the effect of organic Zn and bio-organic sources on fruit length of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	1,046.46			
Treatment	8	149.206	18.651	8.762	0
Error	40	85.142	2.129		
Total	53	1,280.81			

ANOVA for the effect of organic Zn and bio-organic sources on fruit breadth of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	592.864			
Treatment	8	225.217	28.152	7.769	0
Error	40	144.944	3.624		
Total	53	963.025			

ANOVA for the effect of organic Zn and bio-organic sources on shape index of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.037			
Treatment	8	0.001	0	0.152	0.99571
Error	40	0.042	0.001		
Total	53	0.08			

ANOVA for the effect of organic Zn and bio-organic sources on fruit weight of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	25,262.86			
Treatment	8	10,642.55	1,330.32	50.923	0
Error	40	1,044.96	26.124		
Total	53	36,950.37			

ANOVA for the effect of organic Zn and bio-organic sources on fruit volume of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	25,339.74			
Treatment	8	14,726.29	1,840.79	85.649	0
Error	40	859.688	21.492		
Total	53	40,925.72			

ANOVA for the effect of organic Zn and bio-organic sources on specific gravity of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.017			
Treatment	8	0.012	0.002	4.3	0.00084
Error	40	0.015	0		
Total	53	0.044			

ANOVA for the effect of organic Zn and bio-organic sources on fruit firmness of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	18.271			
Treatment	8	8.605	1.076	268.421	0
Error	40	0.16	0.004		
Total	53	27.037			

ANOVA for the effect of organic Zn and bio-organic sources on TSS content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	13.8			
Treatment	8	25.245	3.156	43.05	0
Error	40	2.932	0.073		
Total	53	41.977			

ANOVA for the effect of organic Zn and bio-organic sources on titratable acidity content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.146			
Treatment	8	0.186	0.023	119.061	0
Error	40	0.008	0		
Total	53	0.34			

ANOVA for the effect of organic Zn and bio-organic sources on reducing sugars content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	1.451			
Treatment	8	14.392	1.799	70.909	0
Error	40	1.015	0.025		
Total	53	16.857			

ANOVA for the effect of organic Zn and bio-organic sources on non-reducing sugars content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	1.241			
Treatment	8	1.781	0.223	4.082	0.00126
Error	40	2.181	0.055		
Total	53	5.204			

ANOVA for the effect of organic Zn and bio-organic sources on total sugars content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	5.983			
Treatment	8	26.586	3.323	47.837	0
Error	40	2.779	0.069		
Total	53	35.347			

ANOVA for the effect of organic Zn and bio-organic sources on ascorbic acid content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	4.865			
Treatment	8	19.693	2.462	60.244	0
Error	40	1.634	0.041		
Total	53	26.193			

ANOVA for the effect of organic Zn and bio-organic sources on anthocyanins content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.727			
Treatment	8	22.958	2.87	44.381	0
Error	40	2.586	0.065		
Total	53	26.272			

ANOVA for the effect of organic Zn and bio-organic sources on total phenols of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	93.808			
Treatment	8	3,459.21	432.402	236.665	0
Error	40	73.083	1.827		
Total	53	3,626.11			

ANOVA for the effect of organic Zn and bio-organic sources on pH of soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	2.6			
Treatment	8	1.213	0.152	122.861	0
Error	40	0.049	0.001		
Total	53	3.863			

ANOVA for the effect of organic Zn and bio-organic sources on electrical conductivity of soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.089			
Treatment	8	0.053	0.007	8.039	0
Error	40	0.033	0.001		
Total	53	0.175			

ANOVA for the effect of organic Zn and bio-organic sources on organic carbon content in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.019			
Treatment	8	0.179	0.022	6.657	0.00002
Error	40	0.134	0.003		
Total	53	0.332			

ANOVA for the effect of organic Zn and bio-organic sources on available N content in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	1,852.30			
Treatment	8	21,666.06	2,708.26	286.967	0
Error	40	377.5	9.438		
Total	53	23,895.85			

ANOVA for the effect of organic Zn and bio-organic sources on available P content in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	878.764			
Treatment	8	9,643.24	1,205.40	1,256.59	0
Error	40	38.371	0.959		
Total	53	10,560.37			

ANOVA for the effect of organic Zn and bio-organic sources on available K content in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	3,860.45			
Treatment	8	39,629.62	4,953.70	388.57	0
Error	40	509.942	12.749		
Total	53	44,000.01			

ANOVA for the effect of organic Zn and bio-organic sources on exchangeable Ca content in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	1,821.04			
Zinc lls	8	21,551.58	2,693.95	778.669	0
Error	40	138.387	3.46		
Total	53	23,511.01			

ANOVA for the effect of organic Zn and bio-organic sources on exchangeable Mg content in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	953.725			
treatments	8	6,109.09	763.636	133.344	0
Error	40	229.072	5.727		
Total	53	7,291.88			

ANOVA for the effect of organic Zn and bio-organic sources on DTPA extractable Zn content in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	31.613			
Treatment	8	727.16	90.895	139.31	0
Error	40	26.099	0.652		
Total	53	784.871			

ANOVA for the effect of organic Zn and bio-organic sources on DTPA extractable Fe content in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	35.183			
Treatment	8	438.825	54.853	14.209	0
Error	40	154.423	3.861		
Total	53	628.431			

ANOVA for the effect of organic Zn and bio-organic sources DTPA extractable Cu content in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	15.625			
Treatment	8	183.987	22.998	216.434	0
Error	40	4.25	0.106		
Total	53	203.862			

ANOVA for the effect of organic Zn and bio-organic sources DTPA extractable Mn content in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	43.883			
Treatment	8	468.01	58.501	77.501	0
Error	40	30.194	0.755		
Total	53	542.086			

ANOVA for the effect of organic Zn and bio-organic sources on total bacterial count in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	97.308			
Treatment	8	2,177.14	272.143	307.431	0
Error	40	35.409	0.885		
Total	53	2,309.86			

ANOVA for the effect of organic Zn and bio-organic sources on fungal count in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	18.102			
Treatment	8	192.034	24.004	207.297	0
Error	40	4.632	0.116		
Total	53	214.767			

ANOVA for the effect of organic Zn and bio-organic sources on actinobacterial count in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	22.693			
Treatment	8	602.127	75.266	251.483	0
Error	40	11.972	0.299		
Total	53	636.792			

ANOVA for the effect of organic Zn and bio-organic sources on PSB count in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	15.13			
Treatment	8	153.892	19.236	108.896	0
Error	40	7.066	0.177		
Total	53	176.087			

ANOVA for the effect of organic Zn and bio-organic sources on KSB count in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	7.103			
Treatment	8	75.963	9.495	110.573	0
Error	40	3.435	0.086		
Total	53	86.501			

ANOVA for the effect of organic Zn and bio-organic sources on ZSB count in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	11.443			
Treatment	8	184.631	23.079	125.026	0
Error	40	7.384	0.185		
Total	53	203.458			

ANOVA for the effect of organic Zn and bio-organic sources on *Azotobacter* count in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	23.843			
Treatment	8	844.211	105.526	360.801	0
Error	40	11.699	0.292		
Total	53	879.752			

ANOVA for the effect of organic Zn and bio-organic sources on AM Fungi count in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	5,619.38			
Treatment	8	72,674.87	9,084.36	848.515	0
Error	40	428.247	10.706		
Total	53	78,722.50			

ANOVA for the effect of organic Zn and bio-organic sources on acid phosphatase activity in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	6,079.37			
Treatment	8	14,607.99	1,826.00	275.128	0
Error	40	265.476	6.637		
Total	53	20,952.83			

ANOVA for the effect of organic Zn and bio-organic sources on alkaline phosphatase activity in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	4,996.60			
Treatment	8	17,101.28	2,137.66	339.413	0
Error	40	251.925	6.298		
Total	53	22,349.80			

ANOVA for the effect of organic Zn and bio-organic sources on dehydrogenase activity in soils of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	327.477			
Treatment	8	643.922	80.49	114.882	0
Error	40	28.025	0.701		
Total	53	999.424			

ANOVA for the effect of organic Zn and bio-organic sources on total chlorophyll content of leaves of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	8.077			
Treatment	8	4.414	0.552	101.173	0
Error	40	0.218	0.005		
Total	53	12.708			

ANOVA for the effect of organic Zn and bio-organic sources on photosynthetic efficiency of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.411			
Treatment	8	59.495	7.437	318.351	0
Error	40	0.934	0.023		
Total	53	60.84			

ANOVA for the effect of organic Zn and bio-organic sources on transpiration rate of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.075			
Treatment	8	5.33	0.666	196.571	0
Error	40	0.136	0.003		
Total	53	5.54			

ANOVA for the effect of organic Zn and bio-organic sources on stomatal conductance of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0			
Treatment	8	0.004	0	86.861	0
Error	40	0	0		
Total	53	0.004			

ANOVA for the effect of organic Zn and bio-organic sources on total carbohydrate content in fruiting shoots of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	48.536			
Treatment	8	1,313.60	164.2	56.579	0
Error	40	116.086	2.902		
Total	53	1,478.22			

ANOVA for the effect of organic Zn and bio-organic sources total carbohydrate content in non-fruiting shoots of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	60.16			
Treatment	8	1,737.28	217.16	104.161	0
Error	40	83.394	2.085		
Total	53	1,880.83			

ANOVA for the effect of organic Zn and bio-organic sources on total carbohydrate content in leaves of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	92.418			
Treatment	8	687.66	85.957	93.554	0
Error	40	36.752	0.919		
Total	53	816.829			

ANOVA for the effect of organic Zn and bio-organic sources on total carbohydrate content in fruits of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	174.781			
Treatment	8	8,222.47	1,027.81	158.94	0
Error	40	258.665	6.467		
Total	53	8,655.91			

ANOVA for the effect of organic Zn and bio-organic sources on leaf N content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.159			
Treatment	8	0.668	0.083	26.792	0
Error	40	0.125	0.003		
Total	53	0.951			

ANOVA for the effect of organic Zn and bio-organic sources on leaf P content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.004			
Treatment	8	0.063	0.008	62.187	0
Error	40	0.005	0		
Total	53	0.072			

ANOVA for the effect of organic Zn and bio-organic sources on leaf K content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.03			
Treatment	8	0.471	0.059	34.901	0
Error	40	0.068	0.002		
Total	53	0.569			

ANOVA for the effect of organic Zn and bio-organic sources on leaf Ca content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.012			
Treatment	8	0.48	0.06	67.306	0
Error	40	0.036	0.001		
Total	53	0.528			

ANOVA for the effect of organic Zn and bio-organic sources on leaf Mg content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.003			
Treatment	8	0.064	0.008	104.345	0
Error	40	0.003	0		
Total	53	0.07			

ANOVA for the effect of organic Zn and bio-organic sources on leaf Zn content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	195.411			
Treatment	8	10,324.89	1,290.61	709.22	0
Error	40	72.79	1.82		
Total	53	10,593.09			

ANOVA for the effect of organic Zn and bio-organic sources on flesh P content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.002			
Treatment	8	0.001	0	61.334	0
Error	40	0	0		
Total	53	0.003			

ANOVA for the effect of organic Zn and bio-organic sources on peel P content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0			
Treatment	8	0.013	0.002	30.597	0
Error	40	0.002	0		
Total	53	0.015			

ANOVA for the effect of organic Zn and bio-organic sources on flesh K content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.396			
Treatment	8	0.124	0.016	32.397	0
Error	40	0.019	0		
Total	53	0.539			

ANOVA for the effect of organic Zn and bio-organic sources on peel K content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.404			
Treatment	8	0.63	0.079	289.38	0
Error	40	0.011	0		
Total	53	1.045			

ANOVA for the effect of organic Zn and bio-organic sources on flesh Ca content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.02			
Treatment	8	0.056	0.007	62.029	0
Error	40	0.005	0		
Total	53	0.081			

ANOVA for the effect of organic Zn and bio-organic sources on peel Ca content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.175			
Treatment	8	0.176	0.022	59.575	0
Error	40	0.015	0		
Total	53	0.365			

ANOVA for the effect of organic Zn and bio-organic sources on flesh Mg content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0			
Treatment	8	0.004	0	66.885	0
Error	40	0	0		
Total	53	0.004			

ANOVA for the effect of organic Zn and bio-organic sources on peel Mg content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.002			
Treatment	8	0.013	0.002	70.487	0
Error	40	0.001	0		
Total	53	0.016			

ANOVA for the effect of organic Zn and bio-organic sources on flesh Zn content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0			
Treatment	8	0.001	0	118.833	0
Error	40	0	0		
Total	53	0.001			

ANOVA for the effect of organic Zn and bio-organic sources on peel Zn content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.001			
Treatment	8	0.004	0	54.571	0
Error	40	0	0		
Total	53	0.005			

ANOVA for the effect of organic Zn and bio-organic sources on flesh K/Ca ratio of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.005			
Treatment	8	0.247	0.031	45.588	0
Error	40	0.027	0.001		
Total	53	0.28			

ANOVA for the effect of organic Zn and bio-organic sources on flesh (K+Mg)/Ca ratio of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.01			
Treatment	8	0.278	0.035	33.361	0
Error	40	0.042	0.001		
Total	53	0.33			

ANOVA for the effect of organic Zn and bio-organic sources on peel K/Ca ratio of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.06			
Treatment	8	0.323	0.04	13.144	0
Error	40	0.123	0.003		
Total	53	0.505			

ANOVA for the effect of organic Zn and bio-organic sources on peel (K+Mg)/Ca ratio of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	0.016			
Treatment	8	0.312	0.039	8.45	0
Error	40	0.185	0.005		
Total	53	0.512			

ANOVA for the effect of organic Zn and bio-organic sources on phytic acid content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	1.168			
Treatment	8	1.909	0.239	76.771	0
Error	40	0.124	0.003		
Total	53	3.202			

ANOVA for the effect of organic Zn and bio-organic sources on fruit PA: Ca ratio of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	7.701			
Treatment	8	11.129	1.391	58.052	0
Error	40	0.959	0.024		
Total	53	19.788			

ANOVA for the effect of organic Zn and bio-organic sources on fruit PA: Mg ratio of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	42.439			
Treatment	8	124.451	15.556	114.382	0
Error	40	5.44	0.136		
Total	53	172.33			

ANOVA for the effect of organic Zn and bio-organic sources on fruit PA: Zn ratio of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	5	4,282.23			
Treatment	8	11,119.93	1,389.99	107.967	0
Error	40	514.969	12.874		
Total	53	15,917.12			

ANOVA for the effect of organic Zn and bio-organic sources on ursolic acid content of apple

Source of Variation	DF	Sum of Squares	Mean Squares	F-calculated	Significance
Replication	2	0.292			
Treatment	8	30,060.81	3,757.60	6,295.59	0
Error	16	9.55	0.597		
Total	26	30,070.65			

Department of Fruit Science
Dr Yashwant Singh Parmar University of Horticulture & Forestry
(Nauni) Solan (HP) – 173 230 India

Title of thesis : **Zinc biofortification through organic Zn and bio-organic supplements in high density plantation of apple (*Malus* × *domestica* Borkh.)**
Name of the Student : Simran Saini
Admission Number : H-2020-45-D
Major Discipline : Fruit Science
Minor Discipline : i) Soil Science
ii) Microbiology
Date of Thesis Submission : 28/08/2024
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Major Advisor : Dr Pramod Kumar

Abstract

Micronutrient malnutrition is a critical health issue affecting people worldwide. Zinc deficiency is particularly widespread due to poor soil health, inadequate dietary intake and low bioavailability leading to significant health consequences. To address this issue, the use of organic Zn and bio-organics emerged as a most recent technology to improve both plant and human health by providing a safer and more efficient nutrient source compared to traditional fertilizers. In this regard, present study was carried out during the years 2022 and 2023 with the objectives to evaluate the effect of organic Zn and bio-organics on the growth, yield, quality and soil properties of apple cv. Gala Schniga Schnico under high density plantation. The first experiment was comprised of foliar application of bio-organic Zn sources namely, Zn glycinate (Zn-Gly), Zn gluconate (Zn-Glu), Zn amino acid chelate (Zn-AAC), nano Zn amino acid chelate (NZn-AAC) were applied at 200 and 400 mg/L each and conventional $ZnSO_4$ at 0.5% (control). The second experiment was composed of foliar application of organic Zn sources and soil application of bio-organic amendments in comparison with RDF of NPK + $ZnSO_4$ (control) in nine treatment combinations (T_1 - T_9). Foliar application of the Zn nutrient sources was done at four stages i.e. 15 days before petal fall, petal fall, 15 days after petal fall and after harvest. All the treatments were replicated thrice. In the first experiment, foliar application of NZn-AAC$_{400}$ registered maximum spur density, fruit set and fruit yield with 92.3, 47.2 and 27.4 per cent increase over the control. TSS and anthocyanins content was also increased by 1.2 times through NZn-AAC$_{400}$. Microbial biomass in terms of total bacterial count (1.50×10^6 cfu/g), soil fungi (1.03×10^3 cfu/g) and actinobacterial count (1.09×10^4 cfu/g) was also recorded highest in treatment NZn-AAC$_{400}$. Similarly, PSB, KSB, ZSB, *Azotobacter* and AMF count were increased by 1.6, 1.3, 1.4, 1.2 and 1.3 times, respectively compared to control. Total carbohydrate content *viz*., fruiting shoots, non-fruiting shoots, leaves and fruits exhibited an increase of 20.8, 13.2, 39.7 and 27.8 per cent through NZn-AAC$_{400}$. Application of NZn-AAC$_{400}$ exhibited maximum decrease of phytic acid (46.7%) and increase in ursolic acid (37.5%) over control. In the second experiment, conjoint application of treatment nano Zn-AAC$_{400}$ + VC$_{75}$ + AZ$_{600}$ + AMF$_{20}$ + PGPR$_{250}$ (T_8) registered the highest increment of 146.1, 40.5 and 27.4 per cent in spur density, fruit set and fruit yield. TSS, and anthocyanins content were also increased by 1.2 times each in treatment combination T_8 compared to control. The superior treatment of T_8 also registered the increase of 16.7, 26.7 and 35.3 per cent in terms of available, N, P and K content compared to control. Soil microbial properties in terms of total bacterial count, soil fungi and actinobacterial count were increased by 20.0, 34.1 and 31.0 per cent through treatment combination T_8. Moreover, the superior treatment exhibited 1.3, 1.2, 1.4, 1.3 and 1.4 times increase in PSB, KSB, ZSB, *Azotobacter* and AMF count over control. Phytic acid content recorded a decrease of 64.3 per cent and ursolic acid exhibited an increment of 64.2 per cent through treatment combination T_8 in comparison to control. PCA analysis in both the experiments identified that based on *Eigen* value (>1), PC1 recorded the maximum variability of 95.70 and 96.97 per cent for vegetative growth, yield contributing and quality parameters, and 97.87 and 97.40 per cent for chemical and microbiological properties of soil of apple. Considering the agronomic, environmental and economic benefits of biofortification of apple with Zn organic and bio-organic complexes can ensure quality fruits along with sustainable production.

Signature of the Student
Name: Simran Saini
Date: 28/08/2024

Signature of the Major Advisor
Name: Dr Pramod Kumar
Date: 28/08/2024

Countersigned

Head of the Department

BRIEF BIO-DATA

Name	**:**	Simran Saini (सिमरन सैनी)
Father's name	**:**	Sh. Nem Chand Saini (श्री नेम चंद सैनी)
Mother's name	**:**	Smt. Shakuntla (श्रीमती शकुंतला)
Date of birth	**:**	22.01.1997
Gender	**:**	Female
Aadhar Number	**:**	7889 6528 0633
Permanent Address	**:**	VPO Panjawar, Sub Tehsil Ispur, District Una, HP - 177 208 India
E-mail	**:**	saini.simi11@gmail.com

Academic qualifications:

Certificate/degree	**Year**	**University/Board**	**Marks (%)**	**Division**
10th	2012	CBSE	87.40	First
12th	2014	CBSE	87.80	First
B. Sc. (Hons.)-Horticulture	2018	Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (HP)	79.40	First
M. Sc. (Horticulture)-Fruit Science	2020	Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (HP)	85.90	First

Title of Thesis in M.Sc.	:	Effect of zinc oxide nanoparticles on growth, fruit yield and quality attributes of strawberry (*Fragaria* × *ananassa* Duch.)
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(Simran Saini)