PREPARATION OF NANOCELLULOSE STABILIZED BIO-AVAILABLE MONOSILICIC ACID AND ITS APPLICATION IN TOMATO PLANTS UNDER WATER DEFICIT CONDITIONS

By

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AUTHOR'S DECLARATION

I, Niharika Sandilya declare that the research work carried out for this thesis was in accordance with the regulations of the Asian Institute of Technology. The work presented in it are my own and has been generated by me as the result of my own original research, and if external sources were used, such sources have been cited. It is original and has not been submitted to any other institution to obtain another degree or qualification. This is a true copy of the thesis, including final revisions.

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ABSTRACT

Silicon (Si) is an important and beneficial nutrient for plants, although it does not fulfil the criteria of essentiality in most higher plants. Si can increase crop yield and productivity by protecting the plants from various biotic and abiotic stresses and providing mechanical resistance to the plant in unfavorable conditions and other stress tolerance. The only form of silicon that plant can take up is monosilicic acid, also known as orthosilicic acid. Although being the second most abundant element on the earth's crust, most of the Si is not available to plants due to rapid polymerization of monosilicic acid to oligosilicic and polysilicic acids under high concentration (>2mM) and high pH (>2) of the surroundings. The present study used nanocellulose, a biocompatible natural polymer, for stabilizing monosilicic acid obtained from tetraethyl orthosilicate at a very low pH level of 2. The product obtained was characterized by SEM, EDS and Vibrational Spectroscopy, which demonstrated the bonding of monosilicic acid to nanocellulose fibers through formation of hydrogen bonds between hydroxyl groups of monosilicic acid and nanocellulose. The product was tested upon Lukthar variety of tomato under water deficit conditions inside a greenhouse setup. The interactive effects of three doses of NCF-MSA (37.5, 75, 112.5 kg/ha) and three soil moisture levels (50%, 75% and 100% Field Capacity) produced significant differences in plant height, number of leaves, leaf area, number of flowers, Leaf Relative Water Content at both flowering and fruiting stages and effective quantum yield of PS II. Most of the significant differences observed in the study is due to individual effects of moisture stress on plants.

Keywords: nanocellulose, monosilicic acid, silicon, nanotechnology, tomato

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LIST OF ABBREVIATIONS

ANOVA	= Analysis of Variance
Chol-sSA	= Choline stabilized orthosilicic acid
CWSI	= Crop Water Stress Index
EDS	= Energy Dispersive X-ray Spectroscopy
FC	= Field Capacity
FTIR	= Fourier Transform Infrared Spectroscopy
GPa	= Gigapascals
LRWC	= Leaf Relative Water Content
MSI	= Membrane Stability Index
NCF-MSA	= Nanocellulose stabilized monosilicic acid
OM	= Organic matter
PEG-sSA	= Polyethylene glycol stabilized orthosilicic acid
RDF	= Recommended dose of fertilizers
SAR	= Systemic Acquired Resistance
sSA	= Stabilized orthosilicic acid
SEM	= Scanning Electron Microscopy
SPAD	= Soil Plant Analysis Development
TSS	= Total soluble solids

CHAPTER 1
INTRODUCTION

1.1 Background of the Study

A mineral plant nutrient is an element that is necessary or beneficial for plant growth and development, as well as for improving the quality of harvested products in natural or cultivated environments. Plant nutrients are critical to ensure optimal plant growth and development because they facilitate vital mechanisms such as cell structure and function, metabolism, energy reactions, osmotic and turgor-related activities, enzyme-catalyzed reactions, and reproduction. The absence of essential plant nutrients can have a negative impact on plant metabolism, emphasizing their importance. Essential minerals for plants are defined using criteria set by Arnon and Stout in 1939, with subsequent adjustments and additions, as detailed in Marschner's "Mineral Nutrition of Higher Plants."(Marschner, 1995).

Currently, 16 elements are recognized as essential for plants, with cobalt and nickel still being debated. Macronutrients (Carbon, Hydrogen, Oxygen, Nitrogen, Phosphorus, Potassium, Calcium, Magnesium, and Sulphur) are those required in plant concentrations greater than 1 ppm, while micronutrients (Iron, Manganese, Copper, Zinc, Molybdenum, Boron, and Chlorine) are those required at concentrations less than 1 ppm. There are some elements that are not considered necessary for plants but are good to their growth and development (aluminum, cobalt, sodium, selenium, and silicon), which are referred to as beneficial elements. Silicon is frequently regarded quasi-essential owing to its necessary for specific plants like horsetail and rice, however evidence supporting its essentiality for most other higher plants is insufficient, according to numerous research (Brown et al., 2022; El-Ramady et al., 2022; Pandey, 2018; Pilon-Smits et al., 2009).

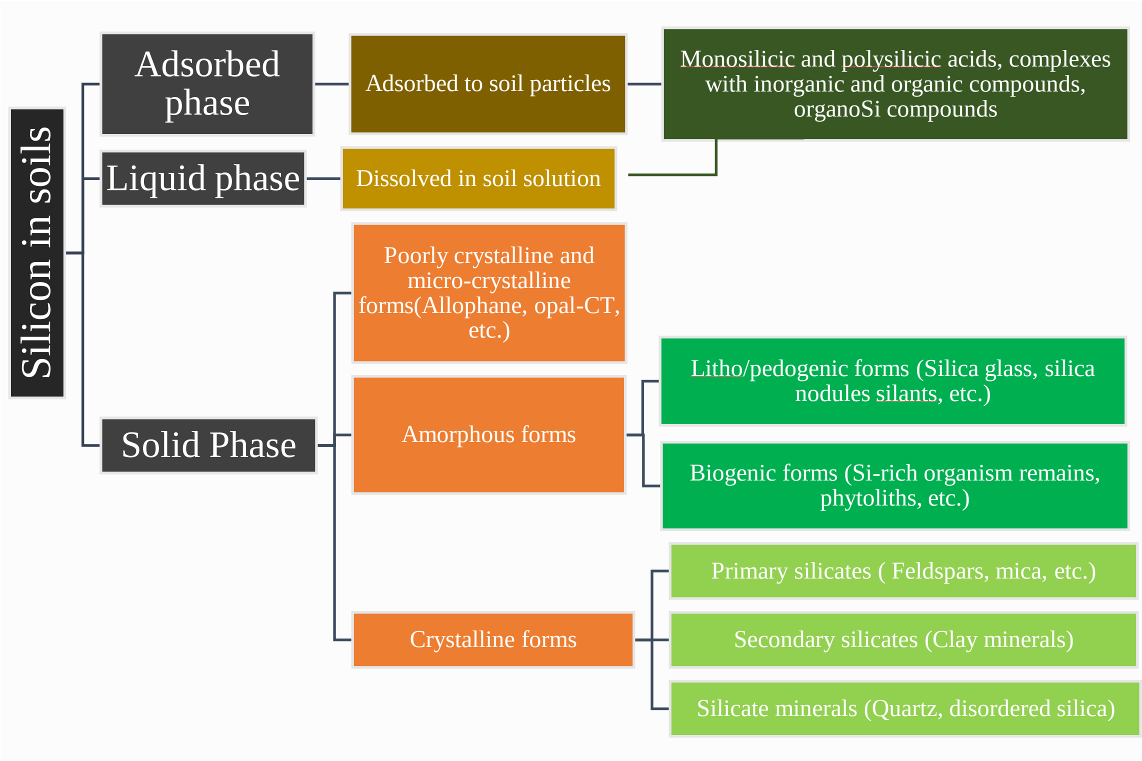
Silicon (Si) is considered as a quasi-essential element (Epstein & Bloom, 2005; Pilon-Smits et al., 2009). It plays an important role in increasing the yield and productivity of a crop. Si protects the plant from both biotic as well as abiotic stresses. Si provides resistance to lodging, different pathogens and infections and improves the exposure of leaves to sunrays. Deposition of Si in the epidermal tissues of the leaves reduces the shading effect on the neighboring plants while deposition in the roots helps modifying the elasticity of the cell wall during root elongation. (Pilon-Smits et al., 2009; Richmond & Sussman, 2003). Another theory says that Si invokes the plant defense mechanism by generating some signals in the plant system that release enzymes (peroxidases, chitinases, etc.), phenolic compounds, phytoalexins, antimicrobial compounds in a process called Systemic Acquired Resistance (SAR) and systemic stress signals like salicylic acid and ethylene to inhibit pathogenic diseases (Marafon & Endres, 2013; Pilon-Smits et al., 2009).

Si can alleviate heavy metal toxicity in plants by regulating root to shoot transport, cation binding capacity of the cell wall, reduction of metal availability in the plant media, complexation of metals with Si, releasing of antioxidants, etc. (Pilon-Smits et al., 2009; Tubaῆa & Heckman, 2015). Si can reduce effects caused by other abiotic stresses like UV radiation, nutrient imbalance, high or low temperatures and salinity by sodium exclusion and reducing liquid membrane peroxidation (Pilon-Smits et al., 2009).

Si is the most abundant element on the earth's crust after oxygen with mean content of 28.8% (weight) and occurrence rate of 0.52 to 47%. Si in soil is found in three phases; liquid phase, solid phase and adsorbed phase, detailed diagram of which is given in Figure 1.1 (Matichenkov & Bocharnikova, 2001; Tubaῆa & Heckman, 2015). Silica formed from silicate parent materials when dissolved with water form nonionic silicic acids rather than ionic silica (Mustoe, 2023). Silicic acids are available either in the monomeric (H_4SiO_4) or in the oligomeric and polymeric forms. Amongst these various forms, the monomeric form of silicic acid; i.e., the monosilicic acid (or, orthosilicic acid, as known commercially) is the only form which can be actively taken up by plants as nutrition. Numerous chains of H_4SiO_4 containing up to ten Si atoms are classified as oligomeric silica (low molecular weight) and those with higher degrees of polymerization (polysilicic acid) are high molecular weight silica. Bigger size of the polymeric chains of silicic acid and its low solubility in water makes it difficult for the plants to take it from soil (Canfield et al., n.d.; Dietzel, 2000; El-Ramady et al., 2022; Marafon & Endres, 2013; Puppe & Sommer, 2018; Richmond & Sussman, 2003; Tayade et al., 2022a; Tubaῆa & Heckman, 2015).

Figure 1.1

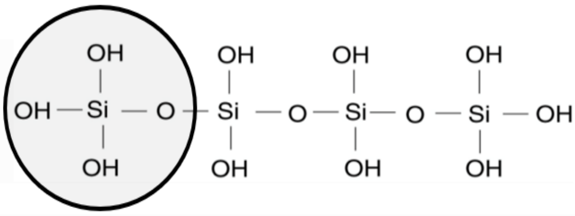
Forms of Silicon in Soil (Adapted from the Study by (Mustoe, 2023))



However, the monosilicic acid or monosilicic acidrapidly changes its form and starts to polymerize forming polysilicic acids (Figure 1.2) owing to several factors, the most important of which are higher concentration of monosilicic acid and higher pH of soil. They also form complexes with soil actions to form silicate ions at alkaline pH (Tubaῆa & Heckman, 2015).

Figure 1.2

Structural Representation of the Formation of Polysilicic Acid from Monosilicic Acid. The Encircled Part Indicates the Monomer Structure, H4SiO4



Si uptake from soil solution to cortical cells in monocotyledons is mediated by a transport mediated component and also by passive transport through diffusion in some. Si uptake rate in higher plants varies from plant to plant depending upon the root Si uptake ability of different plants.

After extracting Si from the soil, different transporters act in different sites of the plant like roots, xylem, etc. to move the element to different parts of the plant (Kaur & Greger, 2019; Ma et al., 2011; Mitani & Jian, 2005; A. Singh & Roychoudhury, 2021). When silicic acid reaches the shoot, it gets converted into a hard immobile silica gel ($SiO_2.nH_2O$), also known as Phytolith. This forms the basis for the prevention of many biotic and abiotic stresses in Si accumulating plants.

1.2 Statement of the Problem

Though Si is the second most abundant element in the soil, the whole of it is not available to plants. Plants can extract Si only in the monomeric form (H_4SiO_4). Heavily weathered, organic, intensively cultivated soils, soils having less mineral weathering microorganisms, other climatic and anthropogenic factors affected soils cannot quickly replenish the lost Si from soils after taken up by plants owing to several factors. Moreover, adsorption of H_4SiO_4 to secondary clay minerals and Al/Fe hydroxides (increases from pH 4 to 9) and the unstable nature of H_4SiO_4 due to polymerization and formation of insoluble complexes with cations reduces its bio-availability in soil. Therefore, to refurbish the depleted Si from soil, Si is applied artificially to crops as fertilizers, biostimulants and other amendments. (Canfield et al., n.d.; Laane, 2018a; Savvas & Ntatsi, 2015; Schaller et al., 2021a; Tubaῃa & Heckman, 2015).

But all silicates fertilizers available are not always soluble (Magnesium silicate, Calcium silicate) and some needs to be refined (Calcium silicate), and natural Si rich products like rice straw and biochar have limitations in terms of uncertainty of quantity and are only effective in the long run and at the same time can cause heavy metal toxicity in soil. Si rich industrial byproducts (slag) increases the pH of the soil and may be toxic (P. Singh et al., n.d.; Tubaῆa & Heckman, 2015). Moreover, these fertilizers are required in a large amount because silicic acid is released in a very low amount from these sources. Also, there is a common thinking that applying more and more fertilizers will increase the efficiency of fertilizers giving more yield and productivity. But it is proved that overfertilization of Si can lead to reduction of H_4SiO_4 in soil. A study describes three phases based on the concentration of fertilizers applied where it is shown that that exceeding the required amount of Si fertilizer reduces the amount of H_4SiO_4 in soil due to formation of long chains of silicic acid. Therefore, continuous application of Si in soil has no effect on the plants. (Matichenkov & Bocharnikova, 2001; Tubaῆa & Heckman, 2015).

Direct application of H_4SiO_4 stabilized by many compounds like choline chloride, polyethylene glycol, etc. have been done both by foliar and soil application methods (Abayisenga et al., 2023; Kleiber et al., 2015; Laane, 2017; Preari et al., 2014; P. Singh et al., n.d.). Foliar sprays of stabilized silicic acid (sSA) are more effective in both dicots and monocots than any other fertilization methods and fertilizer types with very low dozes (Laane, 2018b). But very limited studies have been done on dicots through soil application of sSA. Dicots having no transporters unlike monocots, take up Si through passive diffusion and so are low accumulators (Mitani & Jian, 2005).

Even though foliar application of different types of fertilizers in these plants have given good results in terms of growth and development, but the effectiveness of soil application of fertilizers is not as desired. Many controversies are seen when it comes to the effectiveness of even foliar fertilization (Laane, 2018b; Ma & Yamaji, 2006; Savvas & Ntatsi, 2015). Foliar spraying also leads to wastage in large fields in the long run. It is found in one study that the Si concentration in dicots is more in root cells than in the shoots, which indicates that Si cannot get effectively translocated to shoots (Heine et al., 2005). Also, a couple of studies in grape tomato showed that sSA showed positive results only when the water content of soil was at 75% FC (field capacity) and more or when combined with organic matter (Chakma et al., 2021, 2023).

1.3 Research Questions

Can we stabilize monosilicic acid (H_4SiO_4) using a biocompatible polymer like nanocellulose with no or very less toxic effect to plants and the environment instead of using chemical stabilizers?

1. Can we design a nanocellulose carrier for direct application of H_4SiO_4?
2. Can it show any positive growth and development effects in a low Si accumulator plant like tomato?

1.4 Objectives of the Study

Tomato is a low Si accumulator plant. The main reason is that the xylem loading of Si in tomato is mediated by passive diffusion in the absence of transporters unlike monocots. It is seen that most of the Si is concentrated in the roots than in the aerial parts (Heine et al., 2005; Nascimento-Silva et al., 2022). To increase the Si concentration in the plant and to obtain good growth quality indication parameters, the preparation and use of a biopolymer stabilized H_4SiO_4 is aimed. This ecofriendly biopolymer is nanocellulose, a natural plant derivative nanofiber which is extracted from the cellulose in plant cell wall. Its biodegradable, excellent surface binding properties and hydrophilic nature might make it not only a suitable stabilizer which can stabilize H_4SiO_4 in soil for a longer period but also an efficient carrier of H_4SiO_4 inside the plant (Ghasemlou et al., 2021; Kargarzadeh et al., 2018; Phanthong et al., 2018).

For this study, the objectives are follows:

1. To stabilize monosilicic acid with eco-friendly Nanocellulose Fibres.
2. Application of the nanocellulose and monosilicic acid complex on tomato through soil application and investigating its effects under water deficit stress conditions.

Nanocellulose has many applications in different fields due to its attractive properties like light weight and low density (1.6 g/cm^3), strength, excellent stiffness (up to 220 GPa of elastic modulus), low coefficient of thermal expansion and high tensile strength (up to 10 GPa, more than cast iron). High surface area and presence of many hydroxyl groups facilitates different surface modifications on nanocellulose. It is chemically inert and found abundantly in nature. Nanocellulose has a 3-D hierarchical structure which makes it an important component in many surface chemistry modifications (Phanthong et al., 2018; Trache et al., 2020).

1.5 Scope of the Study

This study considers nanocellulose as a potential stabilizer to form complex with H_4SiO_4, and a dicot plant (tomato) as a model plant. The soil type, greenhouse conditions, growth conditions, field moisture level and recommended fertilizer doses are same for all plant treatments except that the doses of nanocellulose stabilized monosilicic acid complex varies. The study investigates and characterizes the behavior and properties of the proposed nanocellulose and monosilicic acid complex based on the uptake ability by the plant as well as its effects on the plant's growth, yield and other parameters under water deficit soil moisture levels of 50%, 75% and 100% FC.

1.6 Organization of the Study

This report is organized under the following headings:

Chapter 1: Introduction

This chapter gives a background knowledge on the importance of different plants nutrients, more specifically Si, which is the target element of the study. It also contains the problem statements of the study and the proposed solutions in the form of objectives.

Chapter 2: Literature Review

This chapter gives detailed information about Si uptake and accumulation mechanisms along with different fertilizer types available and potential compatibility of nanocellulose and monosilicic acid as a fertilizer.

Chapter 3: Methodology

This chapter gives the methods and techniques used to prepare the target compound and its application to the crops followed by the equipment and methods used to conduct the experiments and data analysis.

Chapter 4: Results and discussion

This chapter gives detailed explanation of the results obtained after successful completion of all experiments and a discussion about the results so obtained is presented.

Chapter 5: Conclusion and future recommendations

This chapter presents an overview of the results and the final remarks on the findings of the study followed by some recommendations and directions to future researches in this area.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Liang et al. defined Si as an "agronomically essential" element (A. Singh & Roychoudhury, 2021).The benefits of Si for plants have been proved and documented in many studies. Si has great positive effects on the vegetative growth, yield, productivity, and mitigating a number of different biotic and abiotic stresses. However, Si is not readily available for plant uptake in most agricultural soils due to the limited presence of the element in its monomeric form. Plants can only be taken up by plants when it is in the monosilicic (or orthosilicic) form i.e., H_4SiO_4 as mentioned in Chapter 1 (Marafon & Endres, 2013; Puppe & Sommer, 2018; Richmond & Sussman, 2003; Tayade et al., 2022a; Tubaῃa & Heckman, 2015).

Mechanisms involved in the uptake and accumulation of Si are studied and proved in may literatures. Moreover, many reasons and factors are responsible for the low availability of H_4SiO_4 to plants. With the increasing population and decreasing agricultural land globally, demand for higher crop productivity has made the use of fertilizers an inevitable solution to meet the food demand. From a long time, different ancient Si application techniques and typical Si fertilizers have been in use. Apart from these, with the advent of science and technology, smart fertilizers (composite fertilizers, bioformulations, nanofertilizers) have paved the way for better growth and productivity in both Si accumulator and non-accumulator plants.

The aim of this chapter is to provide a detailed overview of Si as a plant nutrient, its uptake and release mechanisms in plants, formation and availability of Si in soil, various fertilizer types and conditions that are in use lately and the novel approaches used to increase the efficiency of fertilizers.

2.2 Benefits of Si in Plants

At concentrations of Si greater than ~2 mol/m^3 in the xylem, Si gets condensed into hard silica gel or phytolith ($SiO_2.nH_2O$) in the presence of calcium and pectin ions and deposits on the leaf epidermis below the cuticle. This is known as silicification in plants.

Most of the reduction in plant stresses is due to the formation of this hard outer layer on the cell walls of the shoot giving the plant the much-needed mechanical strength. This property protects the plants from biotic and abiotic stresses and helps them in adapting to unfavorable environmental conditions (Marafon & Endres, 2013; Tubaῆa & Heckman, 2015).

2.2.1 Alleviation of Biotic Stresses

Insect pests, disease pathogens, nematodes, etc. cannot cause damage to plants due to the barrier provided by the hard silica layer (Phytolith) deposited in the cell walls of shoot. The invasion of pests like yellow borer, rice clorops, green leafhopper, brown leafhopper, mites, and aphids etc. is minimized (Tubaῆa & Heckman, 2015). Caterpillars cannot harm plants due to the presence of the hard silica layer. Si prevents many fungal infections owing to cell wall strengthening due to deposition of a cellulose-silica double layer. This provides mechanical resistance to the plants so that fungus cannot penetrate the tissues. Even the grazing animals like rabbits, rodents and locusts prefer to stay away from these Si fertilized plants (A. Singh & Roychoudhury, 2021; Tubaῆa & Heckman, 2015).

Silicon application in sugarcane has been found to reduce pest and disease attacks, including those caused by sugarcane borers (*Diatraea saccharalis* and *Eldana saccharina*), leaf (*Mahanarva posticata*) and root (*Mahanarva fimbriolata*) spittlebugs, as well as diseases such as sugarcane brown rust (*Puccinia melanocephala*) and leaf sheath ringspot (*Leptosphaeria sacchari*) (Marafon & Endres, 2013). In cases where powdery mildew afflicted cucumber plants and Phomopsis damaged asparagus plants, silicon treatment resulted in the production of increased quantities of phytoalexins and pathogenic proteins such as peroxidase and polyphenol oxidase. Furthermore, treating cucumber, sugarcane, and wheat plants infested with white fly (*Bemisia tabaci*) with silicon boosted nymph mortality, resulting in better yields.(A. Singh & Roychoudhury, 2021).

2.2.2 Alleviation of Abiotic Stresses

Silica deposition improves the mechanical strength of the plant making it resistant to lodging during heavy rains and during cyclones. It also keeps the plant safe from other abiotic stresses such as drought by increasing the stomal conductance, water content and water potential of the plant. It also increases the intercellular carbon in the plant. The large thickened leaves due to silica gel deposition reduce transpirational losses by filling of interfibrillar spaces. This deposition of Si on the leaf surfaces also leads to reduction in cuticular transpiration thus saving water during phases of stress. Secondary and tertiary cells of the root endodermis are strengthened thus making the roots resistant to dry soil conditions and increasing the root length to dig deep into the soil (Marafon & Endres, 2013; Tayade et al., 2022a; Tubaῆa & Heckman, 2015).

Si is known to mitigate heavy metal (Al) toxicity in plants by some external and internal mechanisms like formation of metal-phenolic complexes, increase in pH of solution, co-deposition of Si and metals; and complexation and compartmentalization of Si ions in the vacuoles, cytoplasm or cell wall, immobilization of heavy metals, action of anti-oxidants, phytoalexins, phenols, phenylpropanoids, etc. released during Si uptake (SAR), respectively. Exley and Cocker et.al proposed that the mechanism behind mitigation of Al toxicity is the formation of hydroxyl-aluminosilicates (HAS) in soil solution. By suppressing the effect of phenols caused by Mn toxicity, Si can prevent leaf spots and necrosis (Marafon & Endres, 2013; Tubaῆa & Heckman, 2015).

Si deposition on root endodermis and cell wall reduce uptake of metals in plants due to thickening of casparian strips and xylem cells. Si deposition also helps in alleviating sodium accumulation in shoot in low accumulator plants by reducing the peroxidation of membrane lipids by the activation of anti-oxidant enzyme or non-enzyme activities of the plant. Photosynthesis is also maintained with Si uptake due to stimulation of antioxidant defense mechanism and also by removing shading effect of leaves on neighboring plants with the production of erect leaves (Marafon & Endres, 2013; Pilon-Smits et al., 2009; Tubaῆa & Heckman, 2015).

Si has shown to provide many advantages to crops like in rice, Si application can thicken culm wall, increase vascular bundle size and henceforth increasing stem length and resistance to lodging. In maize, it increased water use efficiency by reducing losses through transpiration. Reduction in growth and development due to drought stress in cowpea and kidney beans as a result of sodium chloride (NaCl) toxicity can be alleviated by Si (Tayade et al., 2022a).

Si also protects the plant from lodging and increases the oxidative defense system of plants thereby making them tolerant to floods (Tayade et al., 2022a). Silica gel also removes negative effects of salts on sugarcane plants by reducing sodium (Na) absorption (Marafon & Endres, 2013). The benefits of Si are more expressed in plants facing different stresses than those growing in favorable environmental conditions. (Tubaῆa & Heckman, 2015).

2.3 Uptake Mechanisms and Accumulation of Si in Plants

Si-accumulating plants use transporters like Lsi1, Lsi2, Lsi3, and Lsi6 to move silicon. Lsi1, an influx transporter, transfers silicon from the external solution to the cortical cells of the root. Lsi2 (and potentially Lsi3) is responsible for silicon efflux from root cells, whereas Lsi6, a homolog of Lsi1, unloads silicon from the xylem and transports it to the shoot. (Ma et al., 2011; Ma & Yamaji, 2006; A. Singh & Roychoudhury, 2021).

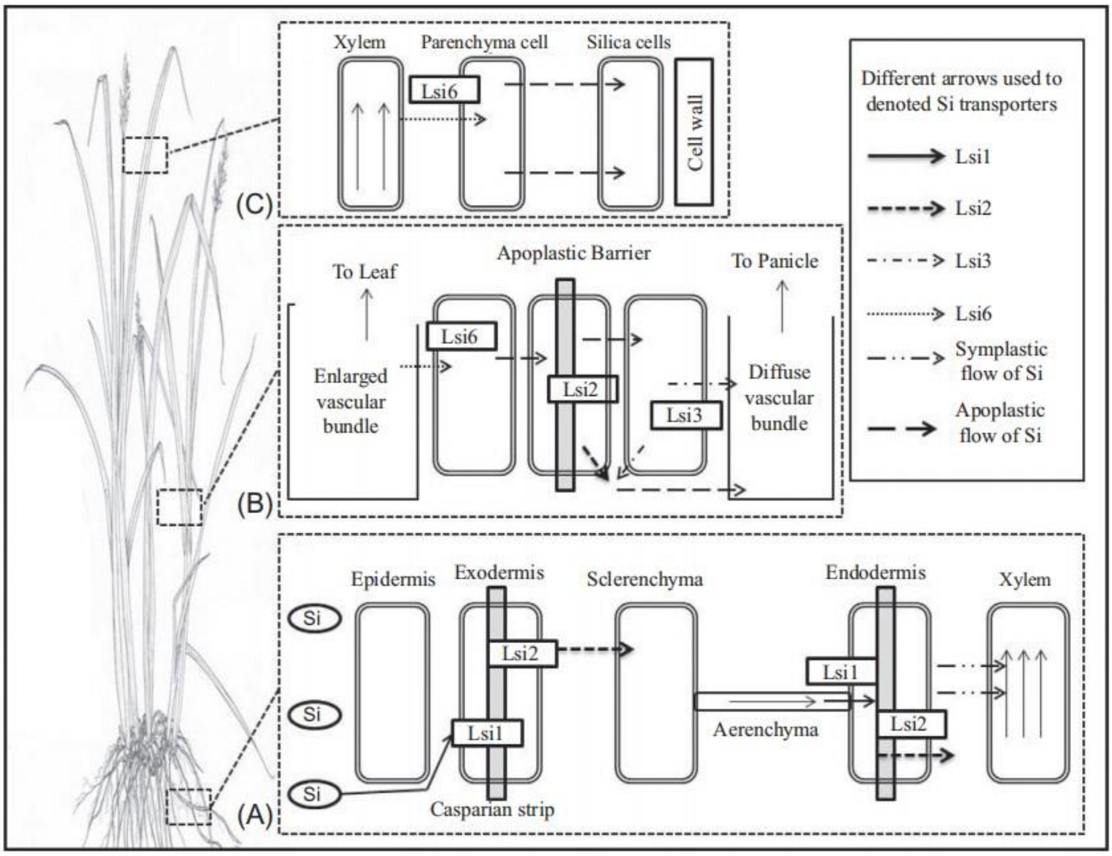
OsLsi1 is the rice influx transporter, whereas HvLsi1 and ZmLsi1 are responsible for barley and maize, respectively. Despite their comparable roles, these transporters have diverse localization patterns, resulting in differences in silicon uptake among species. In rice, influx transporters take silicon from external sources and release it into the apoplast of the aerenchyma via OsLsi2, an efflux transporter found on the proximal side of exodermal cells. As a result, OsLsi1 and OsLsi2 transfer silicon to the plant's stele. (Ma et al., 2011; A. Singh & Roychoudhury, 2021).

Similarly, in the case of efflux transporters- OsLsi2, HvLsi2 and ZmLsi2 are the transporters for rice, barley and maize, respectively. Lsi2, Lsi3 and Lsi6 are expressed near nodes and are involved in the intervascular transport of Si. Lsi6, located in the xylem transfer cells, carries the Si from the roots to the xylem transfer cells and then Lsi2, localized in the bundle sheath cells, transfer it by symplastic pathway. Lsi3 plays an important role here in transporting Si to the deposition site through the parenchyma cells (A. Singh & Roychoudhury, 2021; Yamaji et al., 2015).

Eventually, Lsi6 transports Si out from the xylem parenchyma, by the process called as xylem unloading, to the shoot cells (Figure 2.1) (A. Singh & Roychoudhury, 2021). The silicic acid in the shoots polymerizes to amorphous biogenic silica (Phytolith) due to loss of water when concentration exceeds 2 mM and deposits immediately beneath the thin cuticle layer (deposits as a 2.5 μm layer in rice) on the cell walls of hulls, leaves and stem of the plant. But in xylem sap, the concentration generally is higher than 2 mM even though the major form of Si found in xylem sap is silicic acid. The reason for this might be that silicic acid polymerizes in vitro. Since this element is immobile in plants, therefore, it is accumulated mostly in the older tissues. In rice, silica deposits on the silicified cells of leaf blades. Silicification occurs gradually from silica cells (located in vascular bundles, dumbbell shaped) to silica bodies (located in leaves, bulliform cells), which are two types of silicified cells of the leaf blade (Ma & Yamaji, 2006).

Figure 2.1

Model of Silicon Uptake in Rice Plants (YAN et al., 2018)



Note: *Silicon transport in rice: role of different transporters (A) Silicon pathway through roots; (B) Silicon pathway through nodes; (C) Silicon unloading and deposition pathway*

Based on the uptake ability of Si, plants are categorized into high (Graminae and Cyperaceae have 1-10% dry wt.), intermediate (Cucurbitales, Urticales, Commelinaceae have 0.5-1.5% dry wt.) and low accumulators (most other plant species like Solanaceae have <0.5%) (Mitani & Jian, 2005; Schaller et al., 2021a). Nikolic et al. said that plants like rice, wheat, sugarcane, barley of the Graminae family can take up Si more efficiently from the soil than most other dicots like tomato, beans, etc. (A. Singh & Roychoudhury, 2021). Si concentration in rice is 20 and 100 times more than in cucumber and tomato, respectively.

Comparing rice, cucumber and tomato, transfer of Si from external soil solution to the root cells is an energy dependent process through transporters. Though having equal Km= 0.15 mM, the three plants have different V_{max} values (rice > cucumber > tomato) which means they have different density of transporters. But the second step of xylem loading is through transporters in rice but by passive diffusion in cucumber and tomato. Low density of transporters becomes the reason for low Si accumulation in these plants; absence or defective transporters from roots to xylem being another reason (Mitani & Jian, 2005). A study proves that the absence of effective efflux transporters in tomato is the main reason for tomato being a low accumulator of this beneficial nutrient (H. Sun et al., 2020). Sometimes, elements like $HgCl_2$ can disrupt even the uptake of rice by blocking of water channels through oxidation of cysteine residue, a component of the transporter of Si from soil solution (Mitani & Jian, 2005).

In a study by Christopher Exley, some opposite ideas were proposed saying that instead of some transporters, the transport of Si from the external solution is rather controlled by aquaporins with no specific selectivity for silicic acid and these facilitate Si uptake under the influence of water flow. The degree of super-saturation of silicic acid in tissues are a result of the concentration of silicic acid in the soil water. He proposed guttation as the only mechanism for exit of silicic acid from the shoot tissues. According to him, toxicity-free silicification is possible only when size of silica particles is less than 5 nm, otherwise templating is required (Exley, 2015).

2.4 Si Mechanisms in Soil

2.4.1 Silicic Acid Cycling in Soil

Si is the second most abundant element on the earth's crust after oxygen, comprising more than 25% weight. Silicate minerals and secondary or clay minerals (like vermiculite, kaolinite), amorphous silica are the basic components of soil that provide Si to soil (Matichenkov & Bocharnikova, 2001). Besides, weathering of silicate minerals like nepheline; diopside (7-9 mg/l Si), bioptide; microline (2.3-3.5 mg/L Si), quartz (1.6-1.9 mg/L Si) etc., minerals which are resistant to weathering also contribute to the presence of Si to soil. These forms of Si are the major sources of monosilicic acid (H_4SiO_4) in soils.

In nature, Si is most stable when it is in its +4 form, the different forms of which are solid silicon dioxide or silica (SiO2), mineral silicates and the dissociated anions of H_4SiO_4. Under the liquid phase of Si in soil, comes H_4SiO_4 and its polymerized (oligosilicic and polysilicic acids) and complex forms. H_4SiO_4, the only plant available Si in soil, varies from 3 x 10-3 to 4.5 x 10-3 g Si/kg (Y. Liang et al., 2015). It is the only Si form that is water soluble and hence can be readily absorbed by plants with water.

H_4SiO_4 gets absorbed by the plants and microorganisms and gets deposited as polymerized silica bodies. Eventually, this silica form returns to the topsoil as litter fall and remains of microorganisms. These ultimately contribute to the silica pool of the soil system. Solubilization of silicate minerals by some microorganisms like *Bacillus caldolytyicus, Bacillus mucilaginosus* var *siliceous, Proteus mirabilis, Pseudomonas* and *Penicillium* also contribute to the silica pool. Also, application of silica rich manures and fertilizers also contributes to this pool. In soil, this Si again gets absorbed by plants or gets lost and added through several processes and the cycle continues (El-Ramady et al., 2022; Meena et al., 2014; Tubaῆa & Heckman, 2015).

2.4.2 Fate of Bioavailable Si in Soil

Monosilicic acid (H_4SiO_4) is the only plant available form of Si found in soil. H_4SiO_4 in soils are generally present in acidic pH. In soil solution, Si is found as H_4SiO_4 and its dissociation products. But H_4SiO_4 rapidly polymerizes to oligomeric and polymeric chains of silicic acid. Up to pH 9, H_4SiO_4 continues to remain mostly in its undissociated form. High concentration of silicic acid and high pH (above 9) are the prime factors for polymerization of silicic acid to multiples chains. With increasing degrees of condensation (increase in the number of Si units bound to Si atoms via oxygen), the silicic acid solutions become unstable. Polymers consists of silica tetrahedrons linked by Si-oxygen-Si bonds (Canfield et al., 2005.; Dietzel, 2000; El-Ramady et al., 2022; Schaller et al., 2021b).

pH plays a very important role in the stability of H_4SiO_4 in soil. The minimum level of H_4SiO_4 is found at pH 8-9, low or high which the level of H_4SiO_4 greatly enhances. At pH 2, silicic acid monomers are converted into discrete particles before aggregation and at around pH 5-6, rapid polymerization starts. When the pH of the soil solution drops from 7 to 2, the Si content in the solution can rise dramatically. Various experiments have been proven that the availability of Si in soil is strongly associated with soil pH (El-Ramady et al., 2022; Mustoe, 2023). At pH 9 and above, the H_4SiO_4 dissociates into $H^+ + H_3SiO_4^-$ and then into $2H^+ + H_2SiO_4^{2-}$ at pH values above 11. Polymerization in most natural environment begins when H_4SiO_4 concentration exceeds ~2mM and faster at pH greater than 4. Also, polymerization is much faster at lower pH values than at higher values. Generally, the Si content of soil solutions lies in the range 0.04 to 23.4 mg/L, but it may go up to 46.7 to 93.4 mg/L at pH values 10-11 (El-Ramady et al., 2022; Matichenkov & Bocharnikova, 2001; Schaller et al., 2021b; Tubaῆa & Heckman, 2015).

2.4.3 Other Factors Influencing Bioavailable Si Concentration

pH is the first and foremost factor affecting the concentration of H_4SiO_4 in soil as discussed above as variation of pH affects the polymerization and depolymerization of silicic acid. Many other factors affect the concentration of H_4SiO_4 in the soil. Intensive cropping of Si accumulating crops such as rice, wheat, etc. tends to remove a high amount of Si from the soil. Desilication due to anthropogenic activities tend to cause a loss of 100-500 kg Si/ha of biogenic amorphous silica (bASi) (Schaller et al., 2021b) (Tubaῆa & Heckman, 2015).

Others include weathering of silicate minerals like nepheline; diopside (7-9 mg/l Si), bioptide; microline (2.3-3.5 mg/L Si), quartz (1.6-1.9 mg/L Si), minerals that are insoluble and resistant to weathering like feldspar (a small amount), application of acid producing fertilizers or liming agents (increase and decrease H_4SiO_4, respectively) and other Si rich materials like biochar, slag increasing the concentration of H_4SiO_4 (Tubaῃa & Heckman, 2015). Adsorption of H_4SiO_4 by secondary clay minerals and mainly by Fe/Al hydroxides (pH 4-9) also affects the concentration. Though secondary clay minerals only cause only a minimum loss of Si but Al/Fe hydroxides extracts a significant amount of the same (El-Ramady et al., 2022). Complexation of silicic acid with inorganic and organic ligands also affect the occurrence of silicic acid in soil (Schaller et al., 2021b).

Further factors include soil temperature, redox potential of the soil, organic matter and water content of the soil and also particle size and soil fraction. Dissolved salts also cause aggregation of silicic acid in soil. Over and incorrect fertilization of silicic acid greatly influences the concentration of silicic acid (mentioned in Chapter 1). Different soil types and conditions also affect the concentration of silicic acids in soils. For example; alkaline soils release less H_4SiO_4 than acidic soils and in saline soils, adsorption, coagulation and polymerization of H_4SiO_4 are more. Also, a number of researches have shown that soil having a sandy texture is usually low in the availability of Si and so have very less Si uptake strength, while soils with a hard or appropriate Si are generally clay- textured. Seasons and ecosystems also have a role in this. (Beckwith & Reeve, 1964; Mustoe, 2023; Schaller et al., 2021a; Tubaῃa & Heckman, 2015).

2.5 Si Fertilization

Cultivation of Si accumulator crops like rice, wheat, sugarbeet, maize, and sugarcane extract a large quantity of Si from the soil. With the continuous and intensive cropping of Si accumulator plants results in a significant reduction of plant-available Si from the soils (Guntzer et al., 2012). It is estimated the annual Si removal accounts for about 210 and 224 million tons (Savant et al., 1996). Si is depleted more from the agricultural soils than from soil with other vegetations (Blecker et al., 2006; Makabe et al., 2009; Meyer & Keeping, 2001). But the replenishment of this Si in the soils in nature is very slow, especially when the soils are heavily weathered, organic, or intensely cultivated. Even the accelerated weathering of Si minerals, dissolution of silicate complexes formed with heavy metals, hydroxides and organic matter or depolymerizaton of polysilicic acid are not able to add the required Si in the soils. Hence, the use of Si fertilizers is the only way to add Si in the soil externally (Tubaῃa & Heckman, 2015).

Si fertilizers are generally Si-rich inorganic compounds and they are applied to increase the quantity of the bioavailable form of Si i.e., monosilicic acid (H_4SiO_4). Different forms of Si fertilizers like silicates, silicon dioxides, biogenic silica, diatomaceous earth products, etc. are used to overcome the deficiency of H_4SiO_4. These fertilizers not only increase the soil adsorption surface area and H_4SiO_4, but also increase the amount of polysilicic acids, otherwise important for good aggregation properties of soil (Laane, 2018b; Matichenkov & Bocharnikova, 2001). By increasing the availability of H_4SiO_4, these fertilizers have shown great effects on biotic and abiotic stresses. Recently, Si compounds are better known and classified as biostimulants in EU regulations. But they are also known as amendments or fertilizers (Laane, 2018b).

2.5.1 Forms of Si Fertilizers

Various forms of Si fertilizers are being used till date to enrich soils and plants with Si. Some of the most common types are silicates, silica, slags, biochars, nano Si and bioformulants. Some of the types of Si fertilizers and their chemical compositions are listed below in Table 2.1.

Table 2.1

Different sources of Si-based Amendments Used in Agriculture and their Chemical Composition

Fertilizer type	Chemical composition	References
Wollastonite (E.g.; Canadian wollastonite)	$CaSiO_3$	(Haynes et al., 2013; Sebastian et al., 2013; Tayade et al., 2022b)
Talc	$MgSiO_3$	(Sebastian et al., 2013)
Silica gel	$SiO_2.nH_2O$	(Sebastian et al., 2013; Tubaῆa & Heckman, 2015)
Potassium silicate-liquid (E.g.; SIK Silicato de Potasio)	K_2SiO_3	(Sebastian et al., 2013; Tayade et al., 2022b)

Fertilizer type	Chemical composition	References
Sodium silicate-liquid	Na_2SiO_3	(Abed-Ashtiani et al., 2012)
Silicic acid	H_4SiO_4 / H_2SiO_3	(El-Ramady et al., 2022; Tubaῆa & Heckman, 2015)
Silica blend (monocal or with $FeSO_4$, NH_4NO_3	$CaSiO3_3$	(Sebastian et al., 2013)
Calcium silicate/ Magnesium silicate blend	$CaSiO_3$ / $MgSiO_3$	(Sebastian et al., 2013)
Calcium metasilicate (E.g.; Vansil W-10)	-	(Tayade et al., 2022b)
ASM	Si, acibenzolar-S-methyl	(Assis et al., 2015)
Fused Magnesium potassium phosphate	P_2O_5, K_2O, Ca, Mg, Si	
Thermo-phosphate	P, Ca, Mg	(Gascho, 2001)
Hydrophilic bentonite	$H_2Al_2O_6Si$	(Ma, 2004)
Organo Si fertilizer	OSiF	(Huang et al., 2019)
Si nanoparticles	Si	(El-Ramady et al., 2022)
Nano silica	SiO_2	(El-Ramady et al., 2022)
Choline chloride stabilized monosilicic acid (Chol-sSA)	H_4SiO_4, Choline chloride	(Kleiber et al., 2015)
Polyethylene glycol stabilized monosilicic acid (PEG-sSA)	H_4SiO_4, PEG-400	(Preari et al., 2014)
Carnitine salts stabilized orthosilicic acid	H_4SiO_4, Carnitine dihydrogenphosphate, H_3PO_4 H_4SiO_4, Carnitine hydrochloride, H_3PO_4	(Cepanac et al., 2012)

Fertilizer type	Chemical composition	References
Fulvic acid stabilized orthosilicic acid	H_4SiO_4, Phosphoric/ Phosphorous/polyphosphoric/ pyrophosphoric acid, fulvic acid, PQQ, natural polysaccharides like GA, gum Arabica	(Kumar & Bhagwan, 2019)
Industrial by-product		
Iron/steel slag	$CaSiO_3$	(Haynes et al., 2013)
Electric furnace slag	$CaSiO_3$/$MgSiO_3$	(Sebastian et al., 2013)
Blast furnace slag	$CaSiO_3$/$MgSiO_3$	(Haynes et al., 2013)
Processing mud	-	(Haynes et al., 2013)
Fly ash	-	(Haynes et al., 2013)
Calcium silicate hydrate	-	(Gascho, 2001)
Silico-manganese slag	-	(Gascho, 2001)
Converter slag	-	(Gascho, 2001)
Stainless steel slag	-	(Gascho, 2001)
Ferronickel slag	-	(Gascho, 2001)
Si from organisms		
Miscanthus biochar	SiO_2	(Houben et al., 2014)
Rice hull fresh	SiO_2	(L. Sun & Gong, 2001)
Rice hull ash	SiO_2	(Kalapathy et al., 2002)
Coffee husk	SiO_2	(Tubaῆa & Heckman, 2015)
Diatomaceous earth (E.g.; Agripower Silica)	Amorphous SiO_2	(Sadowska & Świderski, 2020; Tayade et al., 2022b)

In studies by (Artyszak, 2018; Barão, 2023) , they have talked about the benefits of Si fertilization in agricultural crops. Different methods as well trials of Si fertilization and their effects have been explained and discussed. (Artyszak, 2018) have summarized trials on different types of field and horticultural crops and have outlined the advantages of Si fertilizer. He has mentioned the superiority of foliar based Si fertilizers over soil application. Foliar spraying of Si fertilizer act as a biostimulant for the crops especially during various stress conditions and can be considered a standard in the package of practices of crops. The study by (Barão, 2023) discussed the different method trials of Si fertilization and the percentage of positive outcomes.

Nano-Si fertilizers fall under 'smart fertilizer' category because it is a new direction of applying Si to plants. Most applications of nano-Si are done through foliar sprays and sometimes as soil fertilizer. Cucumber, wheat, French bean, broad bean, maize, barley, coriander, tomato, pea, potato, lentil are some crops where nano Si is tested as fertilizer in the form of SiO_2. Plants were seen with increased photosynthetic rate, tillers, plant height, grain and spike productivity, protein content, shoot and root mass, pod number, chlorophyll and carotenoid contents, seed yield, higher nutrient uptake and water use efficiency (Tayade et al., 2022b). However, (Laane, 2018b) presented that stabilized silicic acid (sSA) i.e., bioavailable form of Si is more effective as fertilizer compared to silicates and nano silica.

2.5.2 Novel Methods for Applying Si to Plants

Monosilicic acid (H_4SiO_4) is the simplest form of the soluble silicic acid. It is found naturally in seawater, river water and soils at a concentration of a few ppm. Even though monosilicic acid, also known as monosilicic acidis in dynamic equilibrium with disilicic acid, it is the only form taken up by plants actively (Laane, 2018b). But it is not easily available to plants because of its unstability as discussed before. It rapidly polymerizes to its dimeric, trimeric, oligomeric and eventually polymeric forms, which are not soluble in water unlike the monomeric forms which follows water from the external soil solution up into the shoots.

In the recent years, many have come up with solutions to stabilize H_4SiO_4 with the help of a number of chemical stabilizing agents like Choline chloride, Polyethylene glycol, Carnitine salts, Polyvinylchloride, Fulvic acid, as well as combinations of some of them

(Cepanac et al., 2012; Kleiber et al., 2015; Kumar & Bhagwan, 2019; Preari et al., 2014). These stabilized forms of H_4SiO_4 are implemented and studied in different plant species which yielded both positive and neutral outcomes, depending on different conditions considered. H_4SiO_4 application methods include mostly foliar, while some researches are done through soil application and few by seed priming. The concentrations, dozes and time of application varied according to plant species as well as the method of application in the plants.

Orthosilicic (monosilicic) acid as soil amendment came into market after 2002 when many patents were released on stabilization methods of silicic acids (Laane, 2018b). After that, several studies were conducted to test different application methods as well as to test different conditions in which they work. These include soil, climate, temperature, pH, concentrations and different stresses. The most common monosilicic acidavailable in the market are Choline (Chol-sSA) and PEG (PEG-sSA) stabilized ones. These contain 2-2.5% of concentrated forms of silicic acid having 0.7-0.8% Si content. Most silicic acid products in the market contain oligomers of silicic acid apart from monosilicic acid because oligomers remain in equilibrium with the monomers (Laane, 2018b). Results of some trials given by (Laane, 2018b) (Laane, 2017) are discussed below:

a) **Tomato**: Foliar sprays with 4 ml/l of PEG-sSA increased plant height and stem diameters and uptake of other nutrients like N, P, K, Ca, NO_3 also increased. When compared with Difenoconazole (a commercial fungicide), a 56% reduction in fungal diseases was observed with the 4 ml/l spray.
b) **Potato**: Growth, yield and infection rate studies on potato gave beneficial results when PEG-sSA was applied as foliar spray at varying concentration range of 1-4 ml/l. The positive effects were 6.2% increase in yield in the Netherlands, increase in tuber weight by as much as 39.6% and reduction of late blight and blackleg occurrence in Brazil. Soil and foliar applications in research on pot-grown potato plants resulted in increased leaf area, chlorophyll content, photosynthesis and transpiration rates.
c) **Onion**: A 10.8% increase in yield was seen in the Netherlands when foliar sprays of 4 ml/l of PEG-sSA was used.

d) **Cucumber**: In 2014, a study on organically grown cucumber in Estonia gave taller plants (height increased by 35%) and increased stem diameter (increased by 27%). Also, availability of NO_3, N, P, Mg also increased (Olle, 2014) .

e) **Soybean, common bean and peanut**: 2 ml/l of PEG-sSA improved the oil and protein content of soybean to the maximum extent while in another study, pod numbers and seed yields increased by 14% in soybean, 15% in common bean and 9.6% in peanuts.

f) **Rice**: In Panama, 4 ml/l sprays of PEG-sSA showed great increase in growth and yield parameters and yield increased by 9.6%. Also, a second study revealed that there was not only a reduction in pesticide rates but also, a maximum straw and grain yield (32%) was achieved with 2 and 4 ml/l of PEG-sSA foliar sprays when applied with low-dose boric acid, pesticides and fungicides in different soil conditions.

   Again, another study on the variety MTU 1010 by (Neeru et al., 2019) showed higher chlorophyll content and higher nutrient uptake, increased root volume, tillers and increase in other yield parameters when PEG-sSA was applied as foliar spray (2 ml/l). The study was done both as seed treatment (overnight soaking and wet coating with silicic acid) and in the field. Seed treatment showed better vegetative growth with better root and shoot development with more root hairs. Better results were obtained when seeds were soaked overnight Occurrence of white ear heads also decreased from 10.3/ m^2 to 4.3/ m^2 in fertilized plants.

   In India, 15-45% yield increase was observed along with reduction of infections in rice in the year 2007-2012 when PEG-400 stabilized oligosilicic acid (MSAB) was used (Laane, 2017).

g) **Wheat**: 2 ml/l PEG-sSA showed great improvement in growth and yield parameters like water content, chlorophyll content, root length and seed weight, which increased significantly by more than 10%. K and P content in straw and seed also increased.

h) **Maize**: Combined application of PEG-sSA (3 ml/l) and Si granules through soil and foliar methods improved yield in sandy loam and clay loam soils.

i) **Finger millet:** 2 ml/l and 4 ml/l sprays of PEG-sSA reduced blast disease in finger millet by 50.4-69.8% while the 4 ml/l spray improved the straw and grain yield as well as Si uptake by 54.6%.

j) **Sugarcane**: In comparison to soil application of calcium silicate, foliar application of 4 (26% increase) and 6 ml/l (14% increase) of PEG-sSA showed great yield and growth parameters. However, combination of both methods showed an increase of a whole 33%. In another study, in comparison to a combination of glyphMSAte and sodium metasilicate, foliar PEG-sSA increased the cane yield by 4.6% while increasing both the sugar content and juice purity.

k) **Fruits**: In papaya, an increase in plant height up to 7.8%, stem diameter up to 8.2%, Fruit yield up to 13.2% as well as superiority of flavor was witnessed when PEG-sSA sprays of 4 ml/l was used in Columbia.

In 2009-2010, in Bangalore blue grapes, a noticeable increase in growth parameters like cane length, leaf area, bunches per vine, yield per hectare giving a yield increase of 39% was observed with 4 and 6 ml/l of PEG-sSA sprays (Laane, 2017). 3 ml/l spray of the same in Thompson seedless grapes improved bunch weight and berry quality.

In strawberry, foliar application of 0.1% and 0.2% Chol-sSA lead to increased root length, root mass, leaf area in all types of soil. PEG-sSA sprayed mango plants resulted in increased yield, total soluble solids (TSS) and five days of additional shelf life in comparison to calcium silicate, rice husk ash or foliar silicate sprays.

4 ml/l spray of PEG-sSA improved the juice content in mandarin. Foliar application of a PEG-400 stabilized microcolloidal silicic acid with 2% micronutrients (AB Yellow) in watermelon, 38% yield increase was observed in India while 26% yield increase was observed in Romania in Cardamom (Laane, 2017).

In conclusion, the stabilized form of silicic acid has shown considerable advantages, especially when administered foliarly. Laane explains SAAT (Silicic Acid Agro system), a system that uses stabilized silicic acid in foliar, hydroponic, or soil amendment applications. He observes that because direct silicic acid treatment is used, the amount required for foliar sprays (20-40 g Si/ha/crop cycle) is significantly lower than for silicate sprays (400-5000 g Si/ha/crop cycle). Patented techniques are used to manufacture stabilized non-colloidal silicic acid. The fertilizers used are known to as MSAB (having 2.5% oligomeric silicic acid coupled with 1.2% KCl, 0.8% H3BO3, 47% demi water, and 47.5% PEG-400 as a stabilizer) and AB Yellow® (Laane, 2017).

Plants treated with silicic acid have shown to increase growth, physiological, yield, quality as well as safety parameters. Increase in growth parameters like root length, root mass, stem height and diameter, tiller counts, biomass, physiological parameters like critical nutrient uptake and yield parameters like yield weight, fruit count, seed weight, etc. were commonly visible. Quality parameters like uniformity in production, reduction in wastage, higher vitamin, pigmentation and sugar content also increased significantly. Mitigation of biotic stresses like pathogen attack and abiotic stresses like drought and salinity is observed significantly (Laane, 2017).

It is found that foliar applications of silicic acid are more efficient than any other methods. The methods being that it is added in definite amounts directly on the plants and that different factors in the soil-water environment affect the Si uptake from soil when applied in soil (Laane, 2017). Besides, increase in the root mass and length is the main effect of SAAT technology, which is evident from the study on cowpea where abscisic acid biosynthesis started as a result of Si nutrition (Dakora & Nelwamondo, 2003).

A study conducted by (Abayisenga et al., 2023) in rice in the eastern province of Rwanda applied stabilized monosilicic acid(Silixol MSA) though soil in different combinations with RDF. Results showed that 100% RDF treated with 20 kg/ha MSA increased plant height, tillers, root length and yield increased by 28.4% and 19.9% in the first and second season respectively. When compared to 100% RDF treatment alone, 75% RDF with 20 kg/ha MSA gave better results demonstrating the advantage of silicic acid. Use of MSA can reduce fertilizer use by 25%. He also reported mitigation of diseases and stresses like late blight (Soratto et al., 2012) and insect incidences in potato, early blight in tomato (Gulzar et al., 2021), drought in wheat

(Ratnakumar et al., 2016)and sorghum (Hattori et al., 2005) and improvement of quality in mango (More Ss et al., 2015), grapes (Ramteke et al., 2012) and apple (Javaid & Misgar, 1900).

In a study by (P. Singh et al., 2020), it is demonstrated that foliar application of stabilized silicic acid is advantageous for sugarcane even though it can grow with lesser quantities of silica. Use of MSA improved the growth (increased cane height, cane girth, internode length) and yield parameters of the canes (both plant and ratoon). Also, juice content increased in the Si treated canes. It can increase the yield as well as the sucrose content of both the plant and the ratoon cane. Moreover, it can minimize losses during post-harvest operations. MSA improves rate of photosynthesis and the ability of the plants to take up other essential nutrients efficiently, thus increasing NUE.

A study on tomato plants was conducted in which the effect of Chol-sSA on Mn toxicity plants as well the chemical composition of other nutrients in the leaves and fruits were investigated. It was seen that Chol-sSA was effective in increasing the yield and biomass of plants only in the low Mn toxicity level (9.6 mg/dm^3) and did not influence yield. Also, in case of leaves, chemical composition of leaves depended on Mn, Chol-sSA and cultivar but that was not the case in the fruits, where the nutrients varied with Mn, Chol-sSA and cultivar (Kleiber et al., 2015).

0.4 g/l of monosilicic acid enhanced ascorbic acid, titratable acidity and fruit firmness in a study on tomato conducted by (dos Santos et al., 2022) when compared to other Si sources like potassium silicate, mixture of potassium silicate and sodium silicate and nano silica. Another study showed that 4ml/l of concentrated soluble silicic acid (2.0% Si as H_4SiO_4) obtained from Rexil Agro BV. Chennai enhances nutrient uptake as well as boosts the yield and quality parameters of tomato when applied as soil drenching. (Thimmappa & Nagabovanalli Basavarajappa, 2021). Also, soil drenching of AB Yellow with 2 ml/l increase the growth and yield drastically (Desher et al., 2023). 50 ml of 0.5, 1 and 2% AB Yellow decreased the population of whiteflies and tomato leaf miner (Alyousuf et al., 2021).

In young olive plants, foliar applied 20 mg/l is the best recommended dose to increase Si concentration in the leaves. But it needs to be sprayed from time to time to maintain the Si need of the leaves. It is found that highest accumulation of Si is in the roots, followed by leaves and then the stem.

In another study conducted by (Heine et al., 2005) , they found that tomato is a Si excluder plant when compared to bitter gourd because Si concentration in the xylem sap of tomato was less than the external solution unlike bitter gourd.

A study in Thailand on grape tomato cultivar T309 was done by (Chakma et al., 2021) using Monosilicic acid (MSA or H_4SiO_4) as seed priming and soil application under drought stress conditions considering different soil moisture regimes. The findings they provided stated that irrespective of methods or doses, yield and irrigation water productivity increased only when 0.25 mM MSA (seed priming) and 300 kg/ha (soil application) was applied under soil moisture regimes of 75% FC and 100% FC, i.e. in sufficient moisture conditions. At 50% moisture regime, there was no effect of MSA on growth and development. A similar experiment conducted recently by the same group (Chakma et al., 2023) with Nitrogen, Phosphorus, organic matter and Si gave good results in moderate soil moisture regimes (75% and 100%).

These studies indicate that foliar applied sSA is more effective in maintaining growth and productivity in crops in both monocots and dicots whereas soil applications are more effective in monocots than dicots. Also, sSA works better as a Si source than other sources like silicates, smart Si fertilizers and amendments. Moreover, sSA is an environment-friendly alternative to alleviate biotic stresses caused by insects and disease-causing pathogens. Silicic acid as fertilizer reduces the concentration of sprays to a tremendous extent when compared to other amendments. But, these foliar applied sSA are effective only when several sprays are done at the vegetative stage of the plant within the concentration range of 1-6 ml/l and 4 ml/l being the most effective dose in most plants. (Laane, 2018b).

2.5.3 Cellulose as a Potential Stabilizer of H_4SiO_4 for Use as a Si Source

Cellulose, which makes up the majority of lignocellulosic biomass found within plant cell walls, accounts for 35-50% of the total composition. It is composed of linear homopolysaccharide chains of β-1,4-anhydro-D-glucopyranose units, with two anhydroglucose units producing anhydrocellobiose, which serves as the repeating unit of cellulose molecules. The presence of hydroxy groups facilitates the development of intramolecular and intermolecular hydrogen bonds, resulting in highly structured, three-dimensional crystalline formations. Cellulose is naturally plentiful in materials such as wood, cotton, and hemp, among others, and is widely used in sectors such as papermaking, textiles, and diverse forest products, demonstrating its growing importance. (Kargarzadeh et al., 2018; Phanthong et al., 2018).

Nanocellulose, a derivative of cellulose is in trend in recent researches in order to determine its isolation methods and specific characteristics which arise from the properties of both cellulose (strength, hydrophilicity, prone to chemical modifications) and nanomaterials (high inbuilt surface area). Nanocellulose is a form of cellulose in which one dimension is in the nanomaterial range. The inherent hydrophilic nature of nanocellulose comes from the many hydroxyl groups present on the surface (Kargarzadeh et al., 2018). Cellulose in its nano form is proven non-toxic to plant environment (Barajas-Ledesma et al., 2022; Schiavi et al., 2023).

Direct application of nanocellulose as a stabilizer is not yet discovered. However, a few surface modifications of cellulose using silicic acids were experimented in a patent by (Sunden, 1977) in which monomers and oligomers of silicic acid are prepared and tested on filter papers (made of cellulose), cellulose pulp and textiles to modify their properties like bending endurance, wet strength, resilience, elasticity, stretchability, softness, etc. using different methods. In some methods, binders like latices or cement were used. These methods worked only at very low pH range (1-3) and temperature below 30˚C. The results were very much promising and were effective in modifying these properties. In one method, the silicic acid prepared was divided into three parts and to the second part and third part, tartaric acid and sodium-pyrosulphite, respectively, were added. The three solutions were tested on filter paper and it revealed that the third solution with sodium-pyrosulphite improved the wet strength of the paper by 4,600 m (19 times more than original paper) and the dry tear strength increased by 50-60%. Also, the folding endurance and softness increased and stiffness decreased by 80%.

In his paper, (Mustoe, 2023)described the silicification in fossil wood, also known as petrifaction of wood. The paper talked about the interaction between silicic acid and plant cellulose during the silicification process in order to explain the deposition of silica on organic materials that have OH-functional groups (Figure 2.2). It shows that hydrogen-bonding between silica and cell materials is the first and foremost step in the petrifaction process. Subsequently, further deposition of silica depends on Si-O bonds. The silica bonding to organic materials eventually leads to silica deposition on the inner side of the cell wall (Figure 2.3).

Figure 2.2

Silicic Acid Forms Hydrogen Bonds (-OH) with Plant Organic Material (Cellulose). Subsequent Addition of Silica is Happening by Si-O Bonds. Black Colour Shows Cellulose and Red Colour Shows Silicic Acid and its Polymerized Form Adopted from (Mustoe, 2023)

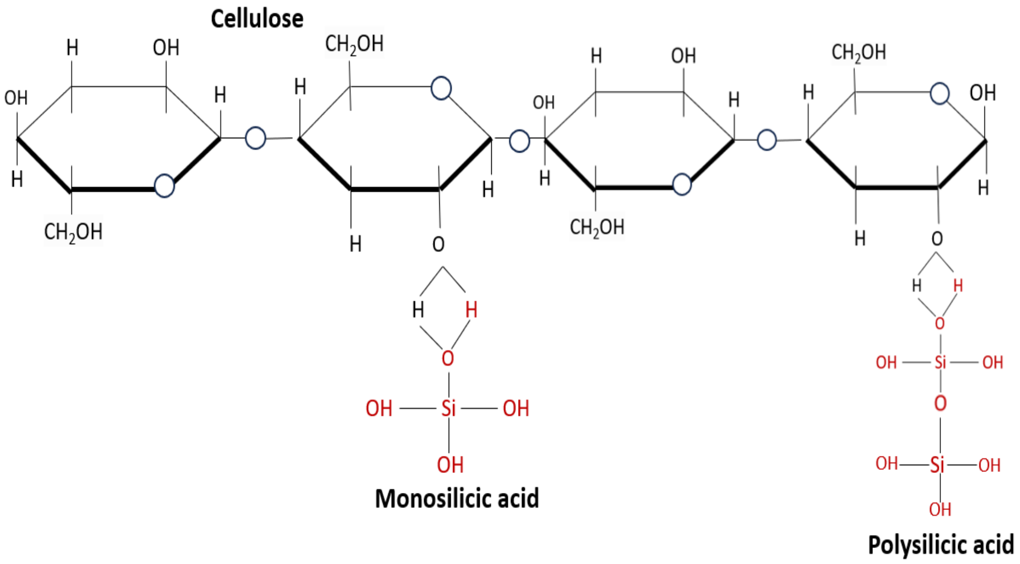
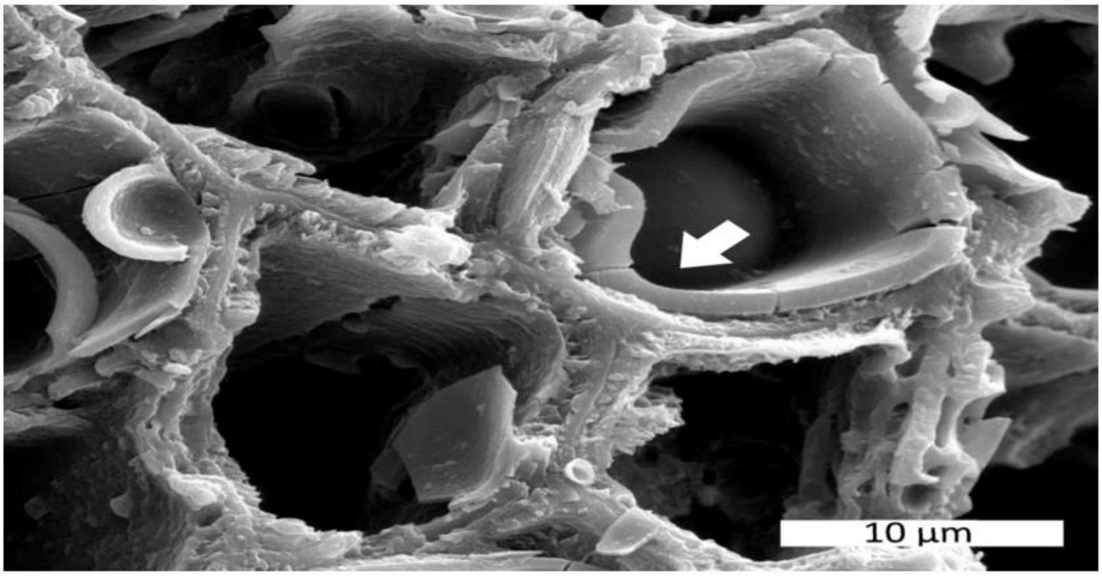


Figure 2.3

Evidence of Silica Layer Formation Inside Plant Cell



Note: *Organic templating is evidenced by silicification of multi-layered cell walls in Late Pliocene fossil wood from Red Hills Lignite Mine, Chocktaw, Missouri USA. The multilayered architecture of the cell walls remains evident, despite the presence of precipitated silica. A continuous layer of silica on an inner cell surface shown by arrow. Specimen provided by David Lang* (Mustoe, 2023)

Silica synthesis from silicic acid includes the polymerization of silicic acid, which is heavily controlled by pH. At pH 2, the concentration of OH- ions influences polymerization rate, but below pH 2, it is determined by the concentration of H+ ions. At pH 7, the rate of dissolution and reprecipitation accelerates. At 25˚C, particles expand to 5 nm diameter before slowing down. At pH values below 7, terminal diameters can reach 2-3 nm. Above pH 6 or 7, negatively charged silica particles resist each other, inhibiting aggregation but enabling diameter growth. In contrast, at low pH, silica particles with little ionic charge may easily interact to create chains, branches, and eventually 3-dimensional networks. When Si concentrations are low, the silicic acid monomer is mostly transformed to discrete particles at pH 2 before beginning to assemble. At pH 5-6, the monomer is quickly converted into particles that begin to aggregate simultaneously, making separation of the two processes difficult. (Mustoe, 2023).

The results obtained by (Perry & Lu, 1992) on the effect of cellulose on the preparation of amorphous silicas from aqueous solutions of an octahedral Si complex $K_2[Si(C_6H_4O_2)_3].2H_2O$ in neutral conditions indicated that cellulose affected the particle growth and aggregation which is the result of interaction between Si and cellulose. They assumed hydrogen bonding as the type of interaction.

2.6 Chapter Summary

This chapter focused on the prime aspects related to this study that consists mainly of silicic acid, its existence and role in nature as the bioavailable form of Si. Also, the recent research trends involving silicic acid as a beneficial fertilizer is discussed. To conclude, there are many studies which have explained the interaction between Si and cellulose interaction, the Si forms being silica, nano silica, silanes, etc. Several studies are being conducted to modify cellulose surface properties to make it a useful component of many commercial products. Because of its biodegradable and eco-friendly nature, cellulose, especially nanocellulose and its forms (cellulose nanocrystals, Nanocellulose Fibres and bacterial nanocellulose (Phanthong et al., 2018)), is in demand in many industries and researches. The combination of Si with nanocellulose is a vast field and has many applications apart from agriculture.

Based on the researches mentioned in this chapter, this study targets the stabilization of monosilicic acid with nanocellulose and investigation of its uptake ability in tomato keeping in mind its hydrophilic property which can prove beneficial for easy uptake of Si and thus enhance the growth and productivity of the plants.

CHAPTER 3
METHODOLOGY

3.1 Materials

For the preparation of a nanocellulose and monosilicic acid complex (NCF-MSA) as a potentially appropriate source of Si for plants and further testing of the complex in a model plant to investigate the effects of the product on the plant, the basic materials that are required are listed in Table 3.1. The plant selected for the study is tomato (variety Lukthar), a dicot plant.

Table 3.1

Materials Required for Preparation and Testing of NCF-MSA in a Model plant

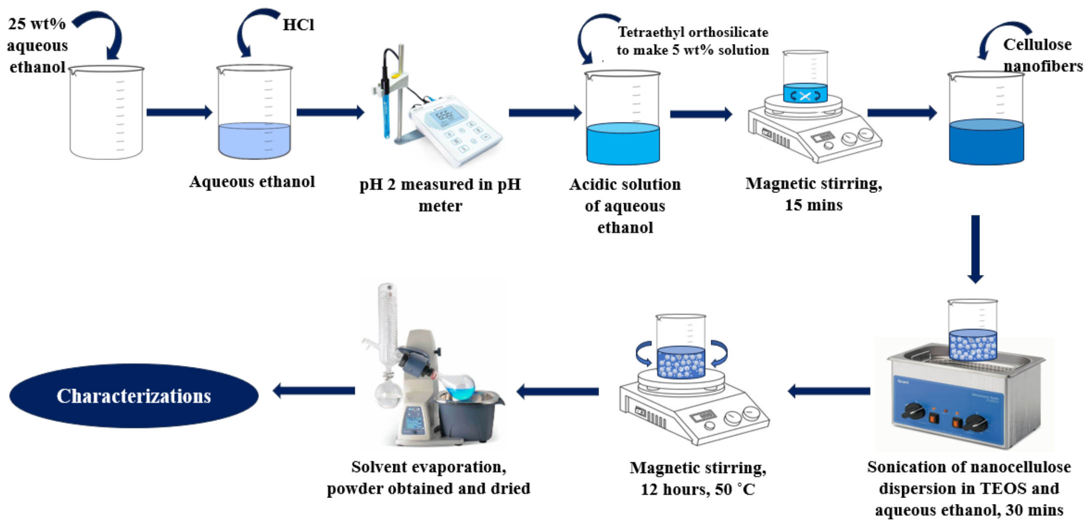
Sl No.	Materials	Purpose
Preparation of NCF-MSA		
1.	Tetraethyl orthosilicate (TEOS)	Si source (from TEOS hydrolysis)
2.	Nanocellulose Fibres (also known as nanofibrillated cellulose)	Carrier of Si in the model plant
3.	Water	For hydrolysis of TEOS
4.	Ethanol	Solvent
5.	Hydrochloric acid	For controlling pH
Application of NCF-MSA during plant testing		
6.	Seeds of tomato (model plant)	Reproductive unit of the plants
7.	Peat moss	Growing medium for seedlings
8.	Fertilizers (Urea, Triple superphosphate, Potassium chloride, Boron, Calcium oxide)	Source of nitrogen, phosphorus, potassium, boron and calcium for the plants
9.	Pots	Container for holding soil for plant growth
10.	Soil	Plant growth medium

3.2 Methodology for Preparation of Nanocellulose Fibres Stabilized Monosilicic Acid (NCF-MSA)

The following methodology was used for the preparation of NCF-MSA complex. A schematic flow diagram of the preparation method is given in Figure 3.1.

Figure 3.1

Schematic Flow Diagram for Preparation of NCF-MSA



Following the methodology given by (Bareiro & Santos, 2014) for the surface modification of hydroxyapatite (HAp) for developing polydimethylsiloxane/ hydroxyapatite composites, a methodology was adopted and modified where Nanocellulose Fibres was used instead of hydroxyapatite. Briefly, 25 wt% aqueous solution of ethanol was made in water, to which hydrochloric acid was added to bring down the pH to 2. Then, tetraethyl orthosilicate (TEOS) was added to make a 5 wt% solution. This was followed by stirring for 15 mins at 50˚C on a magnetic stirrer. Next, 1.25 g (for 500 ml dispersion) of Nanocellulose Fibres was added to the solution. At such low pH, silicic acid that was formed remains in its monomeric form and eventually bind to the Nanocellulose Fibres surface. Monosilicic acid is expected to bind to Nanocellulose Fibres as shown in Figure 2.3 in the previous chapter. Then the dispersion was sonicated in a bath sonicator for 30 minutes and followed by stirring for another 12 hours on a magnetic stirrer at 50˚C. Rotary evaporation of the solvents at

70˚C ultimately gave the powder that have undergone different characterizations to confirm the expected composition, size, etc. of the powder.

3.3 Characterization

Characterization of the NCF-MSA complex was done by the nanomaterial characterization techniques presented in Table 3.2 to get information about the morphology and composition of the same.

Table 3.2

Characterization Techniques for Characterization of NCF-MSA Along with Their Applications

Techniques	Uses
Scanning Electron Microscopy (SEM)	Determination of morphology and surface characteristics
Energy Dispersive X-ray Spectroscopy (EDS)	Elemental analysis for silicon
Fourier Transform Infrared (FTIR)	Determination of bond energy spectrum
Raman spectroscopy	Determination of chemical composition, molecular backbone structure

3.3.1 Scanning Electron Microscopy (SEM)

Morphology of nanocellulose and NCF-MSA was analyzed by using a Environmental Scanning Electron Microscope (ESEM, ThermoFisher, Quattro S) operating at 2-15 kV and under 100-5000X magnification range. The sample preparation conditions include coating with platinum with sputter current of 10 mA and 45 seconds of sputter time. The model of the coating system used was QUORUM Q150R S plus, United Kingdom (UK).

3.3.2 Energy Dispersive X-ray Spectroscopy (EDS)

Elemental analysis of elements in nanocellulose and NCF-MSA was done using Energy Dispersive X-ray Spectroscopy (EDS, Oxford X-Max 20, United Kingdom (UK)) with the help of INCA software.

3.3.3 Vibrational Spectroscopy

3.3.3.1 **Raman Spectroscopy** Analysis of molecular backbone structure and chemical composition of Nanocellulose, NCF-MSA through Raman spectroscopy were performed using Micro HR by Horiba Scientific (Model- MHRS-AMS 5500000085). The data were analyzed using LabSpec-6 software. The laser wavelength used was 785 nm, grating was 600 and Spectro was 699.883 cm^{-1}. There were 20 accumulations for each sample and the acquisition time was 10 seconds. The Detector ADC was 500 kHz. The data were baseline corrected and normalized for better visualization and analysis.

3.3.3.2 **Fourier Transform Infrared Spectroscopy (FTIR)** The functional groups in nanocellulose and NCF-MSA were investigated using a FTIR Spectrometer (FTIR, Nicolet S50, Thermo Scientific, USA) with the help of OMNIC software. The samples for FTIR were prepared using KBr. The instrument was operated in ATR mode using a DTGS ATR detector with a wavenumber range of 400–4000 cm^{-1}, resolution of 4 cm^{-1}, and 64 scans.

3.4 Application of NCF-MSA and Nanocellulose Fibres in Tomato Plant

3.4.1 Experimental Requirements and Conditions for Growth of the Plants

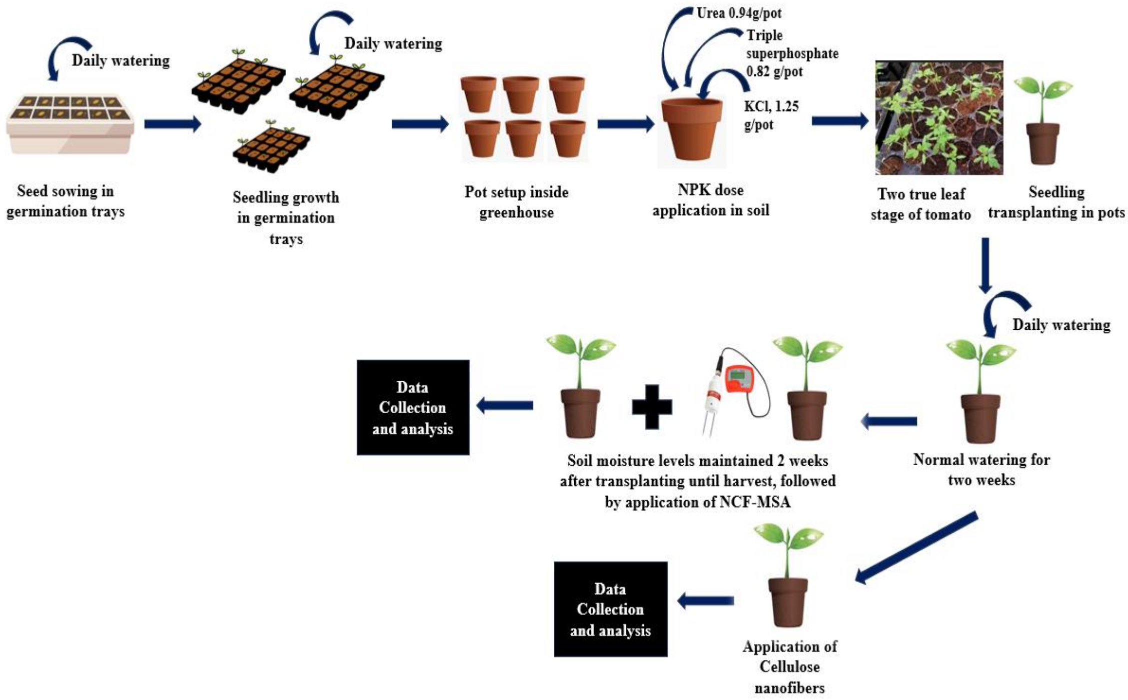
The testing of NCF-MSA complex and Nanocellulose Fibres on tomato plants was conducted in a greenhouse in pots containing 15 kg soil in a completely randomized design. The temperature in the greenhouse condition varied between 30 and 37˚C and relative humidity between 70 and 75%. Transplanted plants required staking as well as ropes up to the net shade above them to facilitate upward growth and resistance to lodging.

3.4.2 Procedure for the Testing of NCF-MSA and Nanocellulose Fibres in Tomato

A schematic diagram of the setup process of nursery and plants in pots followed by treatment of tomato plants with NCF-MSA and Nanocellulose Fibres is given in Figure 3.2.

Figure 3.2

. *Schematic Diagram of Growing Tomato Plants in Greenhouse Followed by Application of NCF-MSA and Cellulose Nanofibres in Tomato Plant*



Tomato seeds were planted in sterilized germination trays containing peat moss as a growth medium and put in a greenhouse to receive enough sunshine. When the seedlings reached the two true leaf stage, they were separated into different pots, with each pot acting as a treatment. 'The Department of Agriculture, Ministry of Agriculture and Cooperatives, Royal Thai Government' suggested that 112.5 kg/ha of N, 50 kg/ha of P_2O_5, and 100 kg/ha of K_2O be applied to the pots seven days before transplanting. This amounts to 0.94 g/pot of urea, 0.82 g/pot of triple superphosphate, and 1.25 g/pot of potassium chloride, giving 50% nitrogen, 100% P2O5, and 100% K2O as a basal dressing for tomato plants. Rest of the 50% N accounting to 0.94 g/pot was applied as top dressing 30 days after transplanting.

Watering was done sufficiently in the first 2 weeks of transplanting after which different water moisture levels (50%, 75% and 100% FC) was maintained in accordance to different treatments throughout the research to study the effectiveness of NCF-MSA in varying soil moisture conditions. The treatment doses for NCF-MSA were applied through soil incorporation after 2 weeks of transplanting. Water stress levels were maintained as per the water content in soil using a soil moisture meter. Soil moisture content was measured every day using the gravimetric method followed by (Chakma et al., 2021; Datta et al., 2009) using a portable soil moisture meter.

Three doses of Nanocellulose Fibres alone were applied to a different set of plants without maintaining water stress levels in the pots to investigate the effects of Nanocellulose Fibres on plants.

3.4.3 Pot Placement and Treatment Combinations for Field Testing

The pots were arranged following a randomized complete block design as shown in Figure 3.3 and 3.4 for NCF-MSA and Nanocellulose Fibres, respectively. The treatment combinations of NCF-MSA that were used as soil incorporation on the transplanted plants for the field testing are given in Table 3.3. The doses were selected after considering those selected by (Chakma et al., 2021) in their study, where they found 300 kg/ha of Monosilicic acid (20% Si content) at 75% and 100% FC levels gave the best results. The doses selected for this study were calculated assuming 80% Si content in NCF-MSA. Each treatment had 3 replications.

The treatment doses for Nanocellulose Fibres were selected based on the amount of Nanocellulose Fibres present in NCF-MSA i.e., 20% of NCF-MSA. Each treatment had 3 replications.

Figure 3.3

Schematic Illustration of Layout of Factorial Pot Experiments Laid Out in a Completely Randomized Design with 16 Treatment Combinations of NCF-MSA and Soil Moisture Levels and Each with 3 Replications.

T1R1	T6R1	T4R2
T11R1	T9R2	T10R1
T12R3	T2R1	T16R3
T15R1	T7R2	T14R2
T6R2	T13R2	T5R1
T16R1	T10R2	T1R2
T5R2	T11R2	T3R3
T2R2	T4R1	T7R1
T8R1	T12R2	T6R3
T1R3	T9R1	T10R3
T3R2	T13R1	T8R2
T7R3	T5R3	T2R3
T4R3	T14R1	T11R3
T15R2	T16R2	T12R1
T13R3	T8R3	T9R3
T14R3	T3R1	T15R3

Table 3.3

Treatment Combinations for NCF-MSA and Soil Moisture

Factors	Treatment doses
NCF-MSA	
T1	NCF-MSA - 0 kg/ha
T2	NCF-MSA - 37.5 kg/ha
T3	NCF-MSA - 75 kg/ha
T4	NCF-MSA - 112.5 kg/ha
Soil moisture levels	
T5	50% FC
T6	75% FC
T7	100%FC
NCF-MSA x Soil Moisture	
T8	NCF-MSA - 37.5 kg/ha + 50% FC
T9	NCF-MSA - 37.5 kg/ha + 75% FC
T10	NCF-MSA - 37.5 kg/ha + 100% FC
T11	NCF-MSA - 75 kg/ha + 50% FC
T12	NCF-MSA - 75 kg/ha + 75% FC
T13	NCF-MSA - 75 kg/ha + 100% FC
T14	NCF-MSA - 112.5 kg/ha + 50% FC
T15	NCF-MSA - 112.5 kg/ha + 75% FC
T16	NCF-MSA - 112.5 kg/ha + 100% FC
No. of replications	3
Total	48

Figure 3.4

Schematic Illustration of Layout of Factorial Pot Experiments Laid Out in A Completely Randomized Design With 4 Treatment Combinations of Nanocellulose Fibres and Each with 3 Replications.

TAR1 (49)	TCR1 (50)	TBR2 (51)
TBR1 (52)	TDR2 (53)	TCR3 (54)
TAR2 (55)	TBR3 (56)	TDR3 (57)
TDR1 (58)	TAR3 (59)	TCR2 (60)

Table 3.4

Treatment Combinations for Nanocellulose Fibres

Treatment number	Treatment doses
TA	Nanocellulose Fibres + 0 kg/ha
TB	Nanocellulose Fibres + 7.5 kg/ha
TC	Nanocellulose Fibres + 15 kg/ha
TD	Nanocellulose Fibres + 22.5 kg/ha
Replications	3
Total	12

This was followed by regular data collection on growth and yield parameters of the plant until harvest for analysis purposes.

3.5 Data Collection and Analysis

Data collection on different parameters of the plant after application of NCF-MSA was proceeded using the equipment and methods as given below.

3.5.1 Growth Parameters

The growth criteria evaluated were plant height, leaf area per plant, number of leaves, Shoot biomass, Root biomass, longest root length, and root volume. Plant height (measured in cm) was measured one day before harvest using a measuring tape to determine the distance between the ground surface and the highest leaf tip. Leaf area per plant (measured in square meters per plant) was obtained non-destructively by measuring the greatest width and length of three leaves using the method described by (KARACA et al., 2021). The total number of leaves on each plant was calculated via eye counting. Shoot and Root biomasss (measured in grams per plant) were determined by weighing shoot and root samples on a precision scale after drying in a hot air oven until constant weight was achieved (Chakma et al., 2021). Length of the longest root of a plant (measured in centimeters) was measured using a ruler and root volume was measured by immersing the roots in a measuring cylinder containing water and recording the difference in water volume.

3.5.2 Yield Parameters

Yield parameters encompassed the number of flowers, fruit yield per plant, number of fruits per plant, and irrigation water productivity. Number of flowers was recorded by eye counting. Fruit yield per plant (measured in grams per plant) was determined by weighing all the fruits from a single plant using a scale. Counting the total number of fruits from one plant provided the number of fruits per plant. Irrigation water productivity (measured in kilograms per cubic meter) was calculated by dividing the fruit yield (in kilograms) by the total volume of irrigation water applied (in cubic meters) as proposed by (Maneepitak et al., 2019) (H. Ullah et al., 2018) (Chakma et al., 2021).

3.5.3 Fruit Quality Parameters

Fruit quality parameters include fruit length, fruit pH, and total soluble solids. Fruit length (cm) was measured with a digital vernier calliper. Fruit pH was measured from the juice of tomato (obtained by sieving through a muslin cloth) with a pH meter

(‘Model FiveGo F2, Mettler-Toledo GmbH, Im Langacher, Greifensee, Switzerland’) (Turhan et al., 2011) . Total soluble solids (TSS) content of the fruit was calculated from the juice of tomato fruits with a handheld refractometer (‘Model HI96801, Hanna Instruments, Woonsocket, RI, USA’) .

3.5.4 Physiological Parameters

Physiological parameters include leaf greenness, Leaf Relative Water Content, Membrane Stability Index, Crop Water Stress Index. Leaf greenness or relative chlorophyll content was measured by a handheld chlorophyll meter from fully emerged new leaves. Leaf Relative Water content (LRWC) (%) was measured using the formula given by (Jones & Turner, 1978) as given below.

$$LRWC = \frac{FW-DW}{TW-DW} \times 100 \quad \text{.................................... Equation 1}$$

where, FW is the ‘fresh weight of leaf’, TW is the ‘turgid weight’ of the leaf after rehydrating the leaf in distilled water and keeping in darkness for one day and DW is the ‘dry weight’ of the leaf after oven drying the turgid leaf for two days at 70˚C.

Membrane Stability Index was measured with a conductivity meter (‘Model Eutech CON 150, Thermo Scientific, Eutech Instruments, Singapore’)) by the following formula given by (Camejo et al., 2005; Lafuente et al., 1991) .

$$\text{Membrane Stability Index} = (1 - \frac{EC1}{EC2}) \times 100 \quad \text{........................Equation 2}$$

Where, EC1 is the ‘electrical conductivity’ of a solution containing leaf tissues incubated at 40 ˚C in a water bath for 30 minutes and EC2 is the ‘electrical conductivity’ of killed leaf tissues by boiling at 100˚C for 15 minutes in a water bath and subsequent cooling at room temperature (Alam et al., 2023).

Crop Water Stress Index (CWSI) was measured using a ‘forward looking infrared (FLIR) camera’ of spectral range 14.0 7.5 µm and resolution of 240 × 180 pixels during 10-11 am. Distance between leaf surface and camera was 1 m and the emissivity value selected was 0.95. Leaf temperature was compared to wet reference using water-wet cotton wool and dry reference was compared with the help of a black paper. Thermal images were analyzed using ‘FLIR Tool 5.1 software’. Thus, CWSI was calculated using the formula given by (Wiriya-Alongkorn et al., 2013) .

$CWSI = (Tleaf - Twet) / (Tdry - Tleaf)$ ....Equation 3

Where, T_{leaf} is ‘leaf temperature’ (˚C) calculated from thermal imagery, T_{dry} is ‘dry reference temperature’ (˚C) and T_{wet} is ‘wet reference temperature’ (Theerawitaya et al., 2023).

3.5.5 Photosynthetic Parameters

Photosynthetic parameters such as net photosynthetic rate (measured in µmol CO_2 m^{-2} s^{-1}), transpiration rate (measured in mmol H2O m^{-2} s^{-1}), stomatal conductance (measured in mmol H2O m^{-2} s^{-1}), etc., were assessed using a portable photosynthesis system (‘LI-6400XT, Li-COR, Lincoln, NE, USA’) on the upper leaf before noon during the fruiting stage. Measurements were conducted within the assimilation chamber under stable conditions, with an ambient temperature maintained at 28 ± 1 °C and a CO2 concentration of 370 ± 20 µmol/mol. Data were recorded once conditions stabilized following chamber sealing. The air flow rate of the infrared gas analyzer (IRGA) micro-chamber was set at 500 µmol· $m^{-2}s^{-1}$. Illumination was provided by a ‘redblue 6400-02B LED’ light source, delivering a photosynthetic photon flux density of 1,000 µmol·$m^{-2}s^{-1}$.

Chlorophyll fluorescence parameters (‘Minimum fluorescence F_0’, ‘Maximum fluorescence F_m’, ‘maximum quantum yield of photosystem II F_v/F_m’ and ‘effective quantum yield of photosystem II Φ_{PSII}’) were measured using a portable fluorescence monitoring system (‘FMS2, Hansatech, King’s Lynn, UK’). Fv/Fm was calculated from the formula given by (Dao & Beardall, 2016):

$Fv/Fm = (Fm - F0)/Fm$..Equation 4

Φ_{PSII} was calculated using the formula given by (Ogawa et al., 2017) -

$\Phi PSII = (Fm' - Fs)/Fm$ ………………………………….………. Equation 5

where Fm′ and Fs are maximum fluorescence and steady-state fluorescence, respectively, measured under ambient light (Ogawa et al., 2017)

3.5.6 Characterization of Root Samples

Morphology of root cross-sections were analyzed by using an Environmental Scanning Electron Microscope (ESEM, ThermoFisher, Quattro S) operating at 2-15 kV and under 100-5000X magnification range. The sample preparation conditions include coating with platinum with sputter current of 10 mA and 45 seconds of sputter time. The model of the coating system used was QUORUM Q150R S plus, United Kingdom (UK).

Molecular backbone structure and presence of silicon in root samples were determined using Raman Spectroscopy using 'Micro HR by Horiba Scientific' ('Model- MHRS-AMS 5500000085'). The data were analyzed using 'LabSpec-6' software. The laser wavelength used was 785 nm, grating was 600 and Spectro was 699.883 cm^{-1}. There were 20 accumulations for each sample and the acquisition time was 10 seconds. The Detector ADC was 500 kHz. The data were baseline corrected and normalized for better visualization and analysis.

3.5.7 Data Analysis

The data collected for the different parameters was analyzed for variations in treatment groups following the Analysis of variance (ANOVA) method using Statistix 10 software. ANOVA gave the statistically significant differences between the means of treatments by partitioning the variations in within-group and between group components. This means that ANOVA gives the variations in means of individual variables which can then generate the interaction of the independent variables with the dependent variable. The F-value at the end of analysis gives the amount of variation between sample means relative to within samples. The larger the F-value, the larger the variation. Again, a p-value lower than 0.05 gives that there is statistically significant difference between groups.

A 2-factorial complete randomized design (CRD) was the experimental design for the analysis. It was analyzed by two-way ANOVA method followed by a Tukey HSD test for all pair-wise comparisons. Data was presented as means of 3 replications ± standard deviations.

CHAPTER 4
RESULTS AND DISCUSSION

This chapter provides the results obtained and analysed after the successful completion of all the experiments conducted as part of the research work followed by a discussion about the consequences of the experiments. The results are analysed, arranged and explained for easy and logical comprehension of the readers. The discussion about the results provides the interpreted reasons and possible scenarios responsible for the outcomes of the experiment. The outcomes, thus, give a view of the different plant parameters which are affected due to the incorporation of the novel nano-based product. The results will provide evidence for the utility of the nano-based product as a silicon source for low silicon accumulator plants. The chapter is divided into 7 parts- 4.1 Synthesis and characterization of Nanocellulose Fibres stabilized monosilicic acid (NCF-MSA) 4.2 Effects of Nanocellulose Fibres stabilized monosilicic acid (NCF-MSA) and nanocellulose on growth parameters under water stress conditions 4.3 Effects of Nanocellulose Fibres stabilized monosilicic acid (NCF-MSA) and nanocellulose on yield parameters under water stress conditions 4.4 Effects of Nanocellulose Fibres stabilized monosilicic acid (NCF-MSA) and nanocellulose on fruit quality parameters under water stress conditions 4.5 Effects of Nanocellulose Fibres stabilized monosilicic acid (NCF-MSA) and nanocellulose on physiological parameters under water stress conditions 4.6 Effects of Nanocellulose Fibres stabilized monosilicic acid (NCF-MSA) and nanocellulose on photosynthetic parameters under water stress conditions and 4.7 Characterization of root samples.

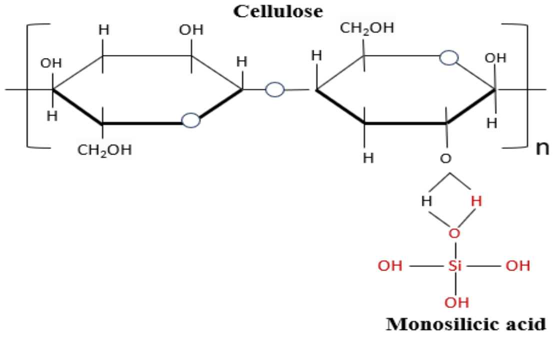
4.1 Synthesis and Characterization of Nanocellulose Fibres Stabilized Monosilicic Acid (NCF-MSA)

4.1.1 Synthesis of NCF-MSA

Our hypothesis talks about formation of monosilicic acid at very low pH (around 2) when TEOS is dispersed in a solvent, which binds with Nanocellulose Fibres to through modification of the hydroxyl groups in both molecules and thus prevents polymerization of the silicic acid (Figure 4.1).

Figure 4.1

Bonding Between Nanocellulose and Monosilicic Acid- A Hypothesis

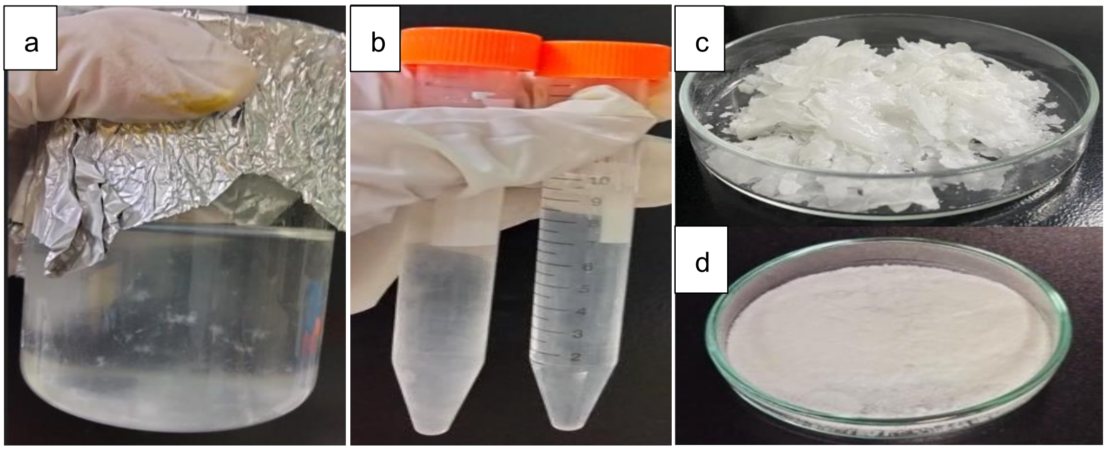


In this study, the author used an adaptation of the sol-gel method followed by (Bareiro & Santos, 2014) to prepare the complex of nanocellulose and monosilicic acid, where he used TEOS as a source of surface modification of hydroxyapatite to form a complex formation of silicic acid and hydroxyapatite. Tetraethylorthosilicate (TEOS) was used as the source of silicon for this study due to the unavailability of reliable monosilicic acid in the market. Since, as per our hypothesis, our goal was to achieve a completely novel complex of nanocellulose with monosilicic acid, a comparable and commercial monosilicic acid for the same was unable to obtain. Therefore, TEOS was used so that the monosilicic acid that is formed when dispersed in aqueous ethanol at a very low pH of 2, gets bonded to the Nanocellulose Fibres through surface modification of the hydroxyl groups of nanocellulose.

Initially, the dispersion of nanocellulose and monosilicic acid was centrifuged with the view of obtaining a precipitate which was to be dried to get the final product. But no precipitate was obtained upon centrifugation. This method was discarded and rotary evaporation was used to replace the centrifugation part (Figure 4.2 a and b).

Figure 4.2

NCF-MSA Preparation A) Nanocellulose and Monosilicic Acid Dispersion B) Nanocellulose and Monosilicic Acid Dispersion After Centrifugation C) White Flakes Of NCF-MSA Obtained After Rotary Evaporation D) Powder Of NCF-MSA Obtained from The Flakes



Rotary evaporation of the nanocellulose and tetraethyl orthosilicate (TEOS) dispersion in aqueous ethanol gave white flakes which were grinded to obtain a fine crystalline powder (NCF-MSA) (Figure 4.2 c and d), which is the final product.

4.1.2 Characterization of NCF-MSA

4.1.2.1 **Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDS)** SEM was performed to get information about the surface changes that occurred when nanocellulose was modified, while, EDS provided information about the atomic weight percentage of silicon found in NCF-MSA.

Figure 4.3

SEM Images for Evidence of Silicon in NCF-MSA a) Nanocellulose Fibres b) Silicon (Represented in Green Colour) Binding with Nanocellulose Fibres

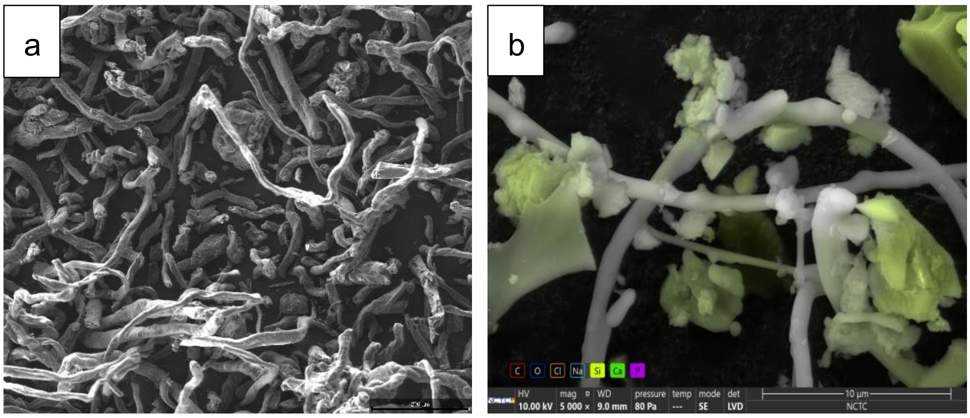
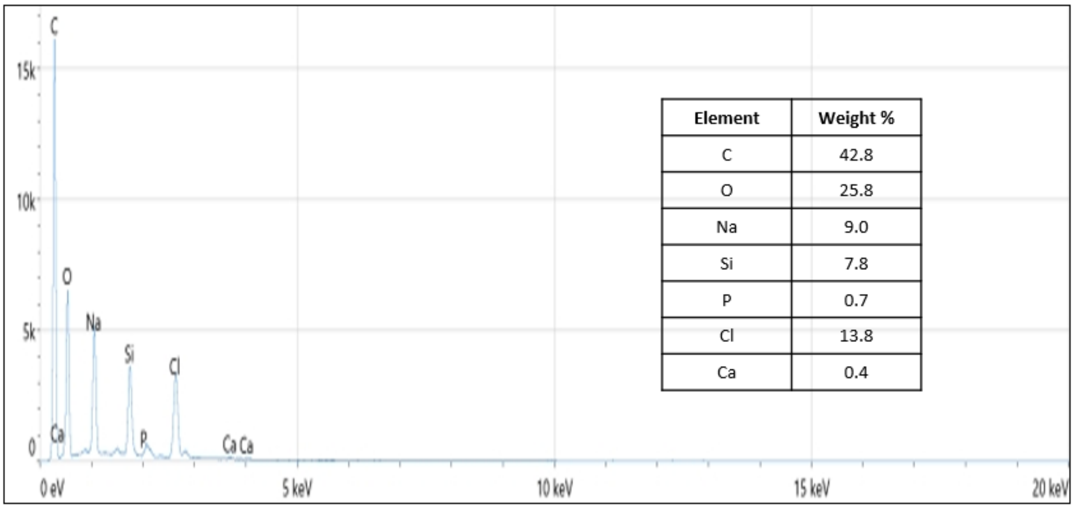


Figure 4.4

EDS Spectra of NCF-MSA



Element	Weight %
C	42.8
O	25.8
Na	9.0
Si	7.8
P	0.7
Cl	13.8
Ca	0.4

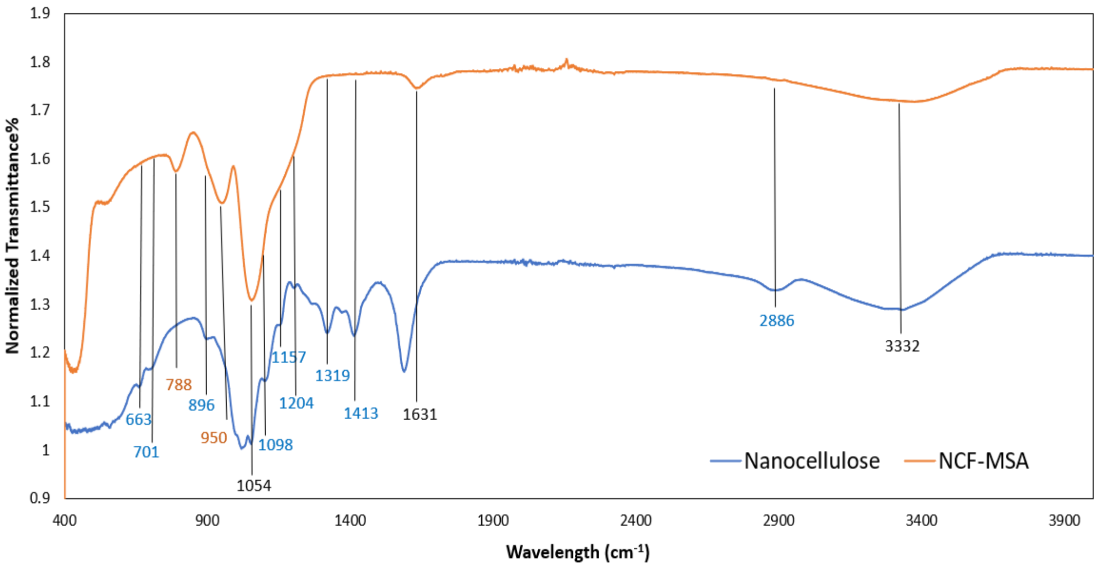
The above SEM images (Figure 4.3) confirms complex formation between Nanocellulose Fibres and silicic acid. However, from the EDS spectra (Figure 4.4), it is seen that compared to other elements, silicon is 6 weight% lower than carbon and about 4 weight% lower than oxygen, which are the main elements found in cellulose. Thus, it can be said that the percentage of silicon in the product is lower than expected.

4.1.2.2 **Vibrational Spectroscopy** Vibrational Spectroscopy, which comprises of Fourier transform Infrared Spectroscopy (FTIR) and Raman Spectroscopy, was performed to determine bond vibrations resulting from surface modification of nanocellulose to form NCF-MSA through hydrogen bonding of monosilicic acid to hydroxyl groups of nanocellulose.

Fourier Transform Infrared Spectroscopy (FTIR) of NCF-MSA (Figure 4.5) was performed to determine the bonds present in NCF-MSA. The FTIR spectrum results showed peaks which depicted some of the nanocellulose bonds as well as bonds which can justify the presence of monosilicic acid bonded with Nanocellulose Fibres.

Figure 4.5

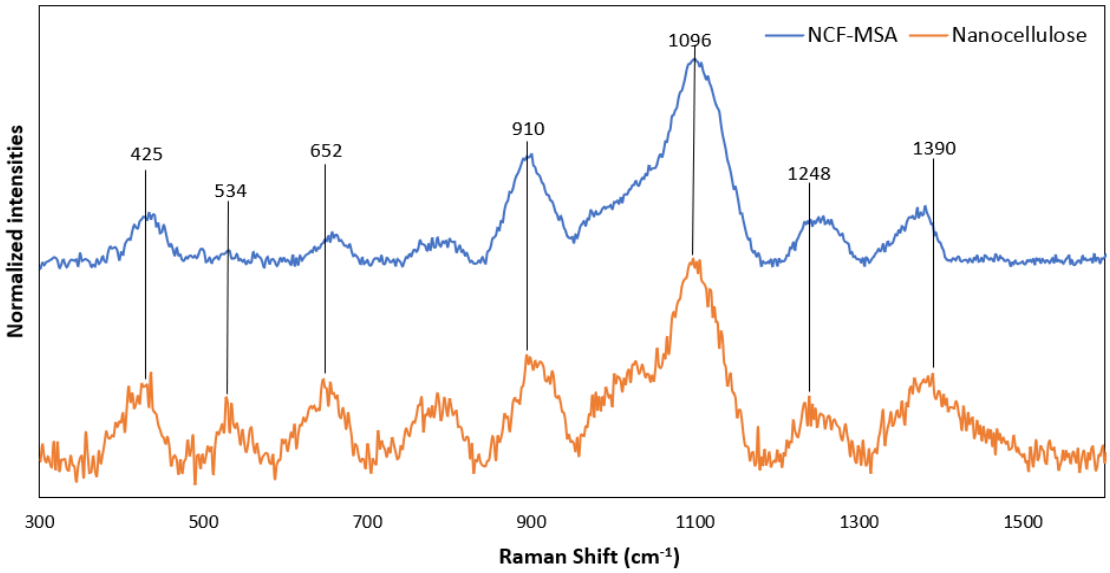
FTIR Spectra of Nanocellulose and NCF-MSA



In the nanocellulose spectrum (Figure 4.5), the peaks at 3332 cm^{-1} and 2886 cm^{-1} relates to stretching of OH groups and HCH stretching of aliphatic groups, respectively. CH bonds in aromatic rings are represented by the peak at 1319 cm^{-1}. 1098 cm^{-1} peak is corresponding to COC anti-symmetric bridge stretching, that at 1053 cm^{-1} is denoting CO stretching and 896 cm^{-1} is denoting COC and CCO deformation and stretching vibrations. HCH vibrations are shown by a small peak at 701 cm^{-1} (Gea et al., 2020). After modification of nanocellulose, i.e., in NCF-MSA, most of the peaks from nanocellulose disappeared. At around 3332, there is a peak shift in NCF-MSA suggesting the formation of H bonded silanol or OH…-H OH with nanocellulose. OH bending of adsorbed water is shown by a peak with reduced transmittance at around 1600 cm^{-1} which can be attributed to loss of water during evaporation process for NCF-MSA preparation. The water adsorbed in NCF-MSA can be explained as the residual water left after evaporation or as water absorbed during storage. The increase in transmittance at peak at 1054 cm^{-1} confirms Si-O stretching due to formation of bonds between nanocellulose and NCF-MSA. Similarly, Si-OH in NCF-MSA is also confirmed by the peak at 950 cm^{-1}. 788 cm^{-1} relates the symmetric stretching of Si-O-Si (Hamelmann et al., 2005; Portaccio et al., 2011).

Figure 4.6

Raman Spectra of Nanocellulose and NCF-MSA to Determine the Molecular Backbone Structure and Molecular Interactions in NCF-MSA which was Compared to that of Nanocellulose.



Raman Spectroscopy results of NCF-MSA (Figure 4.6) showed some peak damping and shifts when nanocellulose was modified to bind with monosilicic acid through hydrogen bonding.

425 cm^{-1} and 652 cm^{-1} shifts in the Raman spectra of nanocellulose and NCF-MSA can be attributed to skeletal-bending modes of CCC, COC, OCC and skeletal stretching modes of CC and CO (Wiley & Atalla, 1987). The cellulose skeletal bending and stretching modes at lower wavenumbers between 400 to 700 cm^{-1} were seen to reduce in terms of their intensities and slightly red-shifted in the NCF-MSA complex. The 534

cm^{-1} peak in the NCF-MSA complex was not detected which might be due to amorphous form of silicon. These observations indicate a possible complex formation of Nanocellulose and NCF-MSA resulting in damping of the skeletal vibration modes of the cellulose structure. Shifts around 910 cm^{-1} refers to bending vibration of HCC and HCO localized at C6 position (Dhar et al., 2016; Djordjevic et al., 2018; Szymańska-Chargot et al., 2017). The intensity of these bendings observed around 900 cm^{-1} was also found to reduce upon NCF-MSA complex formation. Raman shift around 1096 cm^{-1} in NCF-MSA, respectively, represents C−O−C ring stretching modes and the β-1,4 glycosidic linkage (C–O–C) stretching modes in cellulose chains (Gierlinger et al., 2006). These characteristic nanocellulose bands also slightly shifted to higher wavenumbers upon NCF-MSA complex formation. Shifts around 1248 cm^{-1} denotes energy from transition modes due to different types of internal coordinates of cellulose structure which contribute to the modes. Shifts around 1390 cm^{-1}, again, represents COH bending (Wiley & Atalla, 1987). At higher wavenumbers between 1200 to 1400, no significant changes were found in the peak intensities.

4.2 Effects Of Nanocellulose Fibres Stabilized Monosilicic Acid (NCF-MSA) and Nanocellulose on Growth Parameters under Water Stress Conditions

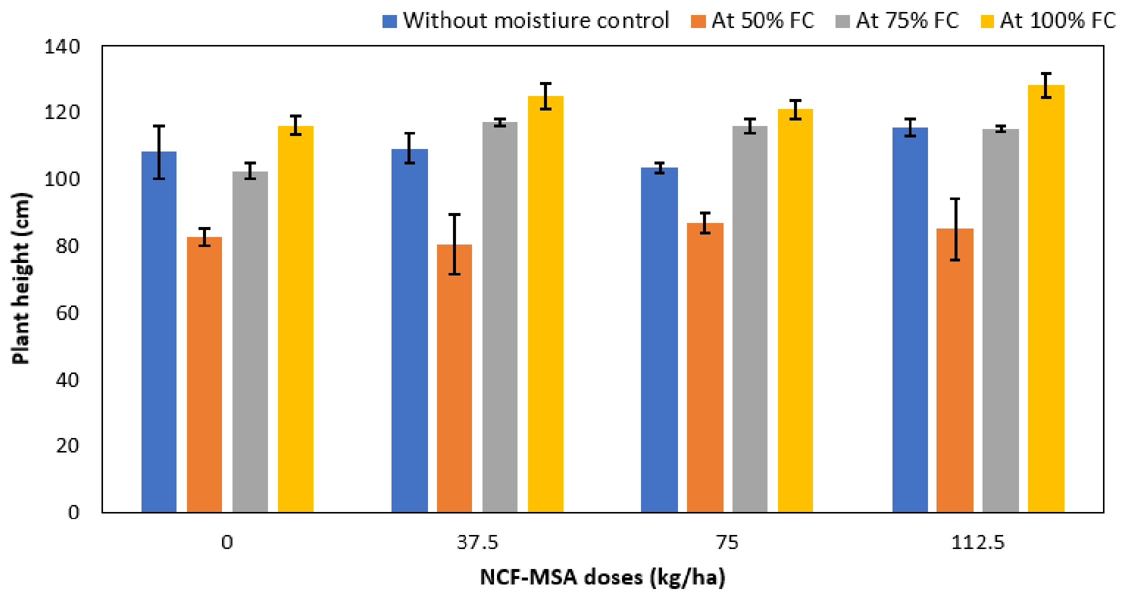
NCF-MSA was applied as soil incorporation in plants and data on growth parameters were recorded. The growth parameters include plant height, number of leaves, leaf area, shoot biomass, root biomass, length of the longest root and root volume. The collected data was analysed and presented as bar graphs, followed by performing an ANOVA on these parameters to investigate significance of data, The means were compared pair-wise using Tukey HSD test. Finally, a discussion on these parameters is presented.

4.2.1 Plant Height (cm)

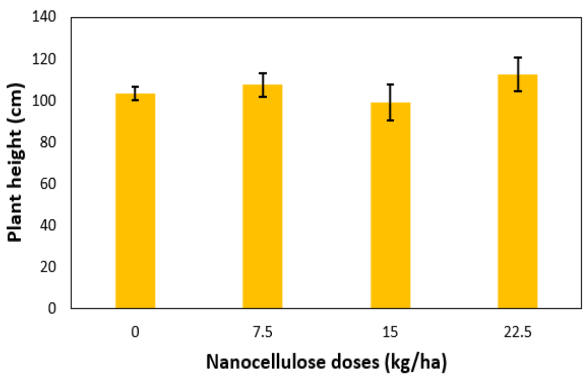
Figure 4.7

Effect of A) NCF-MSA and Soil Moisture B) Nanocellulose on Plant Height

a)



b)



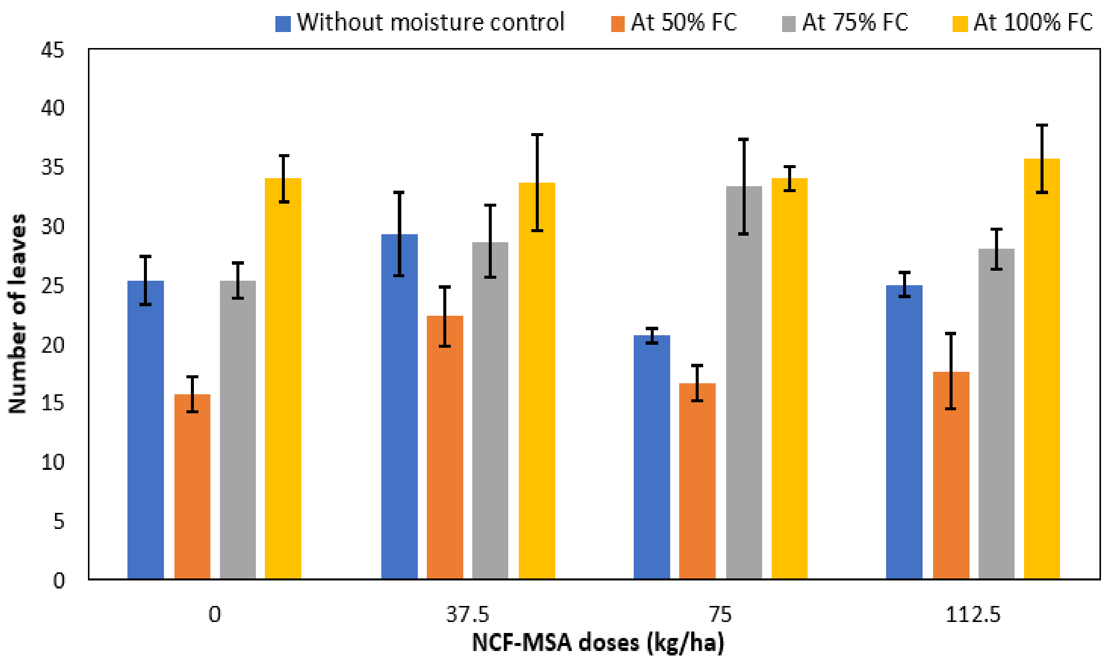
From the figure 4.7, it can be seen that without soil moisture control, plant height was highest (115.33 cm) in 112.5 kg/ha dose of NCF-MSA, which is 6.4% taller than the respective control plant of the group and 5.3% and 10.4% taller, respectively for NCF-MSA doses of 37.5 kg/ha and 75 kg/ha. When compared at 50% FC soil moisture level, plant height was maximum (86.67 cm) at 75 kg/ha dose of NCF-MSA, which is approximately 5% higher than the corresponding control plant and higher than doses 37.5 kg/ha and 112.5 kg/ha by 7.5% and 2%, respectively. Whereas, at 75% FC soil moisture level, 37.5 kg/ha dose of NCF-MSA gave the highest plant height of 117 cm, which is 12.54% more than the respective control plant and 1.2% and 1.7% more that doses 75 kg/ha and 112.5 kg/ha, respectively. Finally, for 100% FC, the maximum height (128 cm) was attained due to dose 112.5 kg/ha. Plants at doses 37.5 and 75 kg/ha were shorter than those at 112.5 kg/ha by 2.6% and 5.6%, respectively. Control plants had approximately 8% less leaves than 112.5 kg/ha dose. Overall, on an average, plants at 100% FC attained the maximum height (122.4 cm), followed by those at 75% FC (112.5 cm) and 50% FC (83.5 cm). Therefore, it can be summarized that moisture plays the foremost role in plant height and visibility of effects of NCF-MSA. With the application of nanocellulose, the maximum plant height (112.67 cm) was shown by nanocellulose dose of 22.5 kg/ha, which is 8.14% higher than the control and 4.6% and 12% higher than 7.5 kg/ha and 15 kg/ha doses, respectively.

4.2.2 Number of Leaves

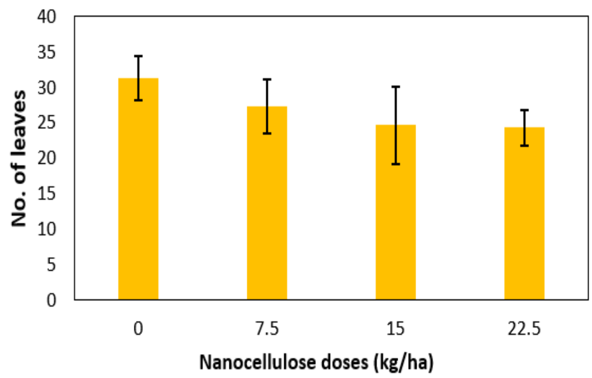
Figure 4.8

Effect of A) NCF-MSA and Soil Moisture B) Nanocellulose on Number of Leaves

a)



b)



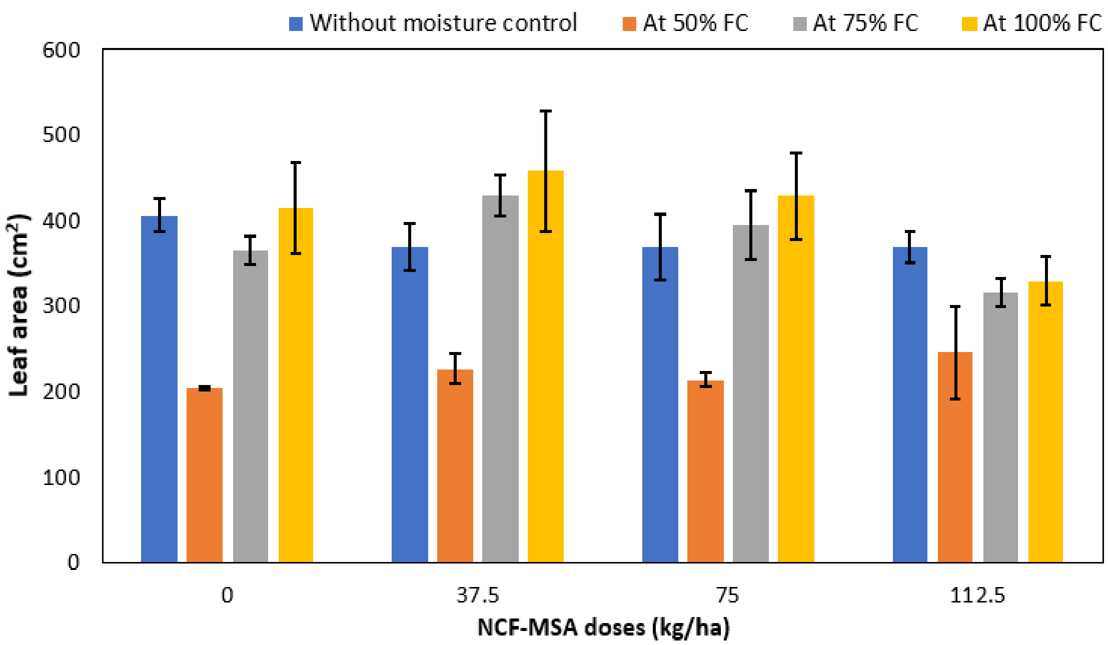
From the figure 4.8, it can be seen that without soil moisture control, number of leaves was highest (29.33) in 37.5 kg/ha dose of NCF-MSA, which is 13.5% more than the respective control plant of the group and 29.5% and 14.8% more, respectively for NCF-MSA doses of 75 kg/ha and 112.5 kg/ha. When compared at 50% FC soil moisture level, number of leaves was maximum (22.33) at 37.5 kg/ha dose of NCF-MSA, which is approximately 30% higher than the corresponding control plant and higher than doses 75 kg/ha and 112.5 kg/ha by 25.3% and 20.9%, respectively. Whereas, at 75% FC soil moisture level, 75 kg/ha dose of NCF-MSA gave the highest number of leaves i.e., 33.33, which is 24% more than the respective control plant and approximately 14% and 16% more that doses 37.5 kg/ha and 112.5 kg/ha, respectively. Finally, for 100% FC, the maximum number of leaves (35.67) was attained due to dose 112.5 kg/ha. Plants at doses 37.5 and 75 kg/ha had lesser leaves than those at 112.5 kg/ha by 5.6% and 4.7%, respectively. Control plants had approximately 5% less leaves than 112.5 kg/ha dose. Overall, plants at 100% FC had maximum number of leaves (34.33), followed by those at 75% FC (28.8) and 50% FC (18.1). Therefore, it can be summarized that moisture is also key for number of leaves in a plant and for showing effects of NCF-MSA. In case of nanocellulose, the maximum number of leaves (31.33) was shown by the control, which is 7.4%, 28.7% and 22.3% higher than 7.5 kg/ha, 15 kg/ha and 22.5 kg/ha doses, respectively.

4.2.3 Leaf Area (cm^2)

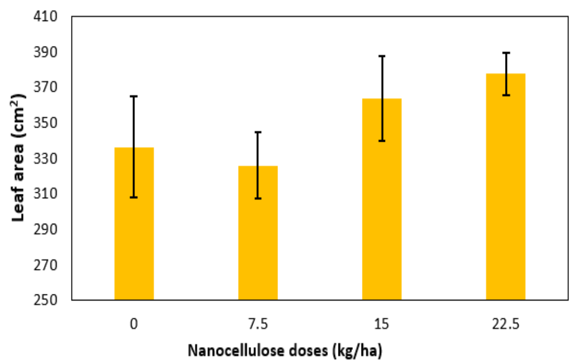
Figure 4.9

Effect of a) NCF-MSA and Soil moisture b) Nanocellulose on Leaf Area

a)



b)



From the figure 4.9, it can be seen that without soil moisture control, leaf area was highest (405.713 cm^2) in the control plant, which is 9.3%, 9.2% and 8.9% higher than the plants of NCF-MSA doses of 37.5 kg/ha, 75 kg/ha and 112.5 kg/ha, respectively. When compared at 50% FC soil moisture level, leaf area was maximum (244.893 cm^2) at 112.5 kg/ha dose of NCF-MSA, which is approximately 17% higher than the corresponding control plant and higher than doses 37.5 kg/ha and 112.5 kg/ha by 7.6% and 13%, respectively. Whereas, at 75% FC soil moisture level, 37.5 kg/ha dose of

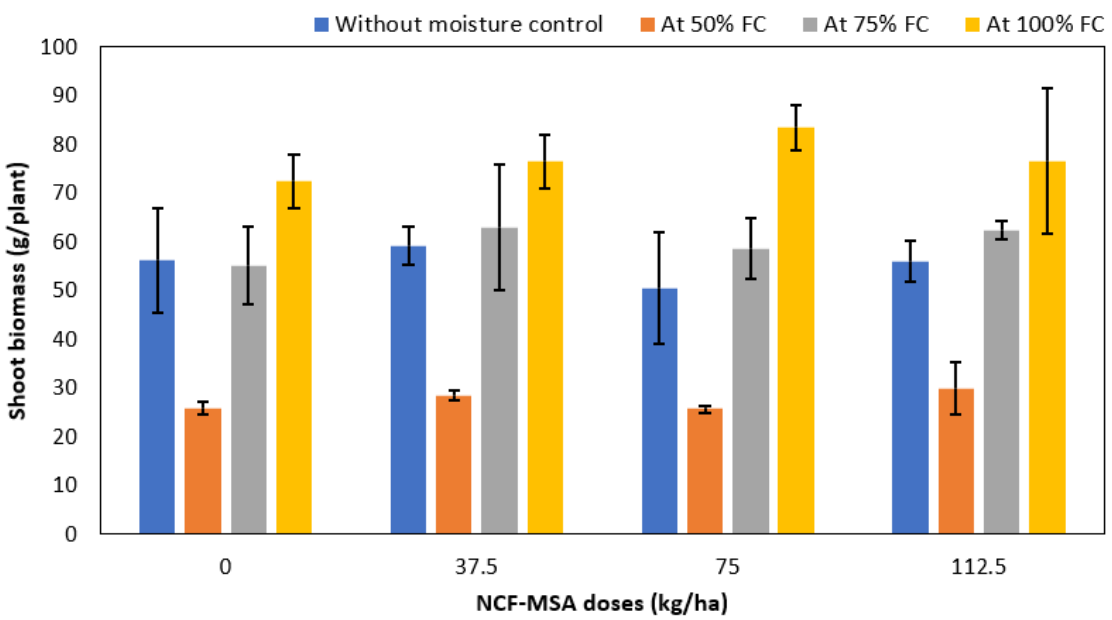
NCF-MSA gave the highest leaf area of 428.91 cm^2, which is 15% higher than the respective control plant and 7.8% and 26.5% more that doses 75 kg/ha and 112.5 kg/ha, respectively. Finally, for 100% FC, the maximum area (457.277 cm^2) was attained due to dose 37.5 kg/ha. Plants at doses 75 and 112.5 kg/ha had smaller leaf area than those at 37.5 kg/ha by 6.3% and 28.1%, respectively. Control plants had approximately 8% less leaves than 112.5 kg/ha dose. Overall, even here, plants at 100% FC attained the maximum leaf area (407.121 cm^2), followed by those at 75% FC (375.812 cm^2) and 50% FC (221.945 cm^2). Inefficient soil moisture gave smaller plants due to which leaf area was affected. Apart from the group without soil moisture control, NCF-MSA have shown increase in leaf area. In case of nanocellulose, the maximum leaf area (377.373 cm^2) was shown by nanocellulose dose of 22.5 kg/ha, which is 11% higher than the control and 13.7% and 3.7% higher than 7.5 kg/ha and 15 kg/ha doses, respectively.

4.2.4 Shoot Biomass (g/plant)

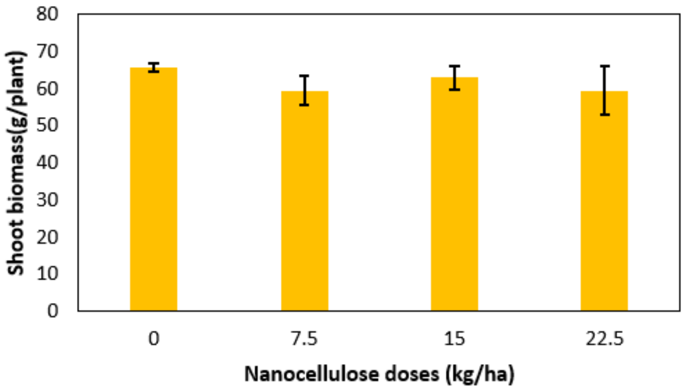
Figure 4.10

Effect of a) NCF-MSA and Soil Moisture b) Nanocellulose on Shoot Biomass

a)



b)



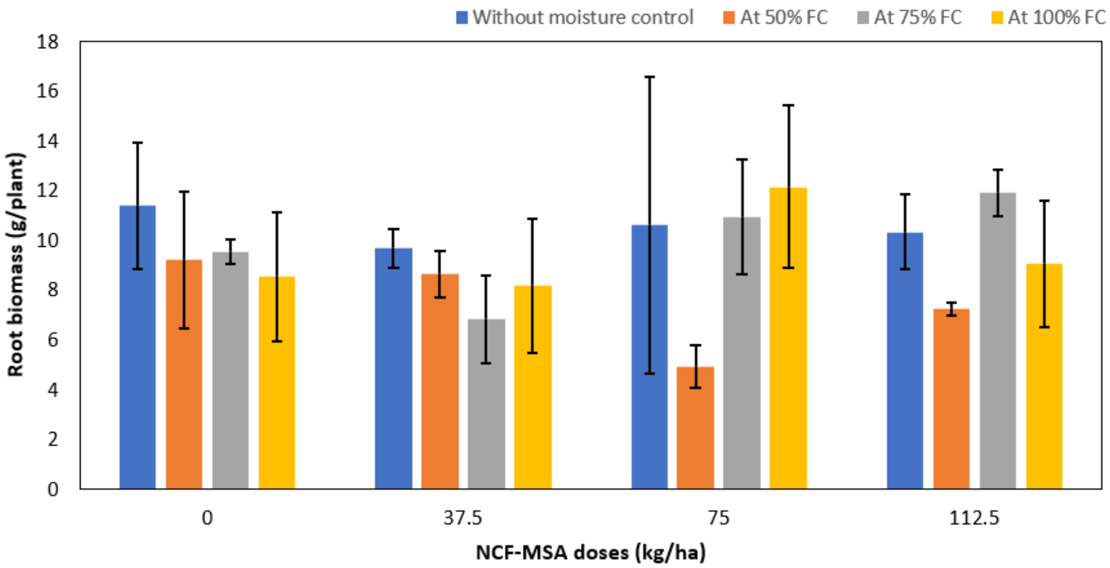
From the figure 4.10, it can be seen that without soil moisture control, shoot biomass was highest (59.14 g/plant) for the 37.5 kg/ha NCF-MSA dose, which is 5.1% more than the control and 14.8% and 5.5% more than 75 kg/ha and 112.5 kg/ha NCF-MSA doses, respectively. When compared at 50% FC soil moisture level, shoot biomass was maximum (29.82 g/plant) at 112.5 kg/ha dose of NCF-MSA, which is approximately 13.7% higher than the corresponding control plant and higher than doses 37.5 kg/ha and 112.5 kg/ha by 4.7% and 14.12%, respectively. Whereas, at 75% FC soil moisture level, 37.5 kg/ha dose of NCF-MSA gave the highest Shoot biomass of 62.83 g/plant, which is 12.4% higher than the respective control plant and 6.7% and 0.7% more that doses 75 kg/ha and 112.5 kg/ha, respectively. Finally, for 100% FC, the maximum Shoot biomass (83.26 g/plant) was attained due to dose 75 kg/ha. Plants at doses 37.5 and 112.5 kg/ha had lesser shoot biomass than those at 75 kg/ha by 8.2% and 8.07%, respectively. Control plants had approximately 13.1% less leaves than 75 kg/ha dose. To conclude, soil moisture affected the dry matter weight of shoots significantly. 100% FC plants had the maximum dry matter weight of 77.15 g on an average which is higher than 75% FC plants by 22.6%, followed by 50% FC plants which has 64.5% lesser weight than 100% FC plants. On application of nanocellulose, the maximum shoot biomass (65.68 g/plant) was shown by the control, which is 9.5%, 4.2% and 9.4% higher than 7.5 kg/ha, 15 kg/ha and 22.5 kg/ha doses, respectively.

4.2.5 Root Biomass (g/plant)

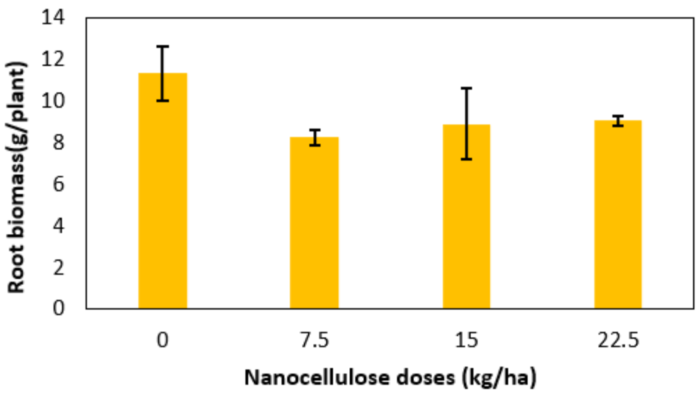
Figure 4.11

Effect of a) NCF-MSA and Soil Moisture b) Nanocellulose on Root Biomass

a)



b)

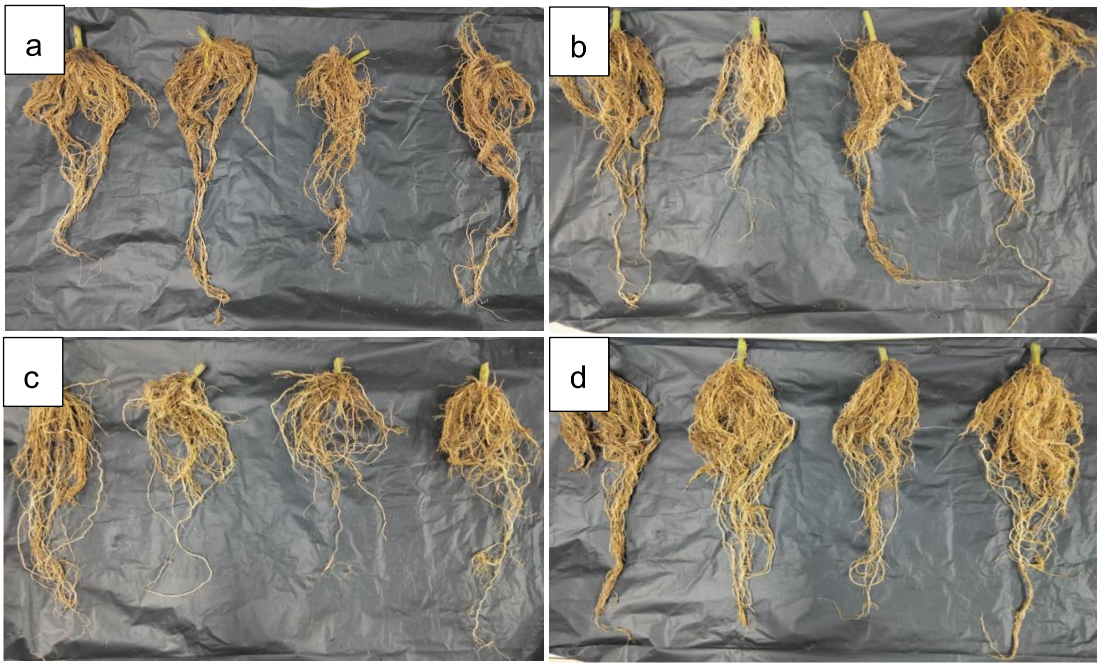


From the figure 4.11, it can be seen that without soil moisture control, root biomass was highest (11.38 g/plant) in the control plant, which is 15%, 7% and 9.2% higher than the plants of NCF-MSA doses of 37.5 kg/ha, 75 kg/ha and 112.5 kg/ha, respectively. When compared at 50% FC soil moisture level, root biomass was maximum (9.22 g/plant) for the control, which is approximately 6.3%, 47% and 21.5% higher than doses 37.5 kg/ha, 75 kg/ha and 112.5 kg/ha, respectively. Whereas, at 75% FC soil moisture level, 112.5 kg/ha dose of NCF-MSA gave the highest root biomass of 11.89 g/plant, which is 20%

higher than the respective control plant and 42% and 8% more that doses 75 kg/ha and 112.5 kg/ha, respectively. Finally, for 100% FC, the maximum root biomass (12.14 g/plant) was attained due to dose 75 kg/ha. Plants at doses 37.5 and 112.5 kg/ha had lesser Root biomass than those at 75 kg/ha by 33% and 25.5%, respectively. Control plants had approximately 29.8% less weight than 75 kg/ha dose. To conclude, although soil moisture affected the dry matter weight of roots significantly; 100% FC and 75% FC plants had the maximum dry matter weight of 9.5 g/plant and 9.8 g/plant, respectively, on an average, which is higher than 50% FC plants by 21% and 23.4% respectively. In case of nanocellulose, the maximum root biomass (11.35 g/plant) was shown by the control, which is 27%, 21.3% and 20% higher than 7.5 kg/ha, 15 kg/ha and 22.5 kg/ha doses, respectively.

Figure 4.12

Roots with the Application of a) NCF-MSA at 50% FC b) NCF-MSA at 75% FC and c) NCF-MSA at 100% FC (Roots of 0, 37.5, 75, 112.5 kg/ha Dose of NCF-MSA from Left to Right in a, b and c) d) Nanocellulose (Roots of 0, 7.5, 15, 22.5 kg/ha from Left to Right)

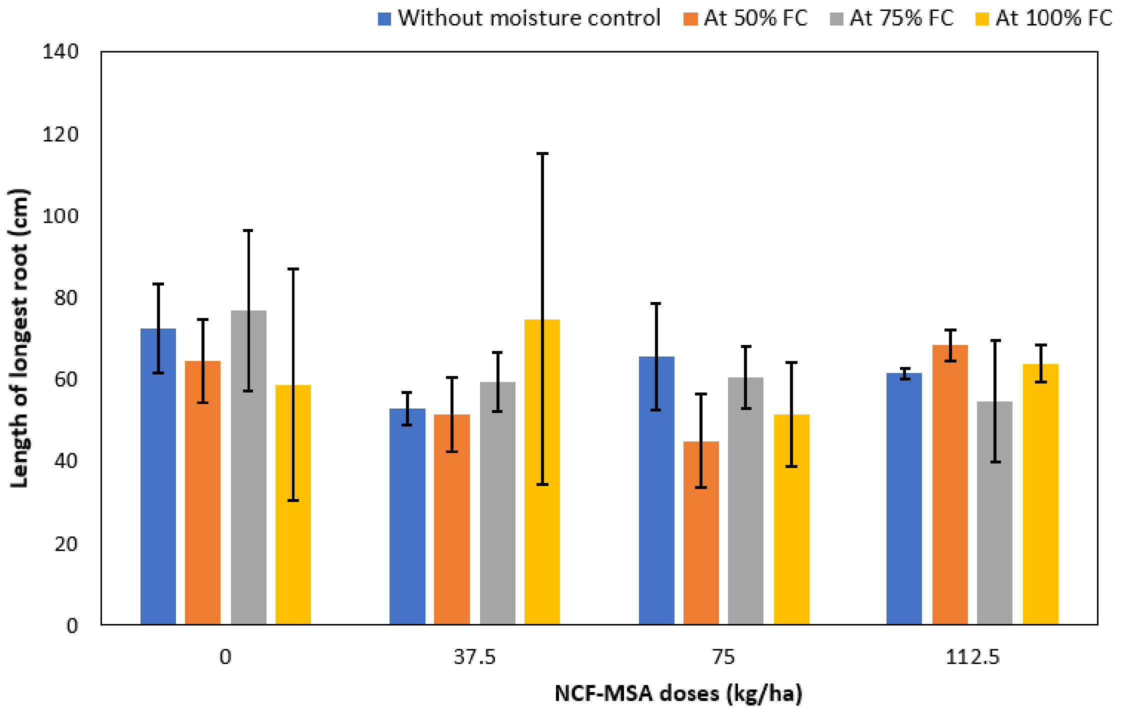


4.2.6 Length of the Longest Root (cm)

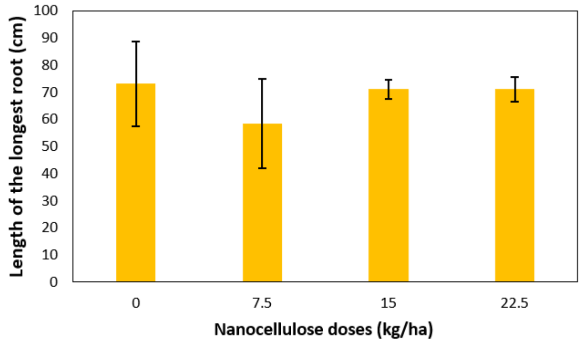
Figure 4.13

Effect of a) a) NCF-MSA and Soil Moisture b) Nanocellulose on Length of the Longest Root

a)



b)

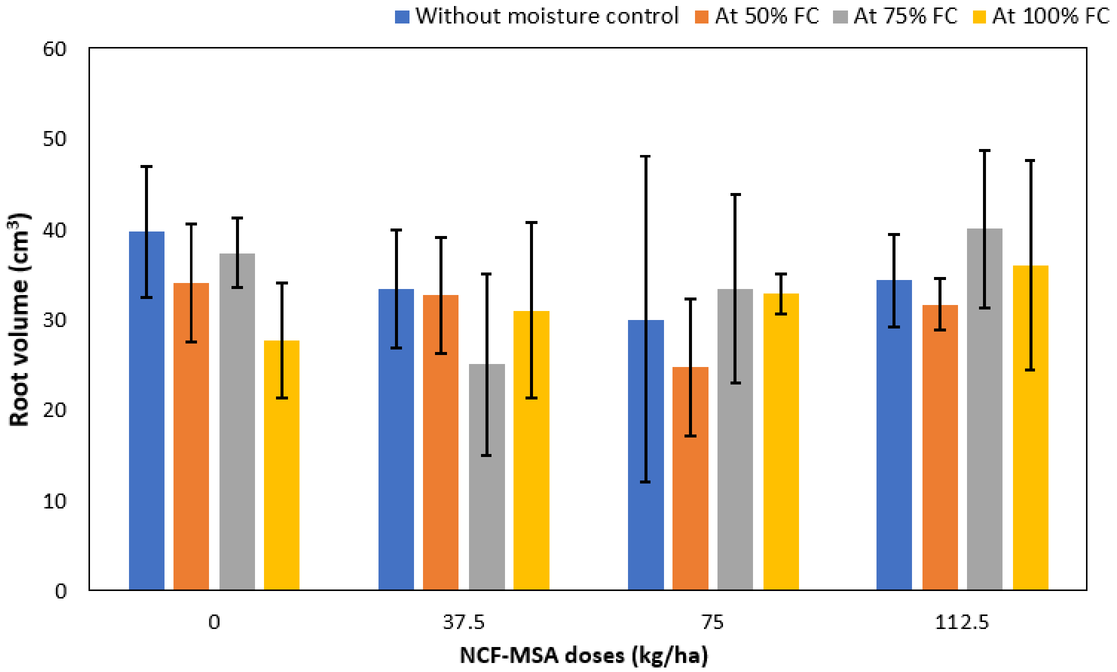


From the figure 4.13, it can be seen that without soil moisture control, length was maximum (72.3 cm) in the control plant, which is 27.1%, 9.7% and 15.2% higher than the plants of NCF-MSA doses of 37.5 kg/ha, 75 kg/ha and 112.5 kg/ha, respectively. When compared at 50% FC soil moisture level, length was maximum (68.3 cm) for NCF-MSA dose 112.5 kg/ha, which is approximately 6% longer than the control at 50% FC, approximately 25% and 34.1% higher than doses 37.5 kg/ha and 75 kg/ha, respectively. However, at 75% FC soil moisture level, the respective control gave the longest root weight of 76.7 cm, which is 22.7%, 21.4% and approximately 30% longer than doses 37.5 kg/ha, 75 kg/ha and 112.5 kg/ha, respectively. Finally, for 100% FC, the maximum Root biomass (74.7 cm) was attained due to dose 37.5 kg/ha. Plants at doses 75 and 112.5 kg/ha were shorter than those at 37.5 kg/ha by 31.3% and 14.7%, respectively. The length of longest root in control plants was 21.4% shorter than 75 kg/ha dose. In case of nanocellulose, the root length was shortest in nanocellulose dose 7.5 kg/ha (58.33 cm) and vice versa in the control (73 cm). 15 kg/ha and 22.5 kg/ha doses produced same length of roots (71 cm), which is 2.7% shorter than the control and 1.8% longer than 7.5 kg/ha dose.

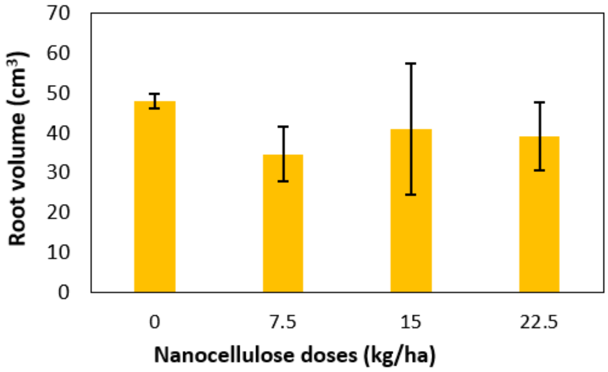
4.2.7 Root Volume (cm^3)

Figure 4.14

Effect of a) NCF-MSA and Soil Moisture b) Nanocellulose on Root Volume



b)



From the figure 4.14, it can be seen that without soil moisture control, volume was maximum (39.7 cm^3) in the control plant, which is 16.1%, 24.4% and 13.6% higher than the plants of NCF-MSA doses of 37.5 kg/ha, 75 kg/ha and 112.5 kg/ha, respectively. When compared at 50% FC soil moisture level, volume was maximum (34 cm^3) in the control plant, which is 3.8%, 27.4% and 6.8% higher than the plants of NCF-MSA doses of 37.5 kg/ha, 75 kg/ha and 112.5 kg/ha, respectively. When compared at 75% FC soil moisture level, volume was maximum (40 cm^3) for NCF-MSA dose 112.5 kg/ha, which was approximately 6.8% longer than the control at 75% FC, 37.5% and 16.8% higher than doses 37.5 kg/ha and 75 kg/ha, respectively. Finally, for 100% FC, the maximum root volume (36 cm^3) was attained due to dose 112.5 kg/ha. Plants at doses 37.5 and 75 kg/ha had lesser root volume than those at 112.5 kg/ha by approximately 14% and 9%, respectively. The root volume in control plants was 23.1% lesser than 112.5 kg/ha dose. In case of nanocellulose, the root volume was lowest in nanocellulose dose 7.5 kg/ha (34.7 cm^3) and vice versa in the control (48 cm^3). 15 kg/ha and 22.5 kg/ha doses produced similar roots of similar volume (41 cm^3 and 39 cm^3, respectively), which is 14.6% and 18.8% lower than the control.

4.2.8 Data Analysis Through ANOVA and Tukey HSD Test.

To investigate the significance in the differences, two way CRD ANOVA was performed for all growth parameters to check interactive effects of NCF-MSA and soil moisture and one-way CRD ANOVA was performed for nanocellulose. This was followed by Tukey HSD test to do multiple comparisons.

Plant height was affected significantly by NCF-MSA, soil moisture and their interaction. Plant height was highly significant (P<0.01) due to individual effects of NCF-MSA and soil moisture and only significant (P<0.05) due to their interaction. Nanocellulose showed statistical significance in plant height with P<0.05. Number of leaves was affected significantly by NCF-MSA, soil moisture and their interaction. Number of leaves was significant (P<0.05) due to individual effect of NCF-MSA and highly significant (P<0.01) due to effects of soil moisture and interaction of NCF-MSA and soil moisture. Nanocellulose showed high statistical significance in number of leaves with P<0.01. Leaf area was affected significantly by NCF-MSA, soil moisture and their interaction. Leaf area was highly significant (P<0.01) due to individual effects of NCF-MSA, soil moisture and their interaction. Nanocellulose did not show statistical significance in leaf area. Shoot biomass was not affected by NCF-MSA and interaction between NCF-MSA. Shoot biomass was highly significant (P<0.01) due to individual effects of soil moisture. Nanocellulose did not show statistical significance in shoot biomass. Root biomass was not affected by NCF-MSA and interaction between NCF-MSA. Shoot biomass was highly significant (P<0.01) due to individual effects of soil moisture. Nanocellulose showed statistical significance in Root biomass with P<0.05. The interaction between NCF-MSA and soil moisture as well as NCF-MSA and soil moisture individually produced no significant difference to length of the longest root of a plant. Nanocellulose did not produced significant effects on the length of the longest root of a plant. The interaction between NCF-MSA and soil moisture as well as NCF-MSA and soil moisture individually produced no significant difference to root volume of a plant. Similarly, nanocellulose had no effect on the volume of roots.

Significance and means for growth parameters are given in Tables 4.1, 4.2 and 4.3.

Table 4.1

Results of ANOVA Analysis with F value for the Effects of NCF-MSA and Moisture (M) for Different Growth Parameters

Growth parameters	NCF-MSA	Moisture	NCF-MSA X Moisture (M)	Nanocellulose
Plant height	7.54**	160.09**	2.65*	4.65*
Number of leaves	8.9*	89.36**	4**	10.82**
Leaf area	5.06**	66.3**	3.07**	3.65ns
Shoot biomass	0.85ns	91.71**	0.64ns	1.51ns
Root biomass	0.88ns	3.32*	1.79ns	4.62*
Length of longest root	1.33ns	0.35ns	1.08ns	0.98ns
Root volume	1.25ns	0.47ns	0.8ns	0.94ns

Note. *, ** and ns represent data is significant at 1% level, significant at 5% level and not significant, respectively

Table 4.2

Effect of Soil Application of NCF-MSA and Soil Moisture Regime (M) on Growth Parameters of Lukthar Tomato
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Factors	PH (cm)	NoL	LA (cm^2)	SDMW (g/plant)	RDMW (g/plant)	RL (cm)	RV (cm^3)
NCF-MSA (P) (kg/ha)							
P-0	102.17 ± 13.56 b	25.083 ± 6.95 b	346.96 ± 92.19 ab	52.3 ± 18.62	9.6608 ± 2.25	68 ± 17.53	34.667 ± 7.05
P-37.5	107.75 ± 18.18 a	28.5 ± 5.09 a	370.09 ± 99.42 a	56.704 ± 19.36	8.3225 ± 1.82	59.5 ± 20.47	30.5 ± 7.88
P75	106.62 ± 13.9 ab	26.167 ± 8.2 ab	351.07 ± 91.86 ab	54.459 ± 22.30	9.6417 ± 4.24	55.5 ± 12.67	30.208 ± 10.15
P-112.5	110.83 ± 17.08 a	26.583± 7.04ab	314.44 ± 54.58 b	56.16 ± 19.05	9.6267 ± 2.22	61.958 ± 8.57	35.5 ± 7.35
Moisture (M) (% FC)							
NMC	108.96 ± 6.07 b	26.583 ± 3.68	377.69 ± 28.62 a	55.38 ± 7.85 b	10.493 ± 2.93 a	62.917 ± 10.51	34.333 ± 9.71

50	83.54 ± 6.23 c	18.083 ± 3.32 d	221.95 ± 29.64 b	27.396 ± 3.02 c	7.502 ± 2.16 b	57.25 ± 12.56	30.75 ± 6.44
75	112.5 ± 6.36 b	28.833 ± 3.83 b	375.81 ± 48.86 a	59.703 ± 7.77 b	9.789 ± 2.39 ab	62.708 ± 14.28	33.917 ± 9.44
100	122.37 ± 5.43 a	34.333 ± 2.46 a	407.12 ± 67.35 a	77.145 ± 8.52 a	9.467 ± 2.9 ab	62.083 ± 23.55	31.875 ± 7.71
P x M							
P-0	108 ± 8.0 bcd	25.333 ± 8.96 cd	405.71 ± 19.57 abc	56.113 ± 10.79	11.38 ± 2.55	72.333 ± 10.79	39.667 ± 7.23
P-0 + 50	82.33 ± 2.52 e	15.667 ± 1.53 f	203.43 ± 2.15 f	25.733 ± 1.33	9.217 ± 2.76	64.333 ± 10.26	34 ± 6.56
P-0 + 75	102.33 ± 2.52 d	25.333 ± 4.16 cd	364.62 ± 16.53 abc	55.017 ± 8.01	9.523 ± 0.49	76.667 ± 19.42	37.333 ± 3.76
P-0 + 100	116 ± 2.65 abcd	34 ± 2 ab	414.07 ± 52.64 abc	72.337 ± 5.56	8.523 ± 2.6	58.667 ± 28.29	27.667 ± 6.43
P-37.5	109.17 ± 4.48 bcd	29.333 ± 4.36 abc	367.99 ± 27.64 abc	59.137 ± 3.84	9.68 ± 0.78	52.667 ± 4.04	33.333 ± 6.51
P-37.5 + 50	80.17 ± 8.98 e	22.333 ± 8.14 cdef	226.21 ± 17.5 def	28.413 ± 0.90	8.643 ± 0.94	51.333 ± 9.07	32.667 ± 6.43
P-37.5 + 75	117 ± 1.0 abc	28.667 ± 4.16 abc	428.91 ± 23.64 ab	62.83 ± 12.85	6.813 ± 1.76	59.333 ± 7.09	25 ± 10
P-37.5 + 100	124.67 ± 3.76 a	33.667 ± 4.04 ab	457.28 ± 71.31 a	76.437 ± 5.51	8.153 ± 2.71	74.667 ± 40.5	31 ± 9.64
P-75	103.33 ± 1.53 cd	20.667 ± 0.58 def	368.44 ± 38.26 abc	50.367 ± 11.40	10.587 ± 5.96	65.333 ± 13.05	30 ± 18.03
P-75 + 50	86.67 ± 3.06 e	16.667 ± 6.66 f	213.25 ± 8.84 ef	25.613 ± 0.73	4.913 ± 0.83	45 ± 11.36	24.667 ± 7.57
P-75 + 75	115.67 ± 2.08 abcd	3.333 ± 9.02 ab	394.32 ± 40.48 abc	58.593 ± 6.28	10.93 ± 2.3	60.333 ± 7.5	33.333 ± 10.41
P-75 + 100	120.83 ± 2.84 ab	34 ± 1 ab	428.27 ±50.44 ab	83.263 ± 4.61	12.137 ± 3.27	51.333 ± 12.5	32.833 ± 2.25
P-112.5	115.33 ± 2.52 cd	25 ± 1 cde	368.61 ± 17.92 abc	55.903 ± 4.23	10.327 ± 1.5	61.333 ± 1.15	34.333 ± 5.14
P-112.5 + 50	85 ± 9.17 e	17.667 ± 3.21 ef	244.89 ± 54.76 def	29.823 ± 5.25	7.237 ± 0.25	68.333 ± 3.79	31.667 ± 2.89
P-112.5 + 75	115 ± 1.0 abcd	28 ± 4.62 bcd	315.4 ± 15.84 cde	62.37 ± 1.86	11.89 ± 0.92	54.5 ± 14.8	40 ± 8.66
P-112.5 + 100	128 ± 3.61 a	35.667 ± 3.46 a	328.87 ± 29.07 bcd	76.543 ± 14.99	9.053 ± 2.53	63.667 ± 4.51	36 ± 11.53

Note. PH, Plant height; NoL, Number of leaves; LA, Leaf area; SDMW, shoot biomass; RDMW, root biomass; RL, Length of longest root; RV, Root volume; NMC, No moisture control; FC, field capacity; means within a column followed by the same letter are not significantly different by least significant difference test at $P < 0.05$; data are means of three replications ± standard deviations.

Table 4.3

Effect of Soil Application of Nanocellulose on Growth Parameters of Lukthar Tomato.

Nanocellulose (kg/ha)	PH (cm)	NoL	LA (cm^2)	SDMW (g)	RDMW (g)	RL (cm)	RV (cm^3)
NC-0	103.5 ± 6.73 ab	31.33 ± 3.06 a	336.22 ± 23.11	65.683 ± 1.14	11.35 ± 1.28 a	73 ± 15.72	48 ± 1.73
NC-7.5	107.5 ± 5.89 ab	29 ± 3.76 ab	325.82 ± 15.19	59.437 ± 4.0	8.29 ± 0.37 b	58.333 ± 16.5	34.667 ± 6.81
NC-15	99.17 ± 1.04 b	22.333 ± 5.51 bc	363.32 ± 19.54	62.953 ± 3.20	8.93 ± 1.68 ab	71 ± 3.6	41 ± 16.52
NC-22.5	112.67 ± 2.08 a	24.333 ± 2.52 ab	377.37 ± 9.74	59.517 ± 6.68	9.07 ± 0.21 ab	71 ± 4.58	39 ± 8.54

Note. PH, Plant height; NoL, Number of leaves; LA, Leaf area; SDMW, shoot biomass; RDMW, Root biomass; RL, Length of longest root; RV, Root volume; means within a column followed by the same letter are not significantly different by least significant difference test at $P < 0.05$; data are means of three replications ± standard deviations.

4.2.9 Discussion

Growth parameters like plant height, number of leaves and leaf area can be related to cell division and cell elongation in plants. Due to water stress, cell division and elongation is slowed or inhibited resulting from lack of water supply from the xylem vessels to the elongating cells, thus reducing turgor pressure in cells. (Chakma et al., 2021) mentioned the role of photosynthesis in the shoot and root biomass weight. Photosynthesis is hampered due to water stress, thus preventing the translocation of carbohydrates to the plant parts. Leaf water potential, transpiration and chloroplast activity is affected which eventually produce small biomass (Chakma et al., 2021, 2023; Farooq et al., 2009; Pervez et al., 2009). (U. Ullah et al., 2016) mentioned that water stress inhibits root proliferation thus disturbing plant-water relations, which eventually leads to inhibition in growth parameters. In this study, plant height, number of leaves, leaf area and plant biomass were affected by water stress conditions. 100% FC had the maximum shoot and root biomass, height, number of leaves and leaf area, followed by 75% FC and the least at 50% FC.

(Chakma et al., 2021) observed increase in growth parameters in 150, 300 and 600 kg/ha doses of monosilicic acid. Plants were taller at the higher doses of the same. Leaf area was maximum at 300 kg/ha dose of the same product. Growth parameters also increased in the study by (Ahmed et al., 2023; Chakma et al., 2023) with the application of NCF-MSA and nanocellulose, significant and highly significant differences were observed in plant height, number of leaves and leaf area. Plant height increased with application of NCF-MSA and 112.5 kg/ha dose gave the tallest plants at 100% FC. Number of leaves was highest at 100% FC when the NCF-MSA dose was 112.5 kg/ha. However, leaf area was maximum at 75% FC with 37.5 kg/ha dose. Nanocellulose, on the other hand, showed directly proportional increase in height and only in last two doses in leaf area but not in number of leaves.

Shoot and root dry matter increased almost proportionally with the application of monosilicic acid in the study by (Ahmed et al., 2023; Chakma et al., 2021, 2023).Shoot biomass increased with the application of NCF-MSA. (Dakora & Nelwamondo, 2003) showed increase in root growth due to application of metasilicic acid. However, root biomass, volume and length did not show any noticeable changes due to NCF-MSA, rather there were reduction in values of these parameters in some. The same goes for nanocellulose in terms of root parameters, but shoot biomass decreased with the application of Nanocellulose.

4.3 Effects of Nanocellulose Fibres Stabilized Monosilicic Acid (NCF-MSA) and Nanocellulose on Yield Parameters under Water Stress Conditions

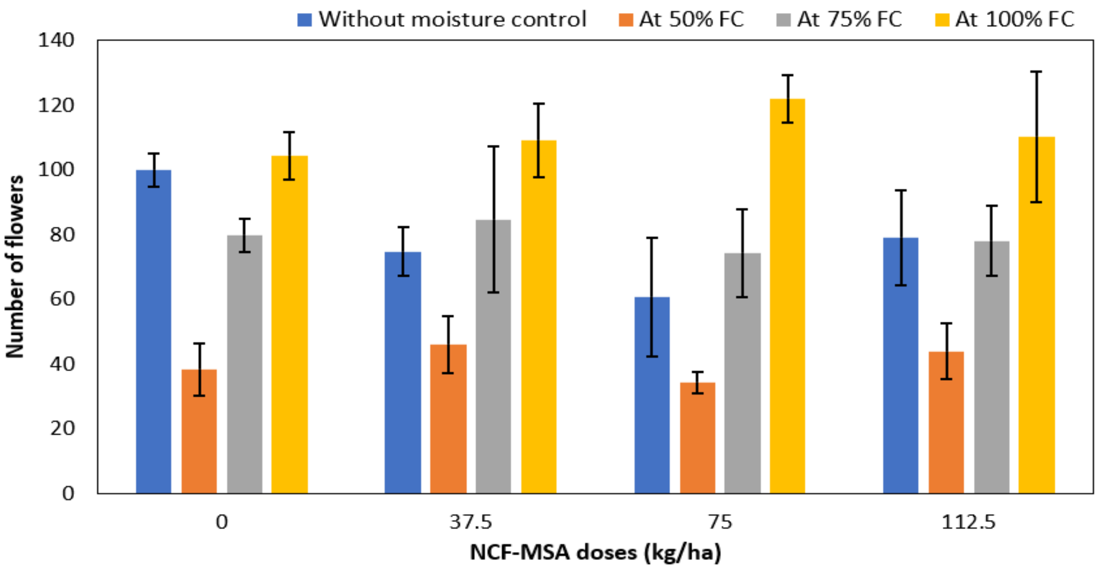
NCF-MSA was applied as soil incorporation in plants and data on yield parameters were recorded. The yield parameters include number of flowers, fruit yield, fruit yield, number of fruits and irrigation water productivity. The collected data was analysed and presented as bar graphs, followed by performing an ANOVA on these parameters to investigate significance of data, The means were compared pair-wise using Tukey HSD test. Finally, a discussion on these parameters is presented.

4.3.1 Number of Flowers

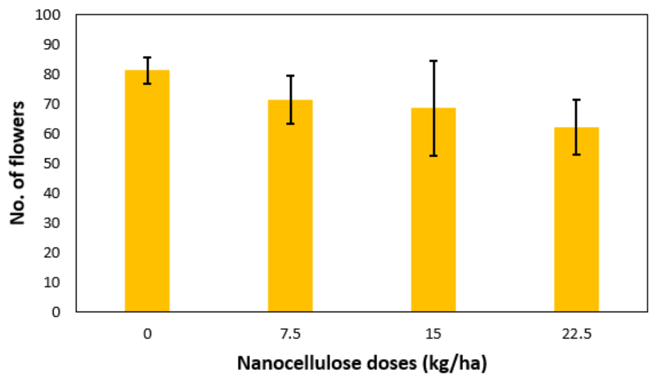
Figure 4.15

Effect of a) NCF-MSA and Soil Moisture b) Nanocellulose on Number of Flowers

a)



b)



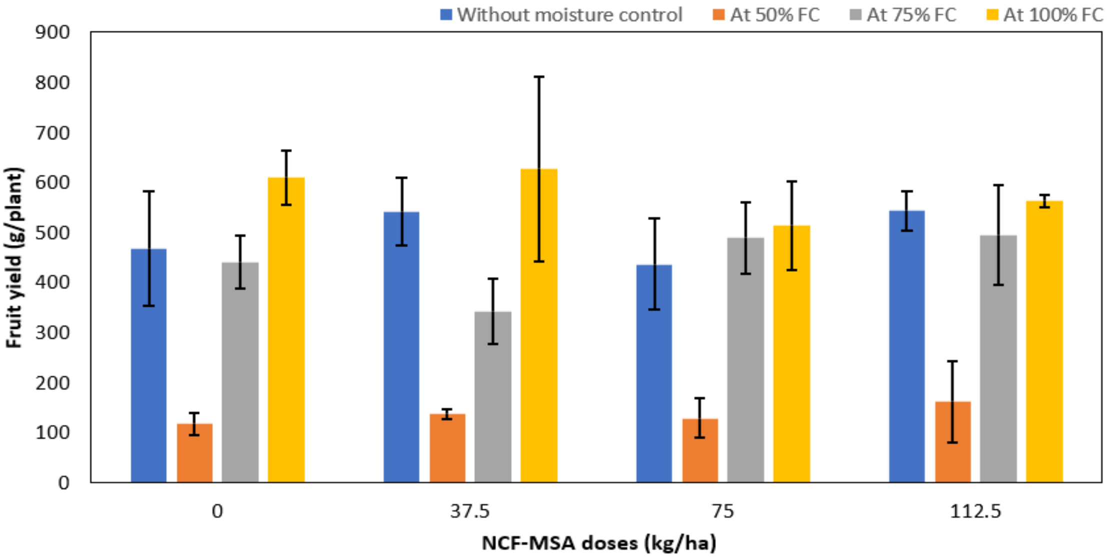
From the figure 4.15, without soil moisture control, number of flowers was maximum (100) in the control, which was 25.3% higher than NCF-MSA doses 37.5 kg/ha, 39.3% higher than 75 kg/ha dose and 21% higher than 112.5kg/ha dose. When compared at 50% FC soil moisture level, number of flowers was maximum (46) in NCF-MSA dose 37.5 kg/ha, which is 16.7% higher than the corresponding control and 25.4% and 4.3% higher than doses 75 kg/ha and 112.5 kg/ha, respectively. Similarly, at 75% FC soil moisture level, 37.5 kg/ha dose of NCF-MSA gave the highest number of flowers i.e., 84.67, which is 5.9% higher than the respective control plant and 12.2% and approximately 8% more that doses 75 kg/ha and 112.5 kg/ha, respectively. Finally, for 100% FC, the maximum number of flowers (122) was attained due to dose 75 kg/ha. Plants at doses 37.5 and 112.5 kg/ha had smaller number of flowers than those at 75 kg/ha by 10.7% and 9.6%, respectively. Control plants had approximately 14.5% less flowers than 37.5 kg/ha dose. To conclude, on an average, 100% FC soil moisture level gave the highest number of flowers (111.42) followed by 75% FC (79.17) and the least at 50% FC (40.67). In nanocellulose applied plants, there was a decrease in the number of flowers with the increase in doses. Contrary to this, fruit yield increased with doses, although number of fruits were almost the same.

4.3.2 Fruit Yield (g/plant)

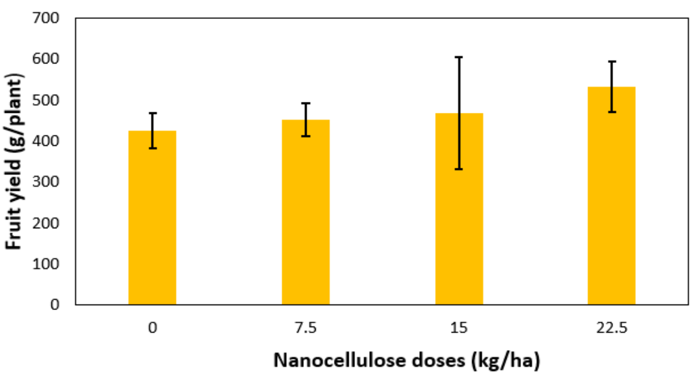
Figure 4.16

Effect of a) NCF-MSA and Soil Moisture b) Nanocellulose on Fruit Yield

a)



b)



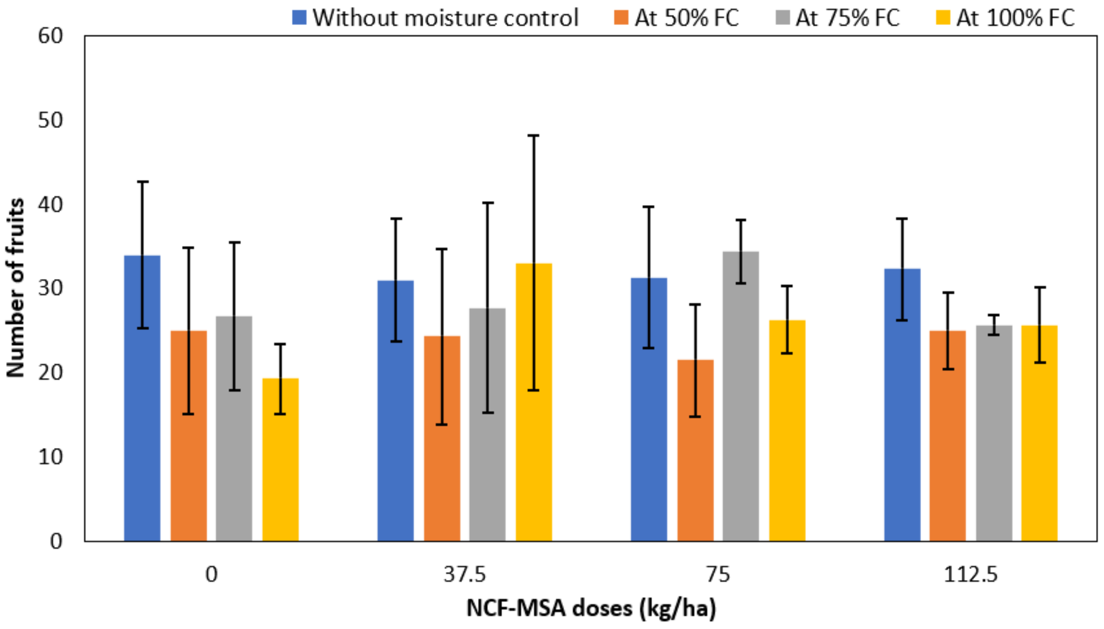
From the figure 4.16, without soil moisture control, fruit yield was maximum (542.76 g) in NCF-MSA dose 112.5 kg/ha, which is 14% more than produced by the control of this group, and 0.3% higher than 37.5 kg/ha dose and 19.6% higher than 75 kg/ha. When compared at 50% FC soil moisture level, fruit yield was maximum (160.99 g/plant) in NCF-MSA dose 112.5 kg/ha, which is 27.4% higher than the corresponding control and 15.3% and 20% higher than doses 37.5 kg/ha and 75 kg/ha, respectively. Similarly, at 75% FC soil moisture level, 112.5 kg/ha dose of NCF-MSA gave the highest yield of 494.67 g/plant, which is 36.4% higher than the respective control plant and 31% and 1.2% more that doses 37.5 kg/ha and 75 kg/ha, respectively. Finally, for 100% FC, the maximum fruit yield (626.57 g/plant) was attained due to dose 37.5 kg/ha. Plants at doses 75 and 112.5 kg/ha had lower yield than those at 37.5 kg/ha by 18% and 10.2%, respectively. Control plants had approximately 2.7% less yield than 37.5 kg/ha dose. To conclude, 100% FC soil moisture level gave the highest produce of 626.57 g/plant at the lowest dose, i.e. 37.5 kg/ha amongst other moisture levels and without moisture control plants. This was followed by a yield of 542.76 g/plant from the highest dose of NCF-MSA without moisture control, then by the highest dose of NCF-MSA at 75% FC moisture level with a yield of 494.67 g/plant and then by the highest dose at 50% FC level with approximately 161 g/plant of fruits. With the application of nanocellulose, although, yield was more in all three nanocellulose doses as compared to control, but yield was not distinctly different statistically. The control plants gave the lowest yield of 425.64 g/plant, which is approximately 20% less than the highest yield of 532.87 g/plant from 22.5 kg/ha dose of nanocellulose. Yield was approximately 15% and 12% lower than 22.5 kg/ha in 7.5 kg/ha and 15 kg/ha doses of nanocellulose, respectively.

4.3.3 Number of Fruits

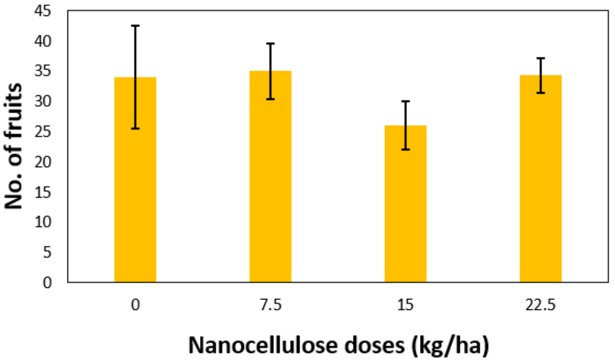
Figure 4.17

Effect of a) NCF-MSA and soil moisture b) Nanocellulose on Number of Fruits

a)



b)



From the figure 4.17, without moisture control, NCF-MSA had no effect on number of fruits. In this group, the control had the highest number of fruits. Higher number of fruits than their respective control was observed at 75% FC and 100% FC moisture levels, where 37.5 kg/ha and 75 kg/ha dose of NCF-MSA gave a greater number of fruits at 75% FC and all three doses gave more fruits than the corresponding control at 100% FC. At 75% FC, number of fruits was 3.6% more in 37.5 kg/ha dose than the control with no NCF-MSA. Whereas, it was 22.2% more in 75 kg/ha dose. Again for 100% FC, highest number of fruits was seen in the lowest dose of NCF-MSA, i.e. 37.5

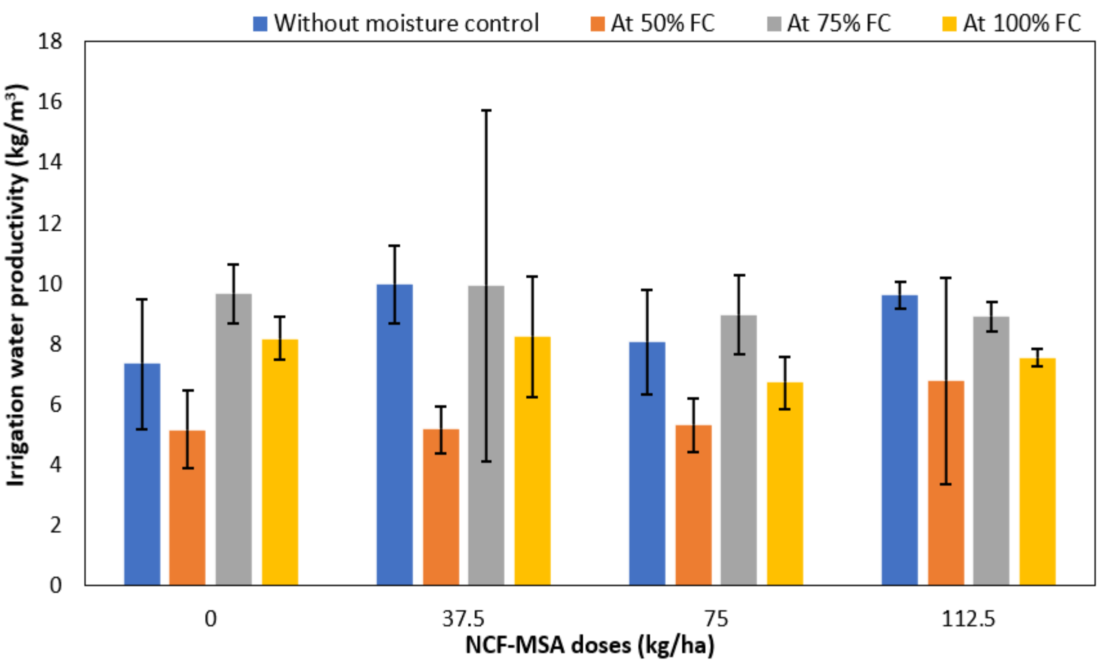
kg/ha, which was 41.5% more than the control and 20.3% and approximately, 22% more than 75 kg/ha and 112.5 kg/ha NCF-MSA doses, respectively. In case of nanocellulose, highest number of fruits was observed in 7.5 kg/ha dose, which was 2.8% more than the control and 25.7% and 2% more than the two higher doses of nanocellulose.

4.3.4 Irrigation Water Productivity (kg/m³)

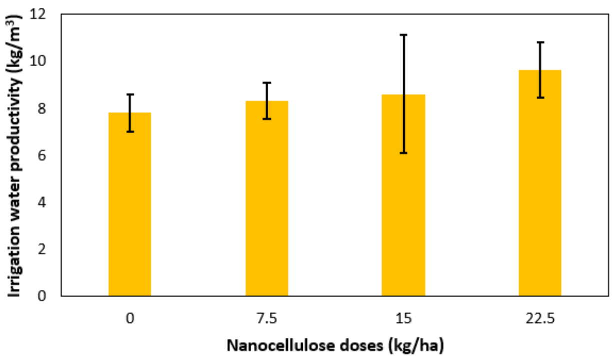
Figure 4.18

Effect of a) NCF-MSA and Soil moisture b) Nanocellulose on Irrigation Water Productivity

a)



b)



From the figure 4.18, without soil moisture control, irrigation water productivity was maximum (9.97 kg/m^3) in NCF-MSA dose 37.5 kg/ha, which is 26.3% more than produced by the control of this group, and 19.3% and 19.6% higher than 75 kg/ha and 112.5 kg/ha, respectively. When compared at 50% FC soil moisture level, irrigation water productivity was maximum (6.79 kg/m^3) in NCF-MSA dose 112.5 kg/ha, which is 24% higher than the corresponding control and 24% and 21.8% higher than doses 37.5 kg/ha and 75 kg/ha, respectively. Similarly, at 75% FC soil moisture level, irrigation water productivity was maximum (9.92 kg/m^3) in NCF-MSA dose 37.5 kg/ha, which is 9.7% more than produced by the control of this group, and 9.7% and 10.3% higher than 75 kg/ha and 112.5 kg/ha, respectively. Finally, for 100% FC, the maximum irrigation water productivity (8.25 kg/m^3) was attained due to dose 37.5 kg/ha. Plants at doses 75 and 112.5 kg/ha had lower irrigation water productivity than those at 37.5 kg/ha by 18.5% and 8.5%, respectively. Control plants had approximately 0.8% less irrigation water productivity than 37.5 kg/ha dose. To conclude, 75% FC soil moisture level gave the highest irrigation water productivity of 9.36 kg/m^3 followed by an irrigation water productivity of 7.67 kg/m^3 at 100% FC moisture level and least at 50% FC (5.61 kg/m^3). There was increase in irrigation water productivity with increase in nanocellulose doses. The highest irrigation water productivity (9.647 kg/m^3) was observed in the highest dose of nanocellulose, i.e., 22.5 kg/ha., which was approximately 19% higher than the control and 13.8% and 10.6% higher than 7.5 kg/ha and 15 kg/ha doses, respectively.

4.3.5 Data Analysis Through ANOVA and Tukey HSD Test.

To investigate the significance in the differences, two way CRD ANOVA was performed for all yield parameters to check interactive effects of NCF-MSA and soil moisture and one-way CRD ANOVA was performed for nanocellulose. This was followed by Tukey HSD test to do multiple comparisons.

Number of flowers was not affected individually by NCF-MSA but was highly significant ($P<0.01$) due to individual effects of soil moisture and significant ($P<0.05$) due the interaction between NCF-MSA and soil moisture. Number of flowers was similar statistically in all doses of nanocellulose. Fruit yield was not affected by NCF-MSA and interaction between NCF-MSA. Fruit yield was significant ($P<0.05$) due to individual effects of soil moisture. Nanocellulose did not produced significant effects on fruit yield. NCF-MSA, soil moisture levels and their interaction had no statistical difference in the number of fruits. Number of fruits did not show any significant differences due to the application of Nanocellulose. Irrigation water productivity was not affected by NCF-MSA and interaction between NCF-MSA. Irrigation water productivity was highly significant ($P<0.01$) due to individual effects of soil moisture Nanocellulose did not produce significant differences in irrigation water productivity.

Significance and means for yield parameters are given in tables 4.4, 4.5 and 4.6.

Table 4.4

Results of ANOVA Analysis with F value for the Effects of NCF-MSA and Moisture (M) for Different Yield Parameters

Yield parameters	**NCF-MSA**	**Moisture**	**NCF-MSA X Moisture (M)**	**Nanocellulose**
Number of flowers	0.89ns	68.41**	2.21*	1.77ns
Fruit yield	1.09ns	69.56*	1.71ns	0.97ns
Number of fruits	0.5ns	2.14ns	0.78ns	1.83ns
Irrigation water productivity	0.75ns	7.91*	0.47ns	0.81ns

Note. *, ** and ns represent data is significant at 1% level, significant at 5% level and not significant, respectively

Table 4.5

Effect of Soil Application of NCF-MSA and Soil Moisture (M) on Yield Parameters of Lukthar Tomato.

Factors	Number of flowers	Fruit yield (g/plant)	Number of fruits	Irrigation water productivity (kg/m^3)
NCF-MSA				
NCF-MSA -0 kg/ha	80.583 ± 27.84	392.24 ± 194.33	25 ± 1.4	7.588 ± 2.07
NCF-MSA -37.5 kg/ha	78.583 ± 26.39	411.4 ± 216.79	29 ± 10.51	8.326 ± 3.38
NCF-MSA -75 kg/ha	72.833 ± 34.81	386.39 ± 182.94	27.417 ± 8.36	7.26 ± 1.79
NCF-MSA -112.5 kg/ha	77.833 ± 27.37	440.18 ± 179.82	27.167 ± 4.91	8.21 ± 1.88
Moisture (M)				
No control	78.58 ± 18.25 b	480.29 ± 96.54 b	30.917 ± 6.65	8.749 ± 1.73 a
50% FC	40.67 ± 8.05 c	130.23 ± 45.27 c	23 ± 7.72	5.607 ± 1.78 b
75% FC	79.17 ± 12.93 b	441.52 ± 90.14 b	28.583 ± 7.57	9.361 ± 2.62 a
100% FC	111.42 ± 12.79 a	578.17 ± 101.52 a	26.083 ± 8.76	7.673 ± 1.17 ab
NCF-MSA X M				
NCF-MSA -0 kg/ha	100 ± 5 ab	401.06 ± 114.75	29 ± 8.72	7.347 ± 2.15
NCF-MSA -0 kg/ha + 50% FC	38.33 ± 8.14 ef	116.93 ± 21.72	25 ± 9.85	5.163 ± 1.29
NCF-MSA -0 kg/ha + 75% FC	79.67 ± 5.13 bcd	441.17 ± 52.18	26.667 ± 8.74	9.665 ± 0.98
NCF-MSA -0 kg/ha + 100% FC	104.33 ± 7.37 ab	609.79 ± 54.33	19.333 ± 15.10	8.179 ± 0.71
NCF-MSA -37.5 kg/ha	74.67 ± 7.51 bcde	541.13 ± 67.09	31 ± 7.21	9.974 ± 1.28

NCF-MSA - 37.5 kg/ha + 50% FC	46 ± 8.89 def	136.29 ± 10.29	24.333 ± 10.41	5.168 ± 0.78
NCF-MSA - 37.5 kg/ha + 75% FC	84.67 ± 22.5 bc	341.6 ± 64.84	27.667 ± 12.42	9.917 ± 5.81
NCF-MSA - 37.5 kg/ha +100% FC	109 ± 11.36 ab	626.57 ± 184.80	33 ± 15.10	8.246 ± 1.98
NCF-MSA -75 kg/ha	60.67 ± 18.45 cdef	436.21 ± 91.24	31.333 ± 8.39	8.053 ± 1.72
NCF-MSA -75 kg/ha + 50% FC	34.33 ± 3.21 f	106.72 ± 39.10	17.667 ± 6.66	5.167 ± 0.89
NCF-MSA @75 kg/ha +75% FC	74.33± 13.65 bcde	488.75 ± 72.17	34.333 ± 3.79	8.96 ± 1.30
NCF-MSA @75 kg/ha +100% FC	122 ± 7.21 a	513.89 ± 88.62	26.333 ± 4.04	6.722 ± 0.89
NCF-MSA - 112.5 kg/ha	79 ± 14.73 bcd	542.76 ± 39.14	32.333 ± 6.03	9.623 ± 0.45
NCF-MSA - 112.5 kg/ha + 50% FC	44 ± 8.54 def	160.99 ± 81.23	25 ± 4.58	6.786 ± 3.41
NCF-MSA - 112.5 kg/ha + 75% FC	78 ± 10.82 bcd	494.57 ± 100.04	25.667 ± 1.15	8.898 ± 0.48
NCF-MSA - 112.5 kg/ha + 100% FC	110.33 ± 20.26 ab	562.42 ± 12.13	25.667 ± 4.51	7.55 ± 0.29

Note. FC, field capacity; means within a column followed by the same letter are not significantly different by least significant difference test at $P < 0.05$; data are means of three replications ± standard deviations.

Table 4.6

Effect of Soil Application of Nanocellulose on Yield Parameters of Lukthar Tomato.

Factors	**Number of flowers**	**Fruit yield (g/plant)**	**Number of fruits**	**Irrigation water productivity (kg/m^3)**
Nanocellulose -0 kg/ha	81.333 ± 4.51	425.64 ± 43.10	34 ± 8.54	7.813 ± 0.79
Nanocellulose -7.5 kg/ha	71.333 ± 8.02	452.6 ± 41.14	35 ± 4.58	8.314 ± 0.77
Nanocellulose -15 kg/ha	68.667 ± 15.89	468.5 ± 136	26 ± 4	8.620 ± 2.51
Nanocellulose -22.5 kg/ha	62.333 ± 9.29	532.87 ± 60.98	34.333 ± 2.89	9.647 ± 1.19

4.3.6 Discussion on Yield Parameters

Fruit yield on weight basis was negatively affected due to water stress conditions. Yield decreased as the moisture level in soil changed from 100% FC (578.17 g/plant) to 50% FC (124.89 g/plant). Some of the common reasons might be a smaller number of trusses and flower abortion and pre-mature fruit drop due to lack of production and immobility of photo-assimilates to the fruits which results from poor photosynthesis due to water stress (Chakma et al., 2021, 2023; Lopez et al., 2012; Pulupol et al., 1996). This leads to a smaller number of fruits and hence weight.

When taken into account the mean data, number of flowers was maximum in plants at 100% FC and least at 50% FC. However, in this study, average number of fruits was highest in 75% FC (28.6) and least in 50% FC (24). There was 76.8% reduction in number of fruits at 100% FC as compared to flowers, followed by approximately 70% reduction at 75% FC and 43.4% reduction at 50% FC. Higher weight of fruits in 100% FC may be attributed to the large size of fruits which gave more Fruit yield per plant. Temperature also played a role in this as increase in temperature above 30˚C led to flower abortion and flower drop leading to reduction in fruit numbers (Berry & Uddin, 1988; Sugiyama et al., 1966). Irrigation water productivity was significantly affected by soil moisture. Irrigation water productivity was highest at 75% FC level, which was due to higher yield of fruits and partly by the moisture input.

The effect of NCF-MSA on Fruit yield was not clearly visible in 50% (Fruit yield increased only doses 75 kg/ha and 112.5 kg/ha doses) and 100% FC (Fruit yield increased only in 37.5 kg/ha dose) but was distinct in 75% FC. The Fruit yield in 75% FC increased with increase in NCF-MSA doses and was maximum in the highest dose of NCF-MSA (494.67 g/plant). (Chakma et al., 2021) showed increase in Fruit yield per plant in 75% FC with the application of monosilicic acid. Weight increased proportionally with the doses of the product. Fruit number distinctly was more than the control in 100% FC with application of NCF-MSA. The reason might be proper translocation of assimilates through xylem to reproductive organs because of the presence of sufficient water and improved plant water relations. Overall, irrigation water productivity was statistically similar with the application of NCF-MSA, however at low moisture level of 50% FC, there was an increase in irrigation water productivity with increase in doses. This indicates the effect of NCF-MSA on water productivity in the low moisture levels.

Fruit yield increased in proportion to nanocellulose doses. On the other hand, fruit number increased only in dose 7.5 kg/ha of nanocellulose. There was increase in irrigation water productivity due to nanocellulose, which is attributed to higher produce of fruits.

4.4 Effects of Nanocellulose Fibres Stabilized Monosilicic Acid (NCF-MSA) and Nanocellulose on Fruit Quality Parameters under Water Stress Conditions

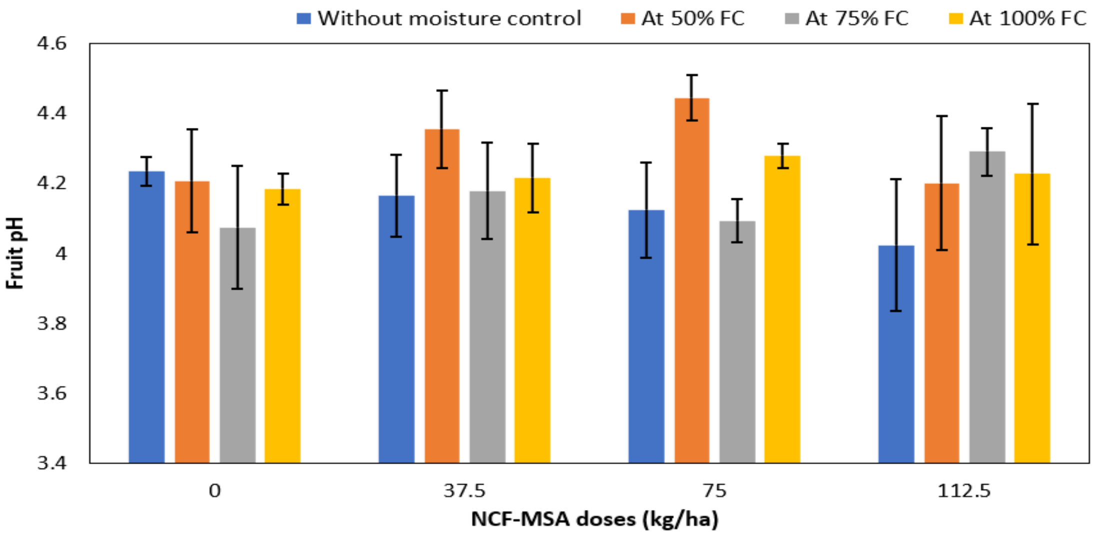
NCF-MSA was applied as soil incorporation in plants and data on fruit quality parameters were recorded. The fruit quality parameters include fruit pH, Total soluble solids and average fruit length. The collected data was analysed and presented as bar graphs, followed by performing an ANOVA on these parameters to investigate significance of data, The means were compared pair-wise using Tukey HSD test. Finally, a discussion on these parameters is presented.

4.4.1 Fruit pH

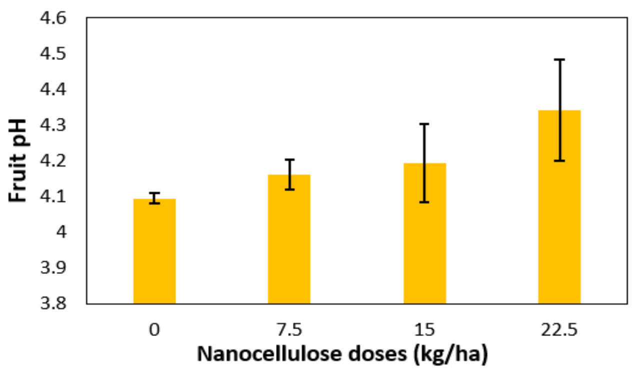
Figure 4.19

Effect of a) NCF-MSA and Soil Moisture b) Nanocellulose on Fruit pH

a)



b)



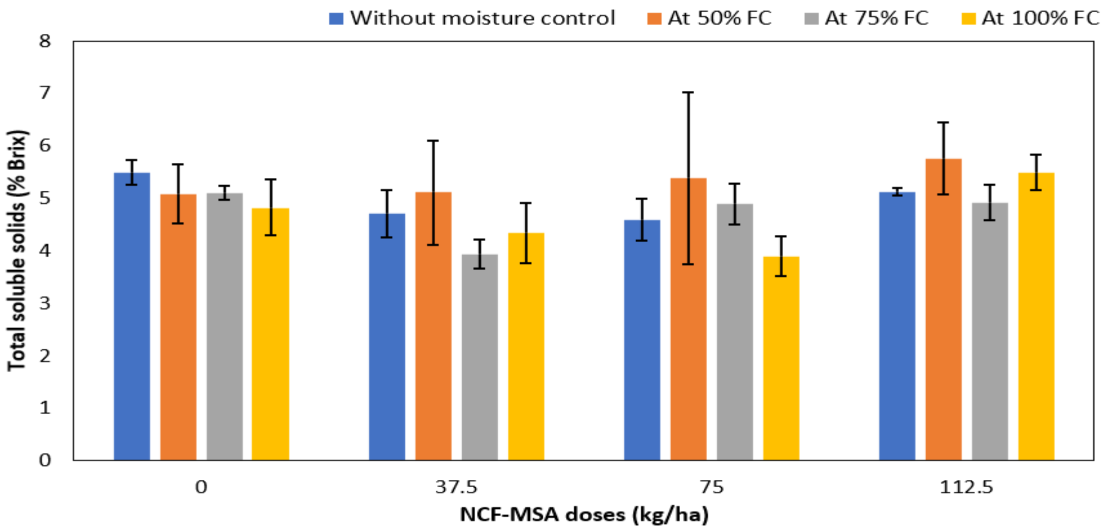
From the figure 4.19, without moisture control, fruit pH decreased with increase in NCF-MSA dose, control having the highest pH (4.24) in the group. At 50% FC moisture level, highest pH (4.44) was observed in 37.5 kg/ha, which is 5.2% higher than the corresponding control at 50% FC and 2% and 5.4% more than 37.5 kg/ha and 75 kg/ha doses of NCF-MSA, respectively. At 75% FC, highest pH value of 4.29 in fruits was observed in the highest dose of NCF-MSA (112.5 kg/ha), followed by 37.5 kg/ha dose (2.6% lower pH value) and then by 75 kg/ha (4.7% lower pH value). The pH of the control at 75% FC had a pH value of 4.07, which is 5.1% lower than highest value. Similarly, at 100% FC, the lowest pH value (4.19) was observed in the respective control, which is 2.1% lower than the highest value (4.28) obtained from 75 kg/ha NCF-MSA dose. pH values of 37.5 kg/ha and 112.5 kg/ha doses were 1.4% and 1.2% lower than that from 75 kg/ha dose of NCF-MSA. Overall, all three doses of NCF-MSA at 75% FC and 100% FC showed increase in pH. When nanocellulose was applied, highest pH value (4.34) was observed in the highest dose, i.e. 22.5 kg/ha, which is 5.8% higher than the respective control, 4.1% higher than 7.5 kg/ha dose and 3.5% higher than 15 kg/ha dose of nanocellulose.

4.4.2 Total Soluble Solids (% Brix)

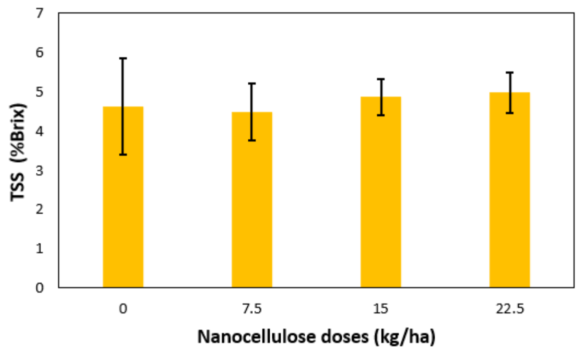
Figure 4.20

Effect of a) NCF-MSA and Soil Moisture b) Nanocellulose on Total Soluble Solids

a)



b)

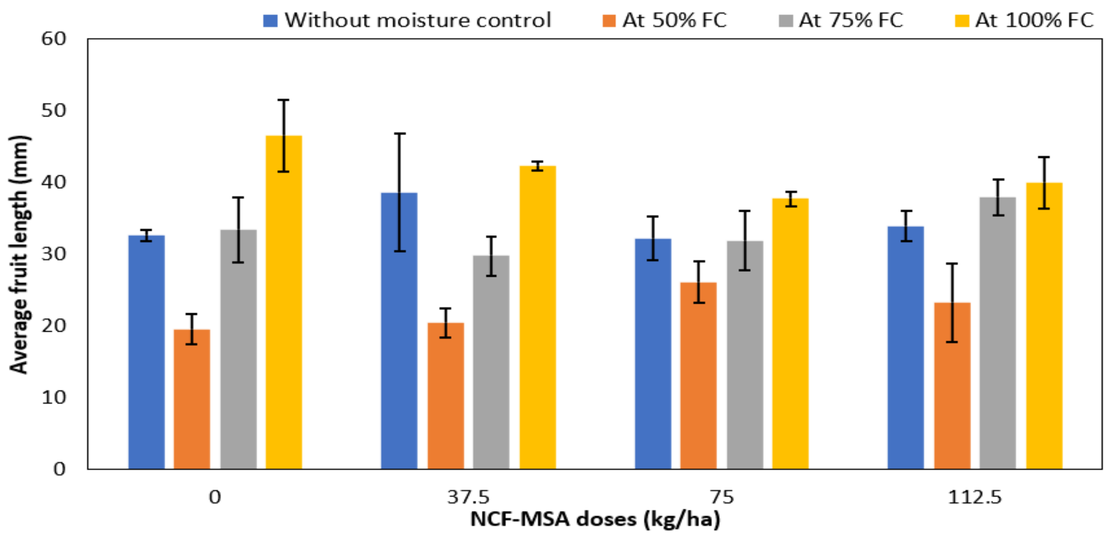


From the figure 4.20, without soil moisture control, highest TSS content (5.5 % Brix) was found in the control plants which was 14.5%, 16.4% and 7.3% higher than plants with NCF-MSA doses 37.5 kg/ha, 75 kg/ha and 112.5 kg/ha, respectively. At 50% FC, highest TSS content (5.76) was found in the highest dose of NCF-MSA (112.5 kg/ha), which is 11.6% higher than the control at 50% FC and 11.3% and 6.4% higher than NCF-MSA doses 37.5 kg/ha and 75 kg/ha, respectively. At 75% FC, TSS content was highest (5.1% Brix) in the control. TSS content NCF-MSA doses 37.5 kg/ha, 75 kg/ha and 112.5 kg/ha were lower than the control by 22.7%, 4% and 3.5%, respectively. Similar to 50% FC, at 100% FC, 112.5 kg/ha gave the highest TSS content of 5.5% Brix, which was 12.4% higher than the control and 21.8% and 29% higher than NCF-MSA doses 37.5 kg/ha and 75 kg/ha, respectively. It can be observed that fruits at 50% FC had a high TSS content for all doses of NCF-MSA, unlike the rest. Again, it was found that the highest dose of nanocellulose, i.e. 22.5 kg/ha had the maximum TSS content of 4.98% Brix. This value is 7% higher than the control and 9.8% higher than 7.5 kg/ha dose and 2.2% higher than 15 kg/ha dose.

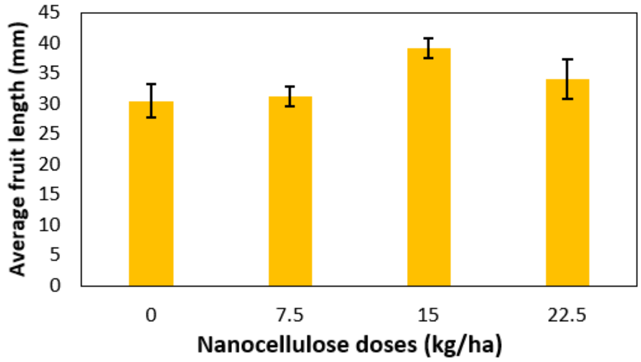
4.4.3 Average Fruit Length (mm)

Figure 4.21

Effect of a) NCF-MSA and Soil Moisture b) Nanocellulose on Average Fruit Length



b)



From the figure 4.21, without soil moisture control, average fruit length increased with the application of NCF-MSA only in doses 37.5 kg/ha and 112.5 kg/ha. 37.5 kg/ha gave the maximum length of 38.6 mm which is 15.5% more than the control. At 50% FC, average fruit length was highest (26.1 mm) at dose 75 kg/ha which is 30% more than the control at 50% FC and 21.5% and approximately 11% more than 37.5 kg/ha and 112.5 kg/ha, respectively. Again at 75% FC, fruits were longest (37.9 mm) when NCF-MSA dose was 112.5 kg/ha, which was 12%, 21.4% and 16.1% longer than control, doses 37.5 kg/ha and 75 kg/ha, respectively. At 100% FC, average fruit length was maximum in the control plant with a length of 46.5 mm and did not increase with NCF-MSA doses. However, on an average, the length of fruits (41.6 mm) at 100% FC was maximum, followed by 75% FC (33.2 mm) and 50% FC (22.3 mm). Average fruit length was similar for NCF-MSA doses at 75% FC and without moisture control. It was seen that when nanocellulose was applied, 15 kg/ha dose gave the longest fruits (39.1 mm) which was 22.3% longer than 7.5 kg/ha and 20.2% and 12.8% longer than 15 kg/ha and 22.5 kg/ha doses.

4.4.4 Data Analysis Through ANOVA and Tukey HSD Test.

To investigate the significance in the differences, two way CRD ANOVA was performed for all fruit quality parameters to check interactive effects of NCF-MSA and soil moisture and one-way CRD ANOVA was performed for nanocellulose. This was followed by Tukey HSD test to do multiple comparisons.

Fruit pH is not statistically significant with respect to individual effect NCF-MSA and interactive effects of NCF-MSA and soil moisture. However, statistically significant differences ($P<0.05$) were observed with respect to individual effect of soil moisture.

Nanocellulose doses produced highly significant differences (P<0.01) in pH values of fruits. The individual effects of NCF-MSA and soil moisture on the Total Soluble Solids (TSS) of fruits are statistically significant with P<0.05. However, the interaction between NCF-MSA doses and soil moisture does not have any significant differences in the TSS content of fruits. Nanocellulose doses did not produce significant differences in Total soluble solids content of fruits. The individual effects of NCF-MSA had no significant differences in average fruit length. However, average fruit length had highly significant differences (P<0.01) due to individual effects of soil moisture and significant differences (P<0.05) due to interaction between NCF-MSA and soil moisture. Average fruit length was found to be highly significant (P<0.01) with the application of nanocellulose.

Significance and means for fruit quality parameters are given in tables 4.7, 4.8 and 4.9.

Table 4.7

Results of ANOVA Analysis with F value for the Effects of NCF-MSA and Moisture (M) for Different Fruit Quality Parameters

Fruit quality parameters	**NCF-MSA**	**Moisture**	**NCF-MSA X Moisture (M)**	**Nanocellulose**
Fruit pH	0.66ns	4.09*	1.82ns	41.09**
Total Soluble solids	4.38*	3.17*	1.2ns	0.23ns
Average fruit length	0.47ns	55.31**	3.01*	7.84**

Note. *, ** and ns represent data is significant at 1% level, significant at 5% level and not significant, respectively

Table 4.8

Effect of Soil Application of NCF-MSA and Soil Moisture Regime (M) on Fruit Quality Parameters of Lukthar Tomato.

Factors	Fruit pH	Total Soluble Solids (% Brix)	Average fruit length (mm)
NCF-MSA			
NCF-MSA -0 kg/ha	4.1756 ± 0.12	5.125 ± 0.43 ab	33.017 ± 10.41
NCF-MSA -37.5 kg/ha	4.2286 ± 0.13	4.5208 ± 0.70 b	32.792 ± 9.59
NCF-MSA -75 kg/ha	4.2353 ± 0.16	4.6889 ± 0.94 ab	31.979 ± 5.0
NCF-MSA -112.5 kg/ha	4.1861 ± 0.18	5.3236 ± 0.49 a	33.763 ± 7.43
Moisture (M)			
No control	4.1372 ± 0.14 b	4.975 ± 0.46 ab	34.33 ± 4.69 b
50% FC	4.3019 ± 0.16 a	5.3375 ± 0.94a	22.352 ± 3.95 c
75% FC	4.1594 ± 0.14 b	4.7125 ± 0.54 ab	33.236 ± 4.36 b
100% FC	4.2269 ± 0.1 ab	4.6333 ± 0.74 b	41.632 ± 4.34 a
NCF-MSA X M			
NCF-MSA -0 kg/ha	4.2344 ± 0.04	5.4889 ± 0.23	32.566 ± 0.80 bcd
NCF-MSA -0 kg/ha + 50% FC	4.2078 ± 0.15	5.0889 ± 0.57	19.582 ± 2.09 e
NCF-MSA -0 kg/ha + 75% FC	4.0744 ± 0.18	5.1 ± 0.13	33.392 ± 4.50 bcd
NCF-MSA -0 kg/ha + 100% FC	4.1856 ± 0.04	4.8222 ± 0.54	46.527 ± 5.05 a
NCF-MSA -37.5 kg/ha	4.1656 ± 0.12	4.7 ± 0.45	38.615 ± 8.20 abc
NCF-MSA -37.5 kg/ha + 50% FC	4.3544 ± 0.11	5.111 ± 0.99	20.49 ± 2.04 e
NCF-MSA -37.5 kg/ha + 75% FC	4.1789 ± 0.14	3.9389 ± 0.28	29.765 ± 2.72 cde
NCF-MSA -37.5 kg/ha +100% FC	4.2156 ± 0.10	4.3333 ± 0.57	42.297 ± 0.59 ab
NCF-MSA -75 kg/ha	4.1244 ± 0.14	4.5889 ± 0.4	32.197 ± 3.07 bcd

Factors	Fruit pH	Total Soluble Solids (% Brix)	Average fruit length (mm)
NCF-MSA -75 kg/ha + 50% FC	4.4444 ± 0.07	5.3889 ± 1.64	26.121 ± 2.93 de
NCF-MSA -75 kg/ha +75% FC	4.0933 ± 0.06	4.8889 ± 0.39	31.878 ± 4.19 bcd
NCF-MSA -75 kg/ha +100% FC	4.2789 ± 0.04	3.8889 ± 0.38	37.719 ± 1.0 abc
NCF-MSA -112.5 kg/ha	4.0244 ± 0.19	5.1222 ± 0.07	33.942 ± 2.13 bcd
NCF-MSA -112.5 kg/ha + 50% FC	4.2011 ± 0.19	5.7611 ± 0.69	23.214 ± 5.43 de
NCF-MSA -112.5 kg/ha + 75% FC	4.2911 ± 0.07	4.9222 ± 0.34	37.909 ± 2.45 abc
NCF-MSA -112.5 kg/ha + 100% FC	4.2278 ± 0.20	5.4889 ± 0.34	39.986 ± 3.62 abc

Note. FC, field capacity; means within a column followed by the same letter are not significantly different by least significant difference test at P < 0.05; data are means of three replications ± standard deviations.

Table 4.9

Effect of Soil application of Nanocellulose on Fruit Quality Parameters of Lukthar Tomato.

Factors	Fruit pH	TSS (% Brix)	Average fruit length (mm)
NCF-MSA			
Nanocellulose -0 kg/ha	4.0944 ± 0.01 b	4.6333 ± 1.23	30.413 ± 2.79 b
Nanocellulose -7.5 kg/ha	4.1611 ± 0.04 b	4.4889 ± 0.72	31.206 ± 1.64 b
Nanocellulose -15 kg/ha	4.1256 ± 0.11 b	4.8667 ± 0.47	39.11 ± 1.64 a
Nanocellulose -22.5 kg/ha	4.4078 ± 0.14 a	4.9778 ± 0.53	34.09 ± 3.24 ab

Note. means within a column followed by the same letter are not significantly different by least significant difference test at P < 0.05; data are means of three replications ± standard deviations.

4.4.5 Discussion on Fruit Quality Parameters

Water stress conditions have shown positive impacts in fruit quality parameters in several studies. In the studies by (Chakma et al., 2021, 2023), total soluble solids content increased with decrease in moisture level. 75% FC and 50% FC had more TSS content than 100% FC. Similarly, in this study significant differences in TSS were observed in terms of soil moisture. 50% FC had higher TSS content compared to 75% FC and 100% FC. (Chakma et al., 2021, 2023) have mentioned that increase in TSS content in fruits at lower moisture levels can be attributed to low dilution of photo assimilates in fruits due to insufficient water transport through xylem vessels. Accumulation of assimilates in fruits that are smaller in size due to water stress results in higher conversion of starch to sugar. Water import inside plants is affected at low moisture levels, however, that of photo assimilates is not (Zegbe et al., 2006; Zegbe-Domínguez et al., 2003).

pH of fruits was affected significantly due to soil moisture, 50% FC fruits had the highest pH, followed by 100% FC and then by 75% FC. Similar to TSS, moisture stress increases pH of fruits due to low dilution (Chen et al., 2013; Nangare et al., 2016). Average fruit length was significantly affected by soil moisture. 100% FC had the longest fruits and 50% FC had the shortest ones. This again can be attributed to adverse effects of moisture in the flowering and fruiting stages and also tolerance of the plants to reduced moisture (Chakma et al., 2021; Gatta et al., n.d.; Nangare et al., 2016; Zegbe et al., 2006).

NCF-MSA had no statistically significant effect on fruit pH. However, there was increase in fruit pH with some doses of NCF-MSA. TSS was negatively affected by NCF-MSA in 75% and 100% FC but the opposite in 50% FC. TSS increased with doses in 50% FC. Average fruit length was significantly affected only by the interactive effect of NCF-MSA and soil moisture. NCF-MSA showed larger sized fruits when doses increased simultaneously with soil moisture. It is mentioned by (Chakma et al., 2021; Ouellette et al., 2017) that silicon cannot transport to fruits and hence shows no effect on the quality of fruits. In a study by (do Nascimento et al., 2020) , it was found that there was no effect of diatomaceous earth (source of silicon) on fruit quality parameters.

Nanocellulose produced highly significant effect in fruit pH. pH of fruits increased with increase in NCF-MSA doses. But TSS was not statistically affected by nanocellulose; yet TSS increased in the highest doses of NCF-MSA. Average fruit length increased with the incorporation of nanocellulose and was maximum at 15 kg/ha dose.

4.5 Effects of Nanocellulose Fibres Stabilized Monosilicic Acid (NCF-MSA) and Nanocellulose on Physiological Parameters under Water Stress Conditions

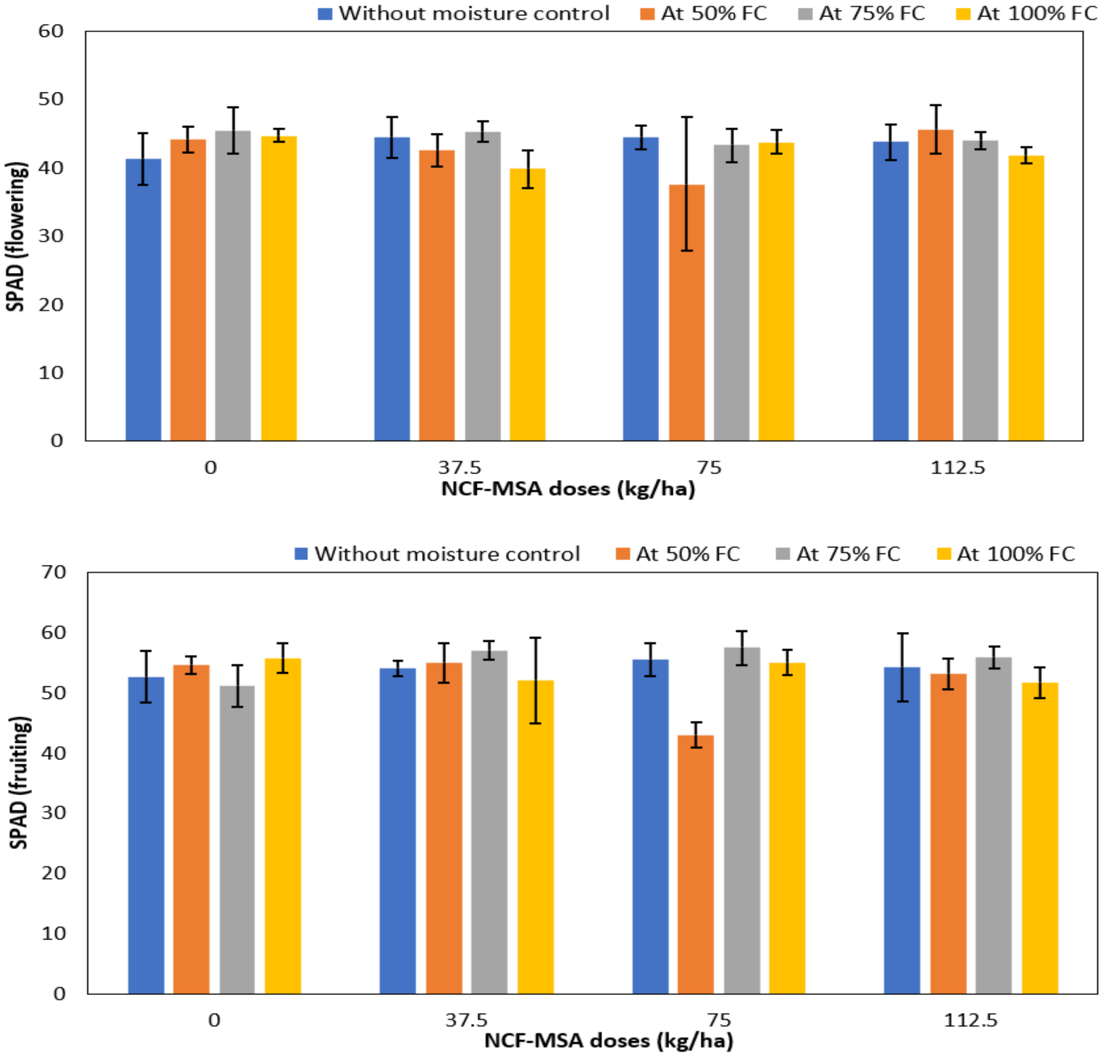
NCF-MSA was applied as soil incorporation in plants and data on physiological parameters were recorded. The physiological parameters include leaf greenness, Membrane Stability Index, Leaf Relative Water Content and Crop Water Stress Index. The collected data was analysed and presented as bar graphs, followed by performing an ANOVA on these parameters to investigate significance of data, The means were compared pair-wise using Tukey HSD test. Finally, a discussion on these parameters is presented.

4.5.1 Leaf Greenness

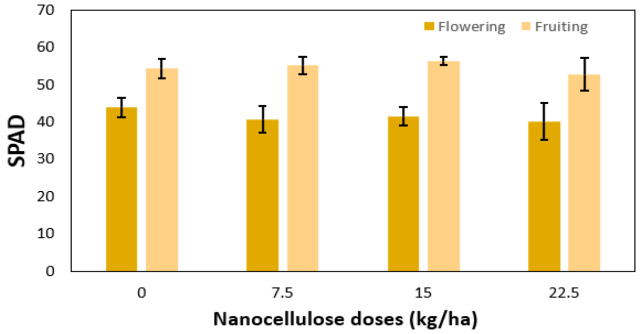
Figure 4.22

Effect of a) NCF-MSA and Soil Moisture b) Nanocellulose on Leaf Greenness at Flowering and Fruiting Stages

a)



b)



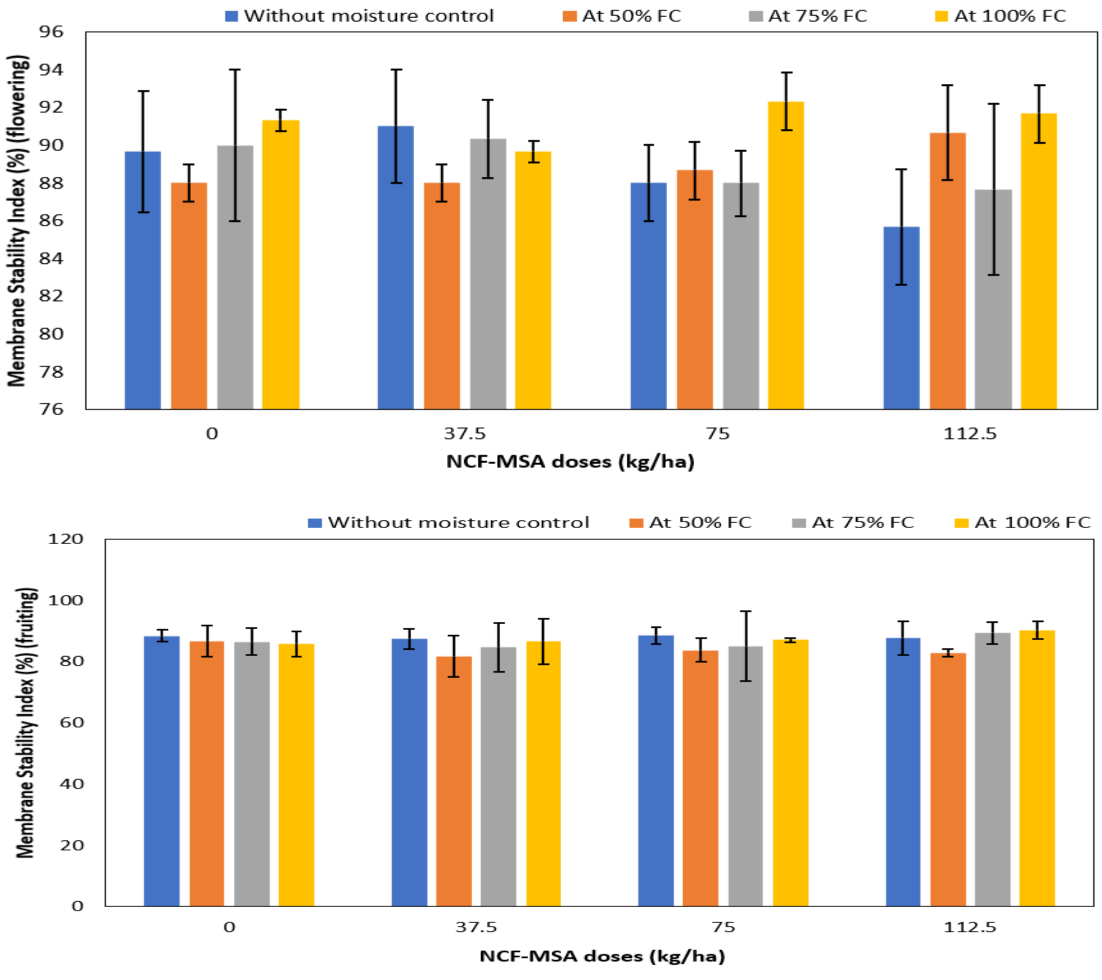
From the figure 4.22, it was noticed that the SPAD values were higher and above 50 in the fruiting stage, whereas the opposite and less than 50 in the flowering stage. The SPAD values with and without moisture control were similar throughout the groups in both flowering and fruiting stages. In case of nanocellulose, the highest SPAD value at flowering stage (43.9) was observed in the control and that in the fruiting stage in 15 kg/ha dose. Moreover, the SPAD value was higher in the fruiting stage (54.8 on an average) than in the flowering stage (41.7 on an average) at all doses.

4.5.2 Membrane Stability Index (%)

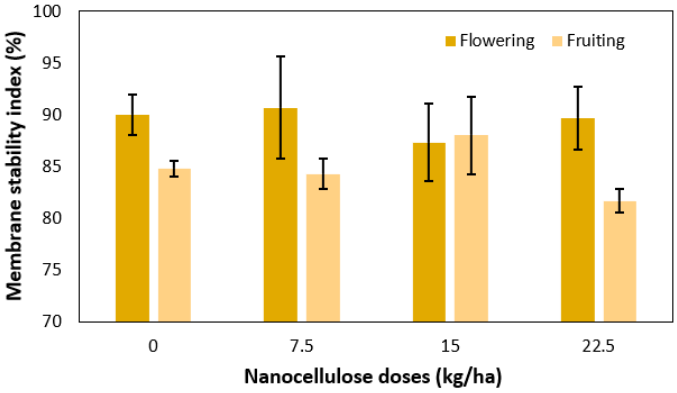
Figure 4.23

Effect of a) NCF-MSA and Soil Moisture b) Nanocellulose on Membrane Stability Index at Flowering and Fruiting Stages

a)



b)



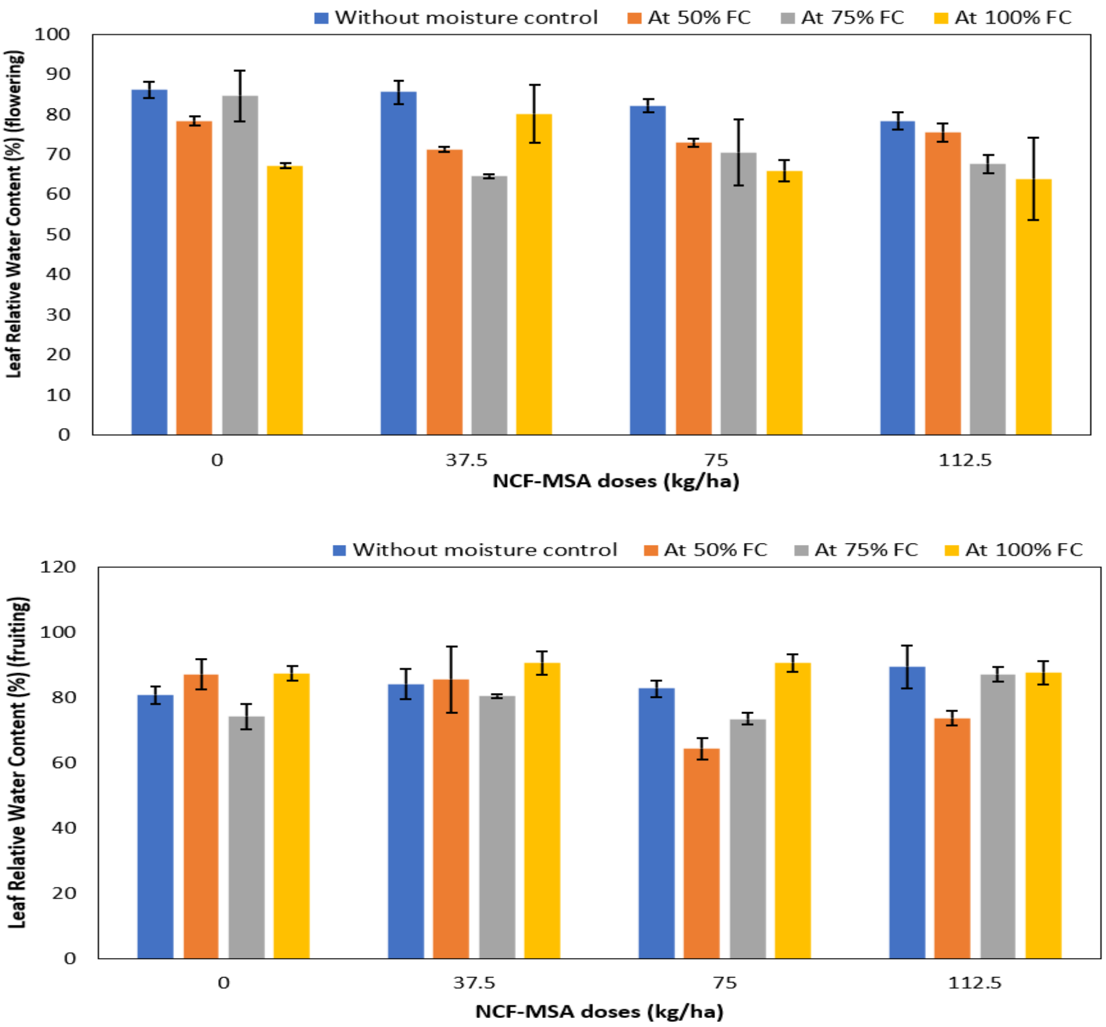
From the figure 4.23, at the fruiting stage, without soil moisture control, MSI values were similar to the control. At 50% FC, MSI value was highest (86.7%) in the control with 50% FC, which was 5.8%, 3.5% and 4.4% higher than doses 37.5 kg/ha, 75 kg/ha and 112.5 kg/ha doses, respectively. At 75% FC, the highest dose of NCF-MSA gave the highest value of MSI (89.4%), which was 3.2% higher than the control and 5.1% and approximately 5% higher than doses 37.5 kg/ha and 75 kg/ha respectively. At 100% FC, similar to above, MSI was highest (90.3%), which was approximately 5% higher than the control at 100% FC and 4.1% and 3.5% higher than doses 37.5 kg/ha and 75 kg/ha, respectively. In case of nanocellulose, Membrane Stability Index was higher at the flowering stage (89.4 on an average) than at the fruiting stage (84.7 on an average). The highest value of MSI (90.7) in the flowering stage was shown by 7.5 kg/ha dose, which was 0.8% higher than control and by 15 kg/ha dose (88) in the fruiting stage, which was 3.6% higher than the control.

4.5.3 Leaf Relative Water Content (%)

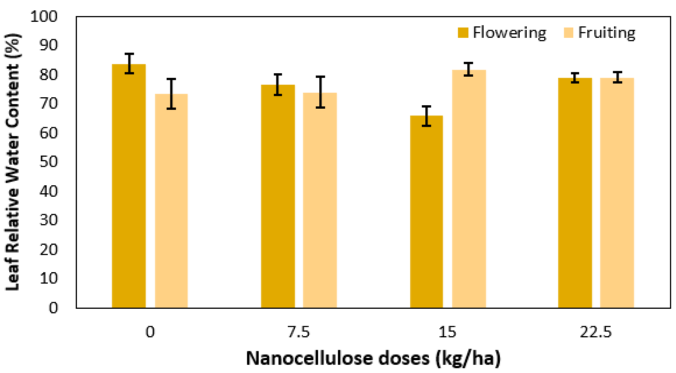
Figure 4.24

Effect of a) NCF-MSA and Soil Moisture b) Nanocellulose on Leaf Relative Water Content at Flowering and Fruiting Stages

a)



b)



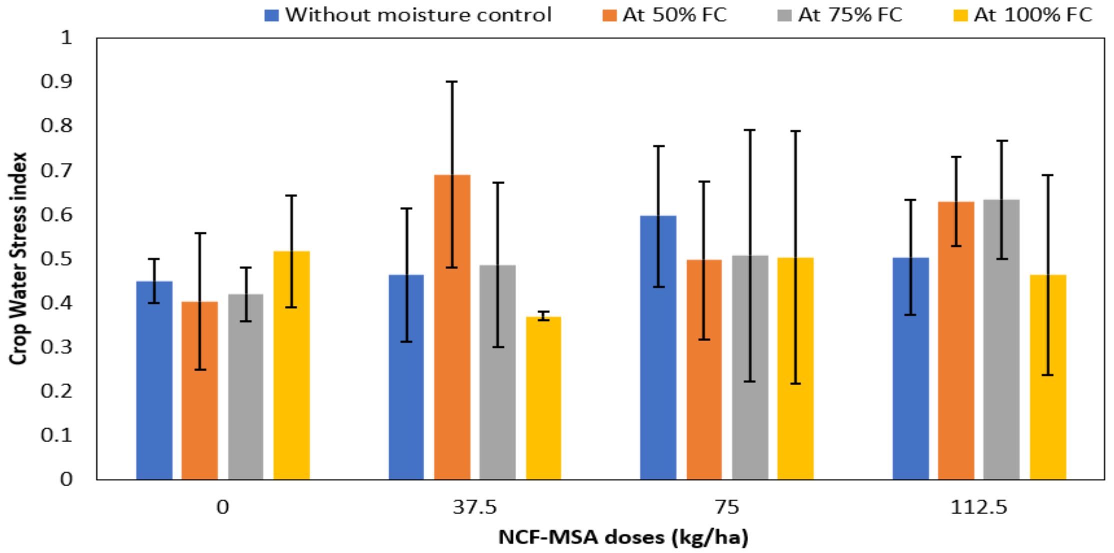
From the figure 4.24, at flowering stage and in all interaction groups, LRWC was lower than the control, except at 100% FC, where 37.5 kg/ha of NCF-MSA gave higher value of LRWC, which was 16.2% higher than the control. However, at the fruiting stage, LRWC was higher than the corresponding control with the application of NCF-MSA in all interaction groups except at 50% FC. At the fruiting stage, without soil moisture control, the highest LRWC (89.5%) was observed in 112.5 kg/ha dose of NCF-MSA, which was 9.7% higher than the control and 5.8% and 7.5% higher than 37.5 kg/ha and 75 kg/ha doses, respectively. At 50% FC, LRWC was highest (87.1%) in the respective control. At 75% FC, LRWC was highest (87.2%) in 112.5 kg/ha dose of NCF-MSA, which was 14.8% higher than the control and 7.6% and 15.7% higher than doses 37.5 kg/ha and 75 kg/ha, respectively. At 100% FC, LRWC was highest in two doses-37.5 kg/ha and 75 kg/ha (about 90.7%), which were 3.4% higher than the control and 112.5 kg/ha dose of NCF-MSA. In nanocellulose applied plants, LRWC at the three nanocellulose doses were lower than the control. On the other hand, LRWC at the three doses increased at the fruiting stage, the highest being 82%, shown by 15 kg/ha dose, which was 10.4% higher than the control (7.5%).

4.5.4 Crop Water Stress Index

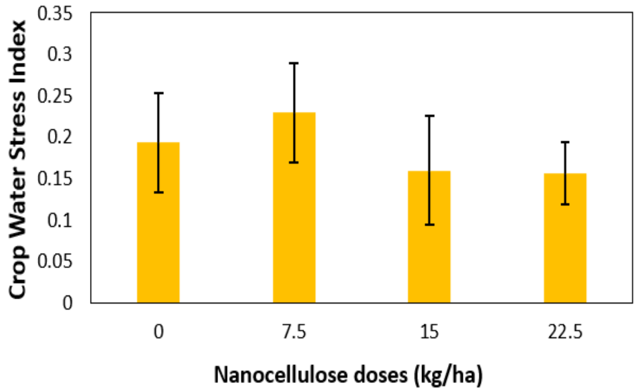
Figure 4.25

Effect of a) NCF-MSA and Soil Moisture b) Nanocellulose on Crop Water Stress Index at Flowering and Fruiting Stages

a)



b)



From the figure 4.25, CWSI increased with the application of NCF-MSA in all moisture groups except 100% FC. However, when compared amongst the soil moisture levels, CWSI decreased with increased moisture. In case of nanocellulose, the highest value was observed in 7.5 kg/ha dose (0.23), which was 17.4% higher than the control.

4.5.5 Data Analysis Through ANOVA and Tukey HSD Test.

To investigate the significance in the differences, two way CRD ANOVA was performed for all growth parameters to check interactive effects of NCF-MSA and soil moisture and one-way CRD ANOVA was performed for nanocellulose. This was followed by Tukey HSD test to do multiple comparisons.

NCF-MSA, soil moisture and their interaction did not produce statistically significant differences in leaf greenness in both flowering and fruiting stages. The differences in leaf greenness (determined by SPAD value) due to the three nanocellulose doses were not statistically significant at both flowering and fruiting stages. NCF-MSA, soil moisture and their interaction did not produce significant differences in Membrane Stability Index in the flowering stage. However, there were statistically significant differences ($P<0.05$) produced by soil moisture in the fruiting stage. Nanocellulose produced significant difference ($P<0.05$) in Membrane Stability Index at fruiting stage but not in the flowering stage. NCF-MSA, soil moisture and their interaction produced highly significant differences ($P<0.01$) in Leaf Relative Water Content at both flowering and fruiting stages. Nanocellulose produced highly significant differences ($P<0.01$) in Leaf relative Water Content at the flowering stage but differences were non-significant in the fruiting stage. The values of Crop Water Stress index were statistically similar and not affected by individual effects of NCF-MSA, soil moisture and their interaction. Crop Water Stress Index values were statistically similar and was not affected by nanocellulose.

Significance and means of physiological parameters are given in tables 4.10, 4.11 and 4.12.

Table 4.10

Results of ANOVA Analysis with F value for the Effects of NCF-MSA and Moisture (M) for Different Physiological Parameters

Physiological parameters	**NCF-MSA**	**Moisture**	**NCF-MSA X Moisture (M)**	**Nanocellulose**
Leaf greenness (flowering)	0.58ns	0.95ns	1.56ns	0.64ns
Leaf greenness (fruiting)	0.17ns	0.86ns	0.15ns	0.85ns
Membrane Stability Index (flowering)	0.35ns	3.17*	1.62ns	2.32ns
Membrane stability Index (fruiting)	0.47ns	1.48ns	0.32ns	4.47*
Leaf Relative Water Content (flowering)	6.4**	26.42**	5.42**	18.41**
Leaf Relative Water Content (fruiting)	7.74**	18.9**	7.29**	3.27ns
Crop Water Stress index	0.92ns	0.58ns	0.86ns	1.09ns

Note. *, ** and ns represent data is significant at 1% level, significant at 5% level and not significant, respectively

Table 4.11

Effect of Soil Application of NCF-MSA and Soil Moisture regime (M) on Physiological Parameters of Lukthar Tomato.

Factors	LG(Fl)	LG(Fr)	MSI(Fl) (%)	MSI(Fr) (%)	LRWC(Fl) (%)	LRWC(Fr) (%)	CWSI
NCF-MSA (P) (kg/ha)							
P-0	43.892 ± 2.86	53.6 ± 3.22	89.75 ± 2.56	86.739 ± 3.11	78.2 ± 8.28 a	82.597 ± 6.12 a	0.446 ± 0.10
P-37.5	43.058 ± 3.03	54.567 ± 3.91	89.75 ± 2.01	85.11 ± 4.81	75.417 ± 9.07 ab	85.265 ± 6.22 a	0.503 ± 0.18
P75	42.3 ± 5.25	52.767 ± 2.35	89.25 ± 2.38	86.074 ± 4.85	72.875 ± 7.24 b	77.862 ± 10.54 b	0.526 ± 0.20
P-112.5	43.817 ± 2.44	53.783 ± 3.37	88.917 ± 3.63	87.577 ± 3.81	71.325 ± 7.69 b	84.544 ± 7.37 a	0.558 ± 0.15
Moisture (M) (% FC)							
NMC	43.5 ± 2.80	54.175 ± 3.44	88.583 ± 3.20 b	87.937 ± 2.91	83.042 ± 3.78 a	84.329 ± 5.05 b	0.503 ± 0.13
50	42.492 ± 5.57	51.458 ± 2.23	88.833 ± 1.80 ab	83.764 ± 3.73	74.517 ± 3.04 b	77.734 ± 10.9 c	0.555 ± 0.18
75	44.517 ± 2.17	55.433 ± 3.39	89 ± 3.07 ab	86.364 ± 5.42	70.958 ± 9.21 bc	79.093 ± 5.96 c	0.51 ± 0.18
100	42.558 ± 2.48	53.65 ± 3.98	91.25 ± 1.42 a	87.434 ± 4.24	69.3 ± 8.65 c	89.111 ± 3.05 a	0.463 ± 0.18
P x M							
P-0	41.267 ± 3.76	52.733 ± 4.28	89.667 ± 3.21	88.133 ± 1.90	86.133 ± 2.01 a	80.646 ± 2.59 abc	0.45 ± 0.05
P-0 + 50	44.133 ± 1.90	54.633 ± 1.47	88 ± 1	86.697 ± 5.12	78.333 ± 1.16 abc	87.173 ± 4.56 ab	0.403 ± 0.16
P-0 + 75	45.433 ± 3.37	51.233 ± 3.46	90 ± 4	86.29 ± 4.42	81.1 ± 6.26 ab	75.064 ± 3.87 bcd	0.413 ± 0.06
P-0 + 100	44.733 ± 0.93	55.8 ± 2.48	91.333 ± 0.58	85.834 ± 4.11	67.233 ± 0.75 cd	87.504 ± 2.27 ab	0.517 ± 0.13
P-37.5	44.467 ± 2.97	54.133 ± 1.31	91 ± 3	87.391 ± 3.27	85.6 ± 2.91 a	84.3 ± 4.53 abc	0.463 ± 0.15
P-37.5 + 50	42.567 ± 2.32	55 ± 3.22	88 ± 1	81.728 ± 6.68	71.333 ± 0.61 bcd	85.538 ± 10.08 abc	0.69 ± 0.19
P-37.5 + 75	45.333 ± 1.54	57.067 ± 1.62	90.333 ± 2.08	84.762 ± 7.95	64.567 ± 0.49 d	80.579 ± 0.68 abc	0.487 ± 0.19

Factors	LG(Fl)	LG(Fr)	MSI(Fl) (%)	MSI(Fr) (%)	LRWC(Fl) (%)	LRWC(Fr) (%)	CWSI
P-37.5 + 100	39.867 ± 2.78	52.067 ± 7.09	89.667 ± 0.58	86.558 ± 7.47	80.167 ± 7.24 ab	90.642 ± 3.57 a	0.37 ± 0.01
P-75	44.467 ± 1.72	55.533 ± 2.76	88 ± 2	88.526 ± 2.78	82.1 ± 1.68 ab	82.846 ± 2.53 abc	0.597 ± 0.0.16
P-75 + 50	37.633 ± 9.76	42.967 ± 2.12	88.667 ± 1.53	83.725 ± 3.84	72.9 ± 1.08 bcd	64.393 ± 3.42 d	0.497 ± 0.18
P-75 + 75	43.333 ± 2.42	57.5 ± 2.85	88 ± 1.73	84.963 ± 11.43	70.533 ± 8.31 bcd	73.544 ± 1.81 cd	0.507 ± 0.29
P-75 + 100	43.767 ± 1.72	55.067 ± 2.12	92.333 ± 1.53	87.082 ± 0.65	65.967 ± 2.78 cd	90.663 ± 2.57 a	0.503 ± 0.29
P-112.5	43.8 ± 2.61	54.3 ± 5.63	85.667 ± 3.06	87.696 ± 5.53	78.333 ± 2.08 abc	89.524 ± 6.52 a	0.503 ± 0.13
P-112.5 + 50	45.633 ± 3.5	53.233 ± 2.55	90.667 ± 2.52	82.908 ± 1.16	75.5 ± 2.36 abcd	73.833 ± 2.18 cd	0.63 ± 0.10
P-112.5 + 75	43.967 ± 1.25	55.933 ± 1.77	87.667 ± 4.51	89.442 ± 3.60	67.333 ± 2.37 cd	87.183 ± 2.20 ab	0.633 ± 0.13
P-112.5 + 100	41.867 ± 1.20	51.667 ± 2.57	91.667 ± 1.53	90.262 ± 2.94	63.833 ± 10.27 d	87.635 ± 3.54 a	0.463 ± 0.23

Note. LG(Fl), Leaf greenness (flowering stage); LG(Fr), Leaf greenness (fruiting stage); MSI(Fl), Membrane Stability Index (Flowering stage); MSI(Fr), Membrane Stability Index (Fruiting stage); LRWC(Fl), Leaf Relative Water Content (Flowering stage); LRWC(Fr), Leaf Relative Water Content (Fruiting stage); CWSI, Crop Water Stress Index; NMC, No moisture control; FC, field capacity; means within a column followed by the same letter are not significantly different by least significant difference test at $P < 0.05$; data are means of three replications ± standard deviations.

Table 4.12

Effect of Soil Application of Nanocellulose on Physiological Parameters of Lukthar Tomato.

Nanocellulose (kg/ha)	LG(Fl)	LG(Fr)	MSI(Fl) (%)	MSI(Fr) (%)	LRWC(Fl) (%)	LRWC(Fr) (%)	CWSI
NC-0	43.9 ± 2.69	54.4 ± 2.59	91.333 ± 2	84.776 ± 0.79 ab	83.867 ± 3.35 a	73.525 ± 5.07	0.193 ± 0.06
NC-7.5	40.767 ± 3.61	55.3 ± 2.57	93 ± 4.93	84.273 ± 1.48 ab	76.633 ± 3.41 a	74.072 ± 5.18	0.23 ± 0.06
NC-15	56.533 ± 2.40	56.533 ± 1.05	89 ± 3.79	88.018 ± 3.77 a	65.933 ± 3.45 b	81.95 ± 2.25	0.16 ± 0.07
NC-22.5	40.2 ± 4.88	52.967 ± 4.36	88.667 ± 3.06	81.652 ± 1.14 b	79.1 ± 1.66 a	79.112 ± 1.74	0.157 ± 0.04

Note. LG(Fl), Leaf greenness (flowering stage); LG(Fr), Leaf greenness (fruiting stage); MSI(Fl), Membrane Stability Index (Flowering stage); MSI(Fr), Membrane Stability Index (Fruiting stage); LRWC(Fl), Leaf Relative Water Content (Flowering stage); LRWC(Fr), Leaf Relative Water Content (Fruiting stage); CWSI, Crop Water Stress Index; means within a column followed by the same letter are not significantly different by least significant difference test at $P < 0.05$; data are means of three replications ± standard deviations.

4.5.6 Discussion on Physiological Parameters

In this study, leaf greenness that is determined by SPAD value from a chlorophyll meter is an indicative of chlorophyll content in leaves. Studies have found increase in SPAD value of leaves with decrease in soil moisture. Some reasons that attribute to this can be greater concentration of chlorophyll in small leaves of water-stressed plants or accumulation of chlorophyll pigments in leaves due to deficit water supply to tissues (Puangbut et al., 2017; Songsri et al., 2009). (A. Ullah et al., 2017) also reported that at water stress conditions, there is oxidative damage to chloroplasts due to production of reaction oxygen species. In our study, no significant differences in leaf greenness were observed at both flowering and fruiting stages amongst the soil moisture levels; however, there was an overall increase in SPAD value in the fruiting stage.

Leaf Relative Water Content (LRWC) is an important parameter that determines presence of required water supply to plant tissues. Decrease in LRWC with decrease in moisture is indicative of increased membrane permeability or leaf relative conductivity.

Due to this, plant loses the ability to prevent water loss leading to decrease in water content in tissues (BAI et al., 2006; Chakma et al., 2021, 2023; Vandegeer et al., 2021). In this study, LRWC was highly significant with different moisture levels at both flowering and fruiting stages. But LRWC was highest in 50% FC and least in 100% FC at the flowering stages but the opposite at the fruiting stage. At fruiting stage, LRWC increased with increased moisture levels.

Similarly, Membrane Stability Index increased with increased soil moisture, indicating electrolyte leakage prevention in cell membrane when there is sufficient water supply. Membrane stability is deteriorated in low moisture levels due to membrane injury (Ahmed et al., 2023; Alam et al., 2023). Although, moisture produced non-significant effects in Crop Water Stress Index (CWSI), it was observed that 100% FC had the lowest CSWI and 50% FC had the highest, which clearly represents water stress in low moisture levels.

Silicon is known to alleviate water stress by optimizing photosynthesis, maintaining membrane stability, cell wall stability, improving the antioxidant defense ability of plants (Cao et al., 2015, 2017, 2020; Chakma et al., 2021, 2023). In the present study, NCF-MSA did not show significant effects in leaf greenness and MSI at both flowering and fruiting stages in plants. Whereas, highly significant effects were noticed in LRWC in both the stages of plant development. In the flowering stage, LRWC mostly decreased with the incorporation of NCF-MSA, however, this was not the case at the fruiting stage. At the fruiting stage, LRWC mostly increased with the use of NCF-MSA. NCF-MSA produced statistically similar results in CWSI, but the effects were negative. CWSI increased after application of NCF-MSA across all moisture levels.

Nanocellulose did produce significant effects in leaf greenness and CWSI. Whereas, differences were statistically in the fruiting stage in MSI, but it increased only in the second dose of nanocellulose. Nanocellulose produced highly significant differences in LRWC in the flowering stage; although the values decreased with increase in doses. But, contrary to flowering stage, LRWC increased with the use of nanocellulose.

4.6 Effects of Nanocellulose Fibres Stabilized Monosilicic Acid (NCF-MSA) and Nanocellulose on Photosynthetic Parameters under Water Stress Conditions

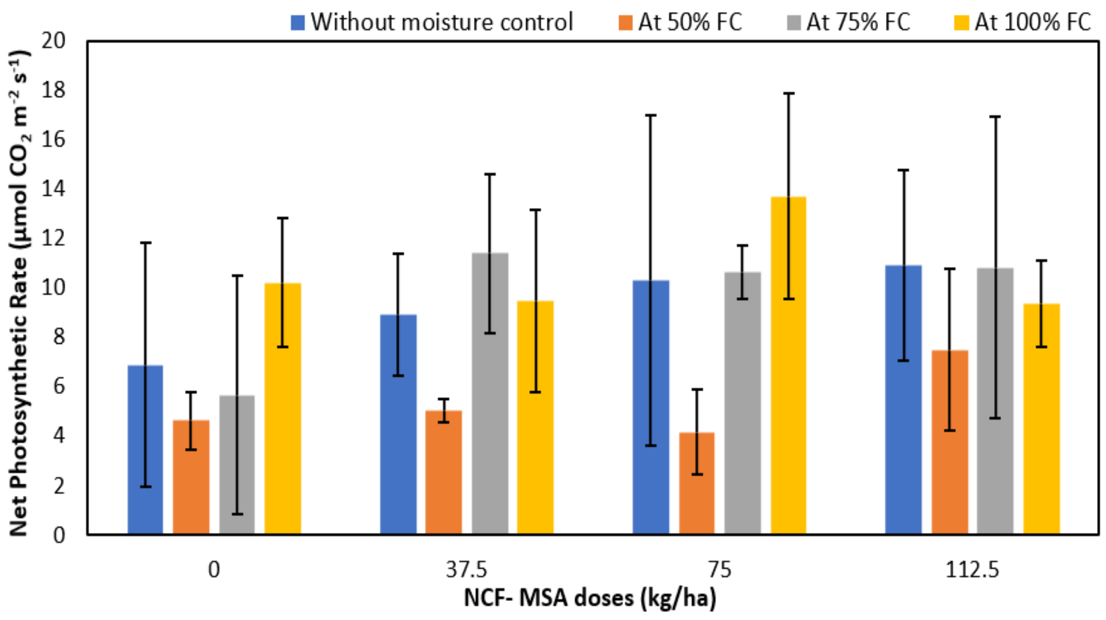
NCF-MSA was applied as soil incorporation in plants and data on photosynthetic parameters were recorded. The photosynthetic parameters include Net Photosynthetic Rate, Stomatal Conductance, Transpiration Rate and Chlorophyll Fluorescence parameters (Maximum quantum yield of Photosystem II and Effective quantum yield of Photosystem II). The collected data was analysed and presented as bar graphs, followed by performing an ANOVA on these parameters to investigate significance of data, The means were compared pair-wise using Tukey HSD test. Finally, a discussion on these parameters is presented.

4.6.1 Net Photosynthetic Rate (μmol CO_2 m^{-2} s^{-1})

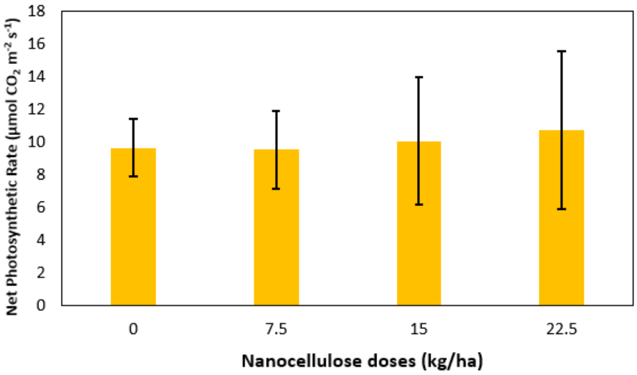
Figure 4.26

Effect of a) NCF-MSA and Soil Moisture b) Nanocellulose on Net Photosynthetic Rate

a)



b)



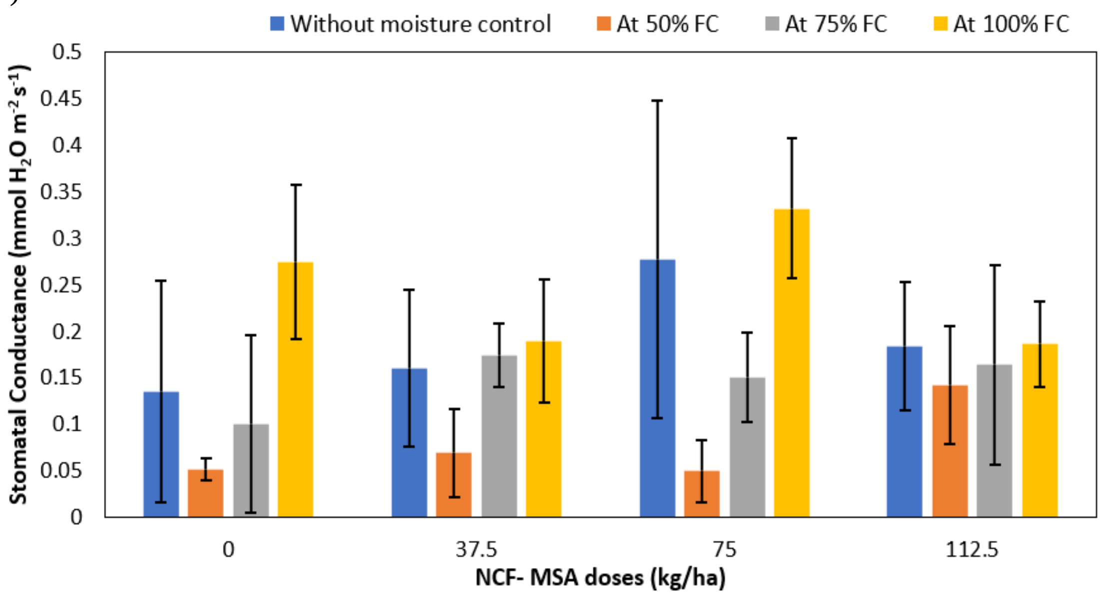
From the figure 4.26, without soil moisture control, Net Photosynthetic Rate was maximum (10.9 µmol CO_2 m^{-2} s^{-1}) in NCF-MSA dose 112.5 kg/ha, which is 36.7% more than the control of this group, and 18.3% higher than 37.5 kg/ha dose and 5.5% higher than 75 kg/ha. When compared at 50% FC soil moisture level, Net Photosynthetic Rate was maximum (7.5 µmol CO_2 m^{-2} s^{-1}) in NCF-MSA dose 112.5 kg/ha, which is 38.7% higher than the corresponding control and 33.3% and 44% higher than doses 37.5 kg/ha and 75 kg/ha, respectively. However, at 75% FC soil moisture level, Net Photosynthetic Rate was maximum (11.4 µmol CO_2 m^{-2} s^{-1}) in NCF-MSA dose 37.5 kg/ha, which was 50% higher than the corresponding control and 7% and 5.3% higher than doses 75 kg/ha and 112.5 kg/ha, respectively. Again, for 100% FC, 112.5 kg/ha dose of NCF-MSA gave the lowest Net Photosynthetic Rate of 9.4 µmol CO_2 m^{-2} s^{-1}, which was 7.8% lower than the respective control plant and 1.1% and 31.4% lower that doses 37.5 kg/ha and 75 kg/ha, respectively. On an average, 100% FC had the highest Net Photosynthetic Rate of 10.7 µmol CO_2 m^{-2} s^{-1}, followed by 75% FC at 9.6 µmol CO_2 m^{-2} s^{-1} and 50% FC at 5.3 µmol CO_2 m^{-2} s^{-1}. In case of nanocellulose, the highest rate of photosynthesis (10.7 µmol CO2 m-2 s-1) was seen at dose 22.5 kg/ha, which was 9.3% higher than the control, and 11.2% and 5.6% higher than doses 7.5 kg/ha and 22.5 kg/ha, respectively.

4.6.2 Stomatal Conductance (mmol H_2O m^{-2} s^{-1})

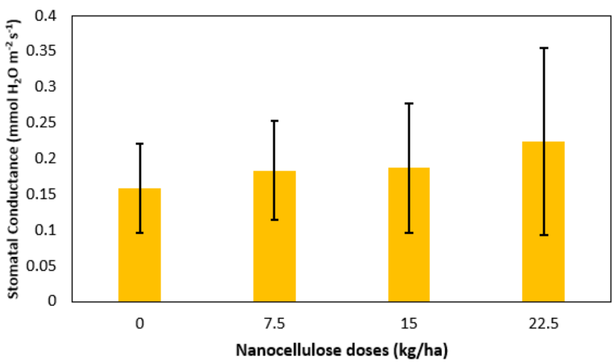
Figure 4.27

Effect of a) NCF-MSA and Soil moisture b) Nanocellulose on Stomatal Conductance

a)



b)



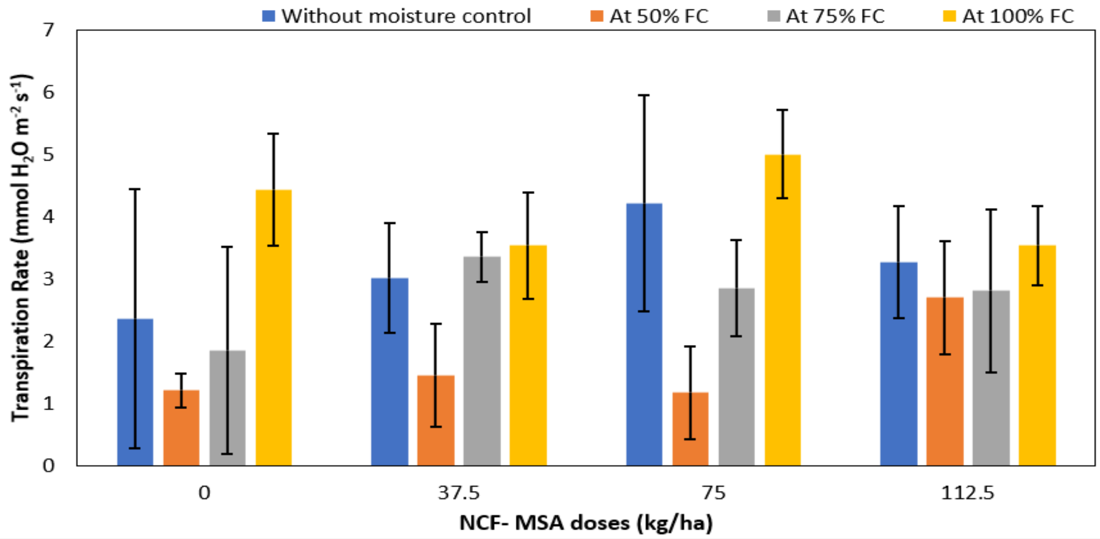
From the figure 4.27, without soil moisture control, stomatal conductance was maximum (0.28 mmol H_2O m^{-2} s^{-1}) in NCF-MSA dose 75 kg/ha, which was 53.6% more than the control of this group, and approximately 43% higher than 37.5 kg/ha dose and 35.7% higher than 112.5 kg/ha. When compared at 50% FC soil moisture level, stomatal conductance was maximum (0.14 mmol H_2O m^{-2} s^{-1}) in NCF-MSA dose 112.5 kg/ha, which was 64.3% higher than the corresponding control and 75 kg/ha and 50% higher than dose 37.5 kg/ha. At 75% FC soil moisture level, stomatal conductance was maximum (0.17 mmol H_2O m^{-2} s^{-1}) in NCF-MSA dose 37.5 kg/ha, which was 41.2% higher than the corresponding control and 11.8% and 5.9% higher than doses 75 kg/ha and 112.5 kg/ha, respectively. Again, for 100% FC, 75 kg/ha dose of NCF-MSA gave the highest stomatal conductance of 0.33 mmol H_2O m^{-2} s^{-1}, which was 18.2% higher than the respective control plant and 42.4% higher that doses 37.5 kg/ha and 112.5 kg/ha. On an average, 100% FC had the highest stomatal conductance of 0.25 mmol H_2O m^{-2} s^{-1}, followed by 75% FC at 0.15 mmol H_2O m^{-2} s^{-1} and 50% FC at 0.08 mmol H_2O m^{-2} s^{-1}. In case of nanocellulose application, the highest stomatal conductance (0.22 mmol H_2O m^{-2} s^{-1}) was seen at dose 22.5 kg/ha, which was 27.3% higher than the control, and 18.2% and 13.6% higher than doses 7.5 kg/ha and 22.5 kg/ha, respectively.

4.6.3 Transpiration Rate (mmol H_2O m^{-2} s^{-1})

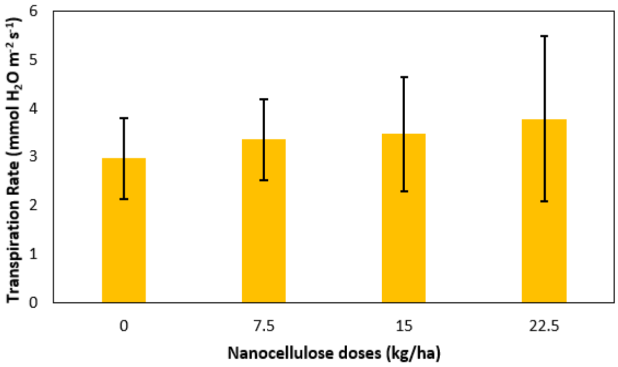
Figure 4.28

Effect of a) NCF-MSA and Soil Moisture b) Nanocellulose on Transpiration Rate

a)



b)



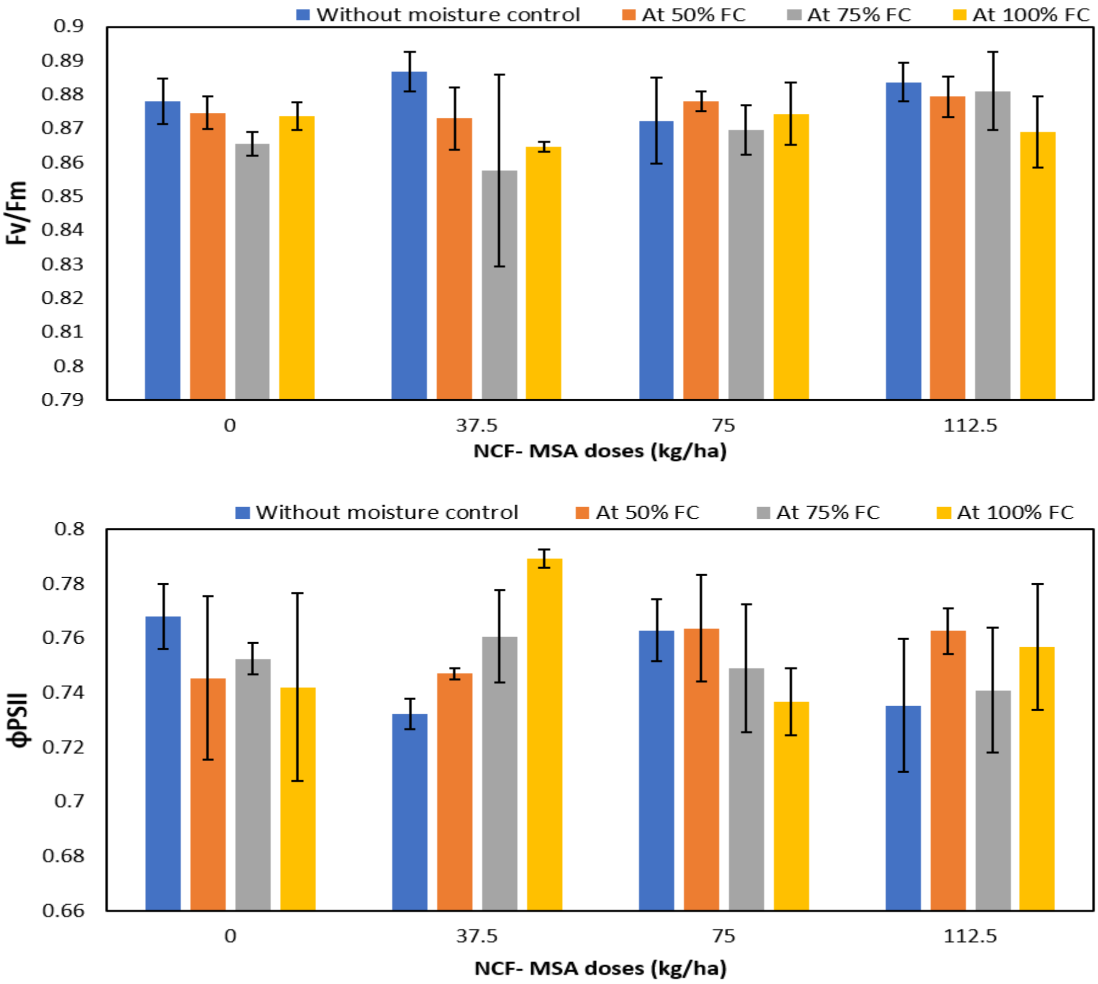
From the figure 4.28, without soil moisture control, Transpiration Rate was maximum (4.21 mmol H_2O m^{-2} s^{-1}) in NCF-MSA dose 75 kg/ha, which was approximately 44% more than the control of this group, and 28.5% higher than 37.5 kg/ha dose and 22.1% higher than 112.5 kg/ha. When compared at 50% FC soil moisture level, Transpiration Rate was maximum (2.7 mmol H_2O m^{-2} s^{-1}) in NCF-MSA dose 112.5 kg/ha, which was 55.2% higher than the corresponding control and 46.7% higher than 37.5 kg/ha and 56.7% higher than dose 75 kg/ha. At 75% FC soil moisture level, Transpiration Rate was maximum (3.36 mmol H_2O m^{-2} s^{-1}) in NCF-MSA dose 37.5 kg/ha, which was approximately 50% higher than the corresponding control and 15.2% and 16.4% higher than doses 75 kg/ha and 112.5 kg/ha, respectively. Again, for 100% FC, 75 kg/ha dose of NCF-MSA gave the highest Transpiration Rate of 5 mmol H_2O m^{-2} s^{-1}, which was 11.4% higher than the respective control plant and 29.2% higher that doses 37.5 kg/ha and 112.5 kg/ha. Overall, on an average, 100% FC had the highest Transpiration Rate of 4.13 mmol H_2O m^{-2} s^{-1}, followed by 75% FC at 2.72 mmol H_2O m^{-2} s^{-1} and 50% FC at 1.63 mmol H_2O m^{-2} s^{-1}. With the incorporation of nanocellulose, the highest rate of transpiration (3.8 mmol H2O m-2 s-1) was seen at dose 22.5 kg/ha, which was 21% higher than the control, and 10.5% and 7.9% higher than doses 7.5 kg/ha and 22.5 kg/ha, respectively.

4.6.4 Chlorophyll Fluorescence

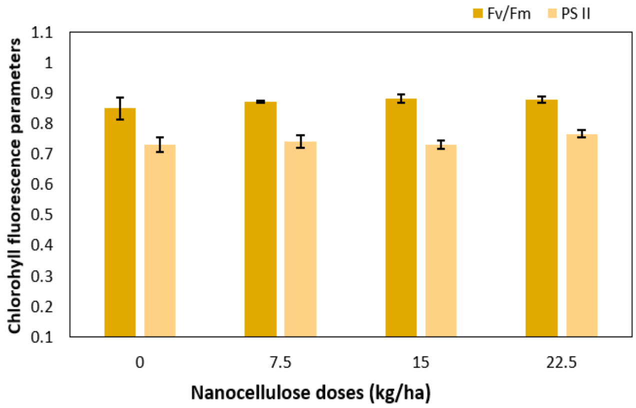
Figure 4.29

Effect of a) NCF-MSA and Soil Moisture b) Nanocellulose on Chlorophyll Fluorescence {(F_v/F_m) and φ_{PSII}}

a)



b)



Maximum Quantum Yield of PS II (F_v/F_m)

From the figure 4.29, without soil moisture control, F_v/F_m was maximum (0.887) in NCF-MSA dose 37.5 kg/ha, which was approximately 1% more than the control of this group, and 1.7% higher than 75 kg/ha dose and 0.3% higher than 112.5 kg/ha. At 50% FC soil moisture level, F_v/F_m was maximum (0.879) in NCF-MSA dose 112.5 kg/ha, which was 0.5% higher than the corresponding control and 0.7% higher than 37.5 kg/ha and 0.1% higher than dose 75 kg/ha. At 75% FC soil moisture level, F_v/F_m was maximum (0.881) in NCF-MSA dose 112.5 kg/ha, which was 1.7% higher than the corresponding control and 2.6% and 1.2% higher than doses 37.5 kg/ha and 75 kg/ha, respectively. Again, for 100% FC, the control and 75 kg/ha dose of NCF-MSA gave the highest F_v/F_m of 0.874, which was approximately 1% higher than dose 37.5 kg/ha and 0.6% higher that dose 112.5 kg/ha. Overall, on an average, 50% FC had the highest F_v/F_m of 0.876, followed by 100% FC at 0.871 and 75% FC at 0.869.

Effective Quantum Yield of PS II (φ_{PSII})

From the figure 4.29, without soil moisture control, φ_{PSII} was maximum (0.768) in the control, which was 4.7%, 0.7% and 4.3% higher than 37.5 kg/ha, 75 kg/ha and 112.5 kg/ha doses of NCF-MSA, respectively. At 50% FC soil moisture level, φ_{PSII} was maximum (0.764) in NCF-MSA dose 75 kg/ha, which was 2.5% higher than the corresponding control and 2.2% higher than 37.5 kg/ha and 0.1% higher than dose 112.5 kg/ha. At 75% FC soil moisture level, φ_{PSII} was maximum (0.761) in NCF-MSA dose 37.5 kg/ha, which was 1.1% higher than the corresponding control and 1.6% and 2.6% higher than doses 75 kg/ha and 112.5 kg/ha, respectively. Similarly, for 100% FC, φ_{PSII} was maximum (0.789) in NCF-MSA dose 37.5 kg/ha, which was approximately 6% higher than the corresponding control and 6.6% and 4.1% higher than doses 75 kg/ha and 112.5 kg/ha, respectively. Overall, on an average, 100% FC had the highest φ_{PSII} of 0.756, followed by 50% FC at 0.755 and 75% FC at 0.751.

In case of nanocellulose, the highest value of F_v/F_m (0.88) was seen at doses 15 kg/ha and 22.5 kg/ha, which were 3.4% higher than the control, and 1.1% higher than dose 7.5 kg/ha. Similarly, the highest value of φ_{PSII} (0.77) was seen at dose 22.5 kg/ha, which was 5.2% higher than the control, and 3.9% and 5.2% higher than doses 7.5 kg/ha and 22.5 kg/ha, respectively.

4.6.5 Data Analysis Through ANOVA and Tukey HSD Test.

To investigate the significance in the differences, two way CRD ANOVA was performed for all growth parameters to check interactive effects of NCF-MSA and soil moisture and one-way CRD ANOVA was performed for nanocellulose. This was followed by Tukey HSD test to do multiple comparisons.

The individual effect of NCF-MSA and the interactive effect of NCF-MSA and soil moisture produced statistically similar results in Net Photosynthetic Rate. However, there were statistically significant differences ($P<0.05$) due to the individual effect of soil moisture. Effects of nanocellulose on Net photosynthetic Rate was statistically similar for all treatments. NCF-MSA and the interaction between NCF-MSA and soil moisture did not produce significant differences in stomatal conductance. However, soil moisture individually produced highly significant differences ($P<0.01$) in stomatal conductance. Effects of nanocellulose on Stomatal Conductance was statistically similar for all treatments. NCF-MSA and the interaction between NCF-MSA and soil moisture did not produce significant differences in Transpiration Rate. However, soil moisture individually produced highly significant differences ($P<0.01$) in Transpiration Rate. Effects of nanocellulose on Transpiration Rate did produce any significant differences statistically. Among the chlorophyll fluorescence parameters, Maximum Quantum Yield of PS II (F_v/F_m) was found to be significant ($P<0.05$) only due to the individual effect of soil moisture while Effective Quantum Yield of PS II (φ_{PSII}) was highly significant ($P<0.01$) due to the interaction between NCF-MSA and soil moisture only. Effects of nanocellulose on Chlorophyll Fluorescence parameters (Maximum Quantum Yield of PS II and Effective Quantum Yield of PS II) was statistically similar for all treatments.

Significance and means of photosynthetic parameters are given in tables 4.13, 4.14 and 4.15.

Table 4.13

Results of ANOVA Analysis with F value for the Effects of NCF-MSA and Moisture (M) for Different Photosynthetic Parameters

Photosynthetic parameters	NCF-MSA	Moisture	NCF-MSA X Moisture (M)	Nanocellulose
Net Photosynthetic Rate	2.09ns	4.41*	0.76ns	0.07ns
Stomatal Conductance	1.38ns	9.01**	1.33ns	0.26ns
Transpiration Rate	1.33ns	11.08**	1.28ns	0.24ns
Maximum quantum yield of PS II	1.36ns	3.1*	1.26ns	1.51ns
Effective quantum yield of PS II	0.4ns	0.3ns	3.03**	2.3ns

Note. *, ** and ns represent data is significant at 1% level, significant at 5% level and not significant, respectively

Table 4.14

Effect of Soil Application of NCF-MSA and Soil Moisture Regime (M) on Photosynthetic Parameters of Lukthar Tomato.

Factors	NPR (µmol CO_2 m^{-2} s^{-1})	SC (mmol H_2O m^{-2} s^{-1})	TR (mmol H_2O m^{-2} s^{-1})	F_v/F_m	Φ_{PSII}
NCF-MSA (P) (kg/ha)					
P-0	6.85 ± 3.86	0.141 ± 0.11	2.466 ± 1.74	0.872 ± 0.01	0.752 ± 0.02
P-37.5	8.7 ± 3.37	0.148 ± 0.07	2.838 ± 1.08	0.871 ± 0.02	0.757 ± 0.02
P75	9.714 ± 5.01	0.202 ± 0.14	3.309 ± 1.78	0.874 ± 0.01	0.753 ± 0.02
P-112.5	9.658 ± 3.75	0.169 ± 0.07	3.08 ± 0.90	0.878 ± 0.01	0.749 ± 0.02
Moisture (M) (% FC)					
NMC	8.712 ± 5.08 ab	0.189 ± 0.11 ab	3.216 ± 1.45 ab	0.879 ± 0.01 a	0.75 ± 0.02
50	5.334 ± 2.14 b	0.078 ± 0.05 c	1.632 ± 0.90 c	0.876 ± 0.01 a	0.755 ± 0.02
75	9.635 ± 4.34 a	0.147 ± 0.07 bc	2.717 ± 1.13 bc	0.868 ± 0.02 a	0.751 ± 0.02
100	10.692 ± 3.29 a	0.246 ± 0.09 a	4.129 ± 0.93 a	0.87 ± 0.01	0.756 ± 0.03
P x M					
P-0	6.9 ± 4.93	0.135 ± 0.12	2.362 ± 2.08	0.874 ± 0.01	0.771 ± 0.01 ab
P-0 + 50	4.637 ± 1.18	0.052 ± 0.01	1.215 ± 0.27	0.875 ± 0.00	0.745 ± 0.03 ab
P-0 + 75	5.671 ± 4.81	0.101 ± 0.10	1.853 ± 1.67	0.864 ± 0.00	0.752 ± 0.01 ab

Factors	**NPR (μmol CO_2 m^{-2} s^{-1})**	**SC (mmol H_2O m^{-2} s^{-1})**	**TR (mmol H_2O m^{-2} s^{-1})**	**F_v/F_m**	**Φ_{PSII}**
P-0 + 100	10.208 ± 2.61	0.275 ± 0.08	4.434 ± 0.90	0.874 ± 0.00	0.742 ± 0.03 ab
P-37.5	8.922 ± 2.48	0.16 ± 0.08	3.013 ± 0.88	0.887 ± 0.01	0.732 ± 0.01 b
P-37.5 + 50	5.016 ± 0.47	0.069 ± 0.05	1.444 ± 0.83	0.873 ± 0.01	0.747 ± 0.00 ab
P-37.5 + 75	11.39 ± 3.23	0.174 ± 0.03	3.356 ± 0.40	0.858 ± 0.03	0.761 ± 0.02 ab
P-37.5 + 100	9.472 ± 3.67	0.189 ± 0.07	3.539 ± 0.86	0.865 ± 0.00	0.789 ± 0.00 a
P-75	10.317 ± 6.67	0.277 ± 0.17	4.214 ± 1.73	0.872 ± 0.01	0.763 ± 0.01 ab
P-75 + 50	4.181 ± 1.73	0.05 ± 0.03	1.170 ± 0.75	0.878 ± 0.00	0.764 ± 0.02 ab
P-75 + 75	10.645 ± 1.10	0.151 ± 0.05	2.853 ± 0.77	0.87 ± 0.01	0.749 ± 0.02 ab
P-75 + 100	13.714 ± 4.18	0.332 ± 0.08	5.001 ± 0.71	0.874 ± 0.01	0.737 ± 0.01 ab
P-112.5	10.92 ± 3.83	0.184 ± 0.07	3.277 ± 0.90	0.884 ± 0.01	0.735 ± 0.02 ab
P-112.5 + 50	7.503 ± 3.28	0.142 ± 0.06	2.7 ± 0.90	0.879 ± 0.01	0.763 ± 0.01 ab
P-112.5 + 75	10.835 ± 6.10	0.164 ± 0.11	2.805 ± 1.31	0.881 ± 0.01	0.741 ± 0.02 ab
P-112.5 + 100	9.376 ± 1.73	0.186 ± 0.05	3.540 ± 0.64	0.869 ± 0.01	0.757 ± 0.02 ab

Note. NPR, Net Photosynthetic Rate; SC, Stomatal Conductance; TR, Transpiration Rate; F_v/F_m, Maximum quantum yield of PS II; Φ_{PSII}, Effective quantum yield of PS II; NMC, no moisture control; FC, field capacity; means within a column followed by the same letter are not significantly different by least significant difference test at $P < 0.05$; data are means of three replications ± standard deviations.

Table 4.15

Effect of Soil Application of Nanocellulose on Photosynthetic Parameters of Lukthar Tomato.

Nanocellulose (kg/ha)	**NPR (µmol CO_2 m^{-2} s^{-1})**	**SC (mmol H_2O m^{-2} s^{-1})**	**TR (mmol H_2O m^{-2} s^{-1})**	**F_v/F_m**	**Φ_{PSII}**
NC-0	9.68 ± 1.75	0.159 ± 0.06	2.966 ± 0.83	0.85 ± 0.04	0.732 ± 0.02
NC-7.5	9.553 ± 2.38	0.183 ± 0.07	3.356 ± 0.83	0.873 ± 0.00	0.741 ± 0.02
NC-15	10.077 ± 3.89	0.187 ± 0.09	3.47 ± 1.18	0.881 ± 0.01	0.731 ± 0.02
NC-22.5	10.727 ± 4.82	0.224 ± 0.13	3.777 ± 1.7	0.879 ± 0.01	0.766 ± 0.01

Note. NPR, Net Photosynthetic Rate; SC, Stomatal Conductance; TR, Transpiration Rate; F_v/F_m, Maximum quantum yield of PS II; Φ_{PSII}, Effective quantum yield of PS II; means within a column followed by the same letter are not significantly different by least significant difference test at $P < 0.05$; data are means of three replications ± standard deviations.

4.6.6 Discussion on Photosynthetic Parameters

Soil moisture showed significant results in all the photosynthetic parameters except φ_{PSII}. Soil moisture stress affects photosynthetic parameters negatively. With the increase in moisture stress, photosynthetic rate decreases due to stomatal or non-stomatal activities or both (Ghotbi - Ravandi et al., 2014; Kebbas et al., 2015; Varone et al., 2012; Zhang et al., 2018). Net Photosynthetic Rate, Stomatal Conductance, Transpiration Rate decreased with decrease in soil moisture. All these parameters produced noticeable differences when the soil moisture decreased from 100% FC to 50% FC. The values were lowest at the least moisture level and vice versa. (G. Liang et al., 2020) observed decrease in F_v/F_m and $\varphi_{PSII,}$ which was attributed to closure of the Photosynthesis II system thus disabling electron transfer and light energy for effective photosynthetic reactions.

NCF-MSA did not affect the photosynthetic parameters statistically. However, it was seen that Net Photosynthetic rate, Stomatal Conductance and Transpiration Rate increased at 75% FC when NCF-MSA was used. At other moisture levels, these parameters showed increase only in some doses of NCF-MSA. These parameters also increased in plants where no moisture control was done. (Haghighi & Pessarakli, 2013) found improvement in photosynthetic parameters in some doses of silicon which they mentioned was due to improvement in stomatal activities. (ZHANG et al., 2018) showed increase in chlorophyll fluorescence parameters when exogenous silicon was applied in water-stressed tomato plants. In our study, only one or two doses of NCF-MSA, depending on the moisture level, showed increase in chlorophyll fluorescence parameters. It was seen that φ_{PSII} increased with increase in NCF-MSA at 50% FC.

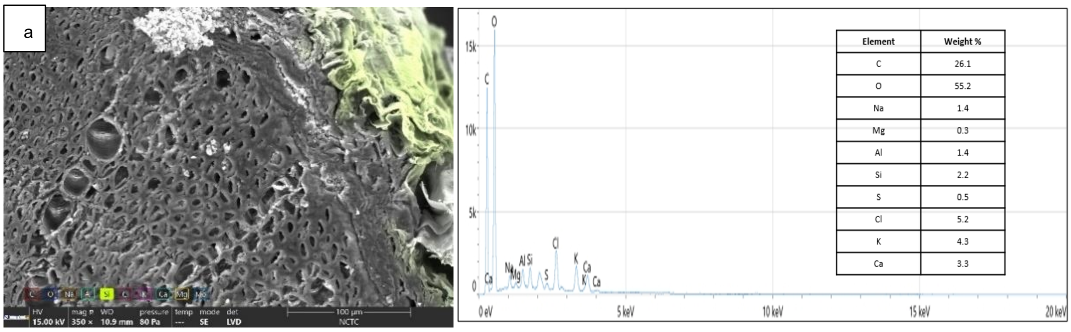
Nanocellulose did not produce significant effects on the photosynthetic parameters. Yet, there was an increase in photosynthetic rate, stomatal conductance and transpiration rate. Also, there was increase in F_v/F_m and φ_{PSII} with the application of nanocellulose.

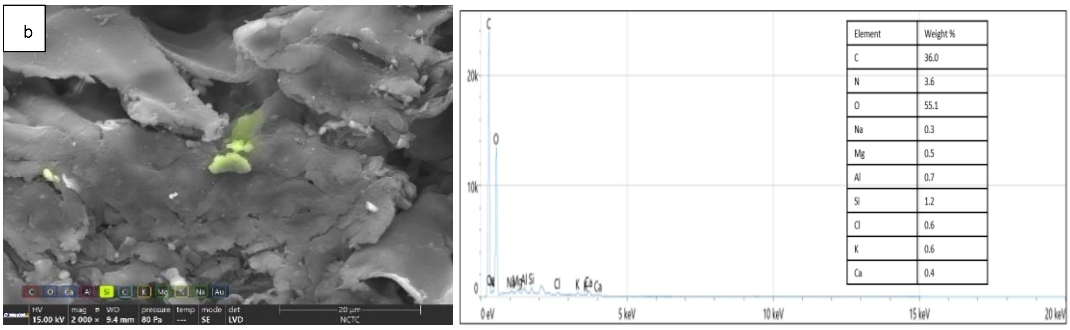
4.7 Characterization of Root Samples

In order to investigate the presence of silicon in the roots of the model plant, SEM, EDS and Raman spectroscopy was performed on root cross-sections. SEM images gives evidence on the morphology of the surface while EDS talks about the elemental composition of the surface. SEM images proves the presence of silicon in the epidermal region. EDS results gives the weight% of silicon present in a definite region with a comparison of other elements. Raman spectroscopy gives the backbone structure and molecular interactions in the sample. However, silicon was not evident in Raman shifts observed.

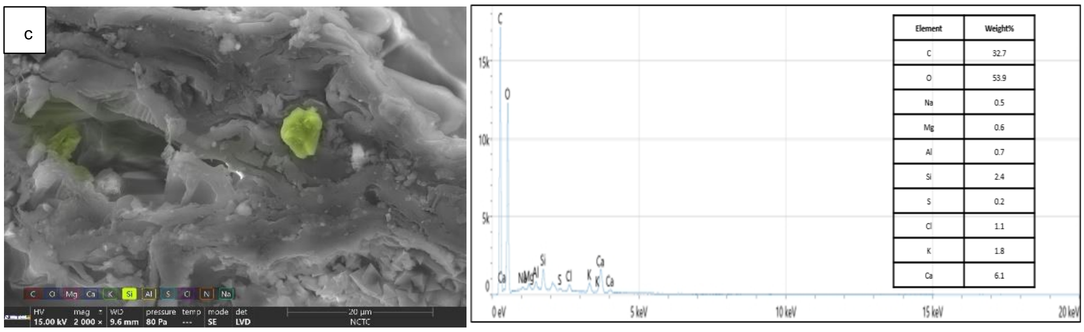
Figure 4.30

SEM Images and EDS Spectra of Root Cross-Sections with NCF-MSA and Soil Moisture Treatments a) Control b) 112.5 kg/ha at 50% FC c) 112.5 kg/ha at 75% FC d) 112.5 kg/ha at 100% FC e) 75 kg/ha at 100% FC and f) 37.5 kg/ha at 100% FC (Light Green Colour in All Images Indicate Silicon)

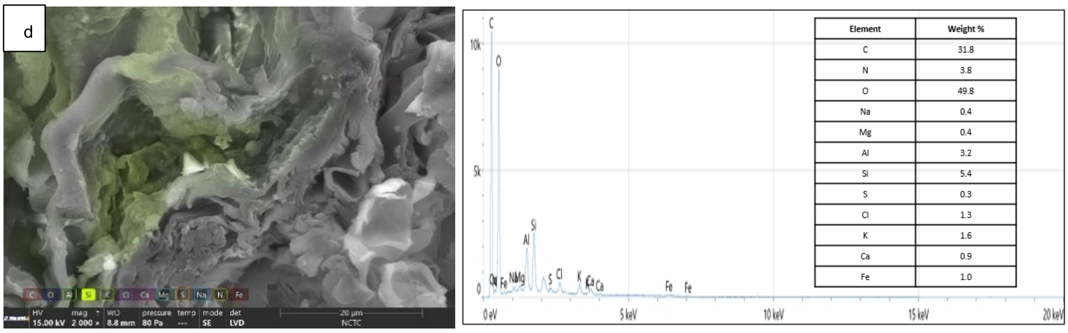




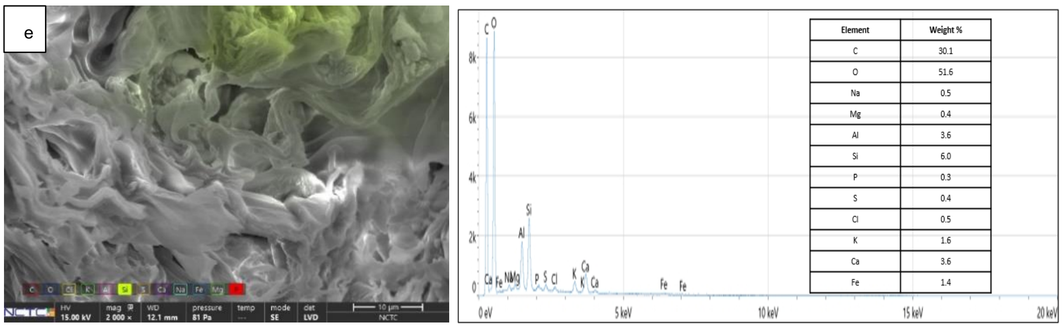
Element	Weight %
C	36.0
N	3.6
O	55.1
Na	0.3
Mg	0.5
Al	0.7
Si	1.2
Cl	0.6
K	0.6
Ca	0.4



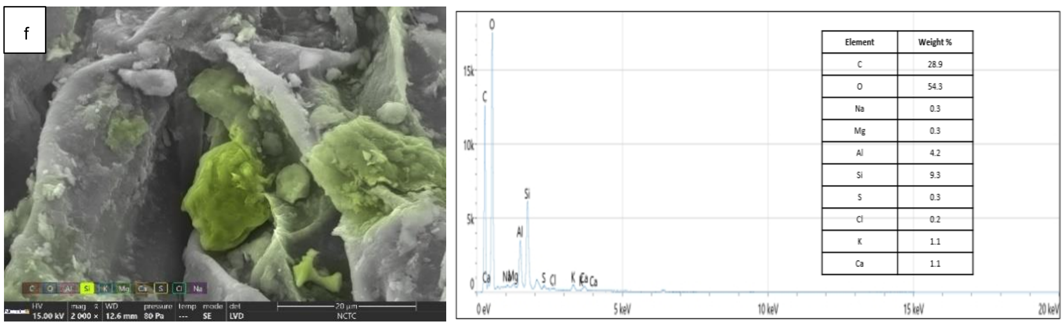
Element	Weight%
C	32.7
O	53.9
Na	0.5
Mg	0.6
Al	0.7
Si	2.4
S	0.2
Cl	1.1
K	1.8
Ca	6.1



Element	Weight %
C	31.8
N	3.8
O	49.8
Na	0.4
Mg	0.4
Al	3.2
Si	5.4
S	0.3
Cl	1.3
K	1.6
Ca	0.9
Fe	1.0



Element	Weight %
C	30.1
O	51.6
Na	0.5
Mg	0.4
Al	3.6
Si	6.0
P	0.3
S	0.4
Cl	0.5
K	1.6
Ca	3.6
Fe	1.4

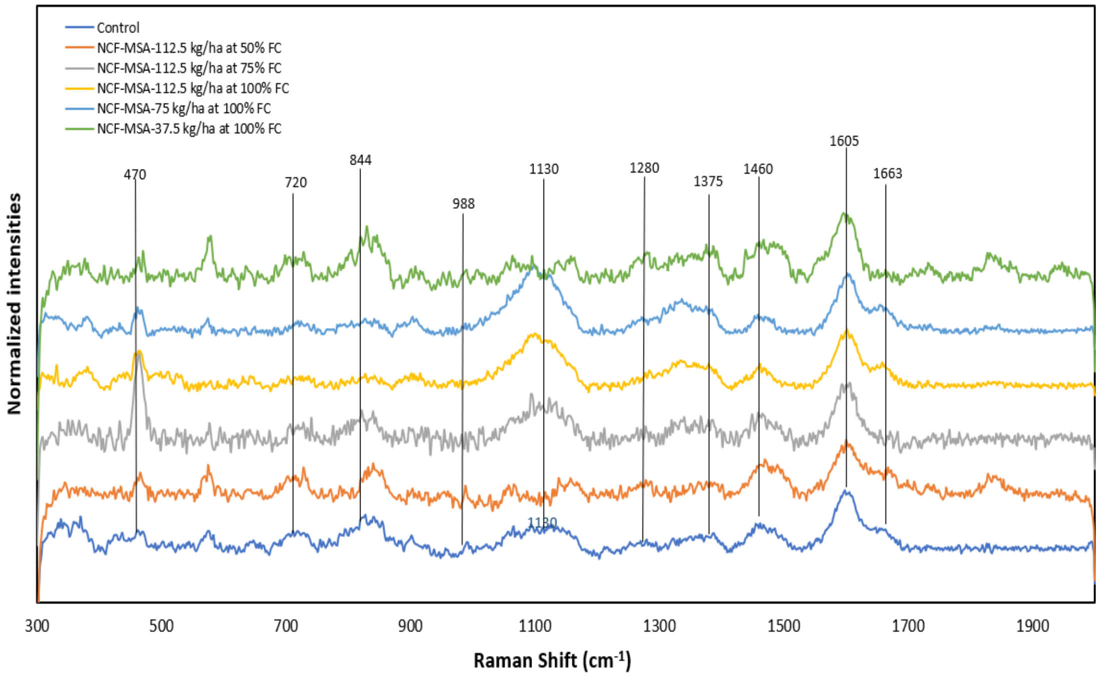


Element	Weight %
C	28.9
O	54.3
Na	0.3
Mg	0.3
Al	4.2
Si	9.3
S	0.3
Cl	0.2
K	1.1
Ca	1.1

From the figure 4.30, it is clear that silicon is present in the epidermal region of the root tissues. In the control, silicon was found on the lateral surface of the root cross section. At low moisture levels (50% FC and 75% FC), the weight% of silicon was low (1.2 and 1.4 weight%). However, at 100% FC, the silicon was 5.4 weight% at the highest dose of NCF-MSA, 6 weight% at the second dose and 9.3% in the lowest dose. Thus, it can be known that low moisture levels of silicon affect the silicon composition in root tissues. However, the reason behind higher composition of the same in low doses of NCF-MSA is still unclear.

Figure 4.31

Raman Spectra of Root Cross-Sections for Investigation of Effect of NCF-MSA and Soil Moisture in Silicon Uptake by Roots



From figure 4.31, it can be seen that the peaks that appeared around 470 represents amorphous silicon (Mueller et al., 2010). Because of the presence of amorphous silicon, the intensity of silicon peaks was very low, thus peaks were not found around 530 cm^{-1}, which is characteristic for silicon. The intensities of this peak increased with NCF-MSA at 50% FC and 75% FC but remained similar in 100% FC for all doses, when compared to control. Peaks around 720 cm^{-1} represents vibration of C-O-H of COOH in pectin (Sanchez et al., 2020). Peaks around 844 cm^{-1} represents C-O-C vibrations in cellulose and Si-O-Si bond (Mueller et al., 2010). Peaks close to 988 refers to cm^{-1} Si-

$(OH)_x$ stretching (Mueller et al., 2010). Peaks corresponding to 1130 cm^{-1} can be attributed to stretching vibrations of CC and CO in cellulose (Zeise et al., 2018). The peaks at around1280 cm^{-1} are due to aryl-O of Aryl-OH and Aryl-O-CH3; guaiacylring mode (with CO-group)) of lignin. Deformation vibrations of HCC and HCO, and that of HOC in cellulose is responsible for appearance of peaks close to 1375 cm^{-1}. Deformation vibrations of O-CH3, CH2 scissoring, guajacyl ring (with C=O group) of lignin and deformation vibrations of HCH and that of HOC in cellulose contributes to peaks at around 1460 cm^{-1}. The peaks that are seen around 1605 $cm^{-1}$are due to symmetrical stretching vibrations of Aryl-Ring in lignin. Conjugated stretching vibrations of Ring C=C of coniferylalcohol, stretching vibrations of C=O of coniferylaldehyde in lignin relates to peaks corresponding to 1663 cm^{-1}. (Zeise et al., 2018).

CHAPTER 5

CONCLUSION AND FUTURE RECOMMENDATIONS

4.8 Conclusion

The first objective of the study was preparation of monosilicic acid that is stabilized by nanocellulose through hydrogen bonding of the hydroxyl groups of both the molecules. The product was characterized with the help of characterization techniques by which the successful preparation of the product was confirmed. SEM images confirm the presence of silicon bonded with nanocellulose fibers but the percentage of silicon in the final product was lower than expected. While, peak shift at 3332 cm^{-1} in FTIR Spectroscopy indicates OH-H bonding and reduction in intensity of peaks at 1054 cm^{-1} indicate formation of Si-OH bond, which is also shown by peak appearance at 950 cm^{-1}. In Raman Spectroscopy, peak damping was observed in the range 400-700 cm^{-1}, which suggests possible complex formation. This is seconded by peak shifts around 1096 cm^{-1} and 1319 cm^{-1}. However, the product requires optimization in terms of preparation conditions and concentration of the sources.

In order to investigate the effects of the product on a low silicon accumulator plant (tomato), the second objective was application of the product in plants. Thus, it was incorporated into the tomato plant soil as three doses and three moisture levels were maintained to see if the product, soil moisture and interactive effects of both improves growth, development and productivity of plants under water deficit stress. The product did produce significant differences in some plant parameters.

In case of growth parameters, significant differences due to interactive effect of NCF-MSA and soil moisture were observed in height, number of leaves and leaf area. Length of longest root and root volume was not affected statistically by any treatment, however soil moisture had individual statistical effects on shoot and root biomass. NCF-MSA showed individual significant differences in plant height, number of leaves and leaf area. However, shoot biomass was seen to increase with use of NCF-MSA. Improvements in these parameters were however not observed with a specific dose of NCF-MSA but high moisture levels were more effective. Nanocellulose showed significant differences in height, number of leaves and root biomass. Height, number of leaves and leaf area improved with its incorporation. Thus, NCF-MSA showed promise in improving growth parameters with sufficient water present for uptake.

In yield parameters, statistical significance was observed most clearly due to individual effect of soil moisture, except number of flowers where there was improvement in this parameter due to interactive effect of NCF-MSA and soil moisture. Number of flowers increased with increase in moisture and use of NCF-MSA. Lower soil moisture deteriorated yield of the plants even with the presence of NCF-MSA. Number of fruits was highest at moderate soil moisture at 75 kg/ha of NCF-MSA. Irrigation water productivity was highest at the moderate soil moisture level in all doses of NCF-MSA. Nanocellulose did not produced any statistical differences however there was improvement in fruit yield and irrigation water productivity.

In case of fruit quality parameters, individual effect of soil moisture produced significant differences in all parameters. But only fruit length was affected statistically by the interaction between NCF-MSA and soil moisture. pH of fruits was high in the lowest moisture level in the lower doses of NCF-MSA. TSS increased at the lowest moisture level but at highest dose of NCF-MSA. Fruit length increased with increased moisture but NCF-MSA was effective only in lower moisture levels. Thus NCF-MSA have potential in improving fruit quality. On the other hand, nanocellulose improved all fruit quality parameters, although only pH and length showed statistical differences.

In physiological parameters, it was observed that NCF-MSA, soil moisture and their interaction produced highly significant differences only in LRWC at both flowering and fruiting stages. While leaf greenness and MSI were found non-significant due to interactive effect of NCF-MSA and soil moisture, soil moisture produced differences in MSI only in the flowering stage. MSI improved with increased moisture levels and some NCF-MSA doses at both stages but the difference was not distinct at the fruiting stage. However, MSI was higher in the flowering stage. LRWC was seen to improve with increase in NCF-MSA doses at the higher moisture levels. CWSI, although, did not produce any difference statistically, increased with decrease in soil moisture. Nanocellulose produced significant differences only in fruiting stage of MSI and flowering stage of LRWC. Similar to NCF-MSA and soil moisture interaction, SPAD value was higher in the fruiting stage with the use of nanocellulose. LRWC increased with the use of nanocellulose at the fruiting stage. Thus, NCF-MSA can essentially improve water uptake by plants.

In case of photosynthetic parameters, only individual effects of soil moisture exhibited significance in all parameters except φ_{PSII}. φ_{PSII} was significant only due to interaction of NCF-MSA and soil moisture. Nanocellulose improved all photosynthetic parameters except F_v/F_m and φ_{PSII}.

From the root sample characterizations with SEM and EDS it can be concluded that silicon was present in the epidermal region of the roots. Raman spectroscopy also provides evidence of the presence of silicon indicated by peak shifts around 470 cm^{-1}, 488 cm^{-1} and 988 cm^{-1}. However, it was unclear how interaction of different doses of NCF-MSA and soil moisture have a role in it.

4.9 Future Recommendations

After the successful completion of this preliminary study on the effect of nanocellulose stabilized monosilicic acid in tomato, some recommendations for future research and directions towards continuation of this study are listed below.

1. The product obtained through combination of Nanocellulose Fibres and monosilicic acid requires optimization. The product preparation was based on some basic experiments and the parameters were chosen as per literature search on similar works. Optimization of the conditions like pH, temperature, quantity and concentration, etc. needs further study to look for their effects on the stabilization process.
2. Use of cellulose nanocrystals instead of fibers can be a better option for the stabilization process because of smaller size of nanocrystals compared to nanofibers whose length is still in the micrometer range.
3. Different doses of the product can be researched based upon the present research.
4. Further studies on product application method on plants. For instance, foliar application is proven to be better method for silicon application and lower environmental temperature is recommended.

5. The product needs to be tested on monocots like rice, wheat, sugarcane as they are high accumulators of silicon. The detection and effects of the product might be more efficient on these plants.
6. Deeper research on the anatomy of plants parts is recommended for investigation of the presence of silicon and nanocellulose in cell tissues of shoots and roots.
7. Studies on the uptake mechanism of the product through roots to shoots are recommended.

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APPENDIX

ANOVA TABLES OF ALL PLANT PARAMETERS

This section consists of the tables obtained after performing ANOVA on the growth, yield, fruit quality, physiological and photosynthetic parameters of tomato.

Table A1 Completely Randomized ANOVA for Effectiveness of Soil Moisture and NCF-MSA on Plant Height

Source	DF	SS	MS	F	P
Treatment	3	463.9	154.64	7.54	0.0006
Moisture	3	9848.1	3282.69	160.09	0
Treatment*Moisture	9	489.4	54.38	2.65	0.0203
Error	32	656.2	20.51		
Total	47	11457.6			

Table A2 Completely Randomized ANOVA for Effectiveness of Nanocellulose on Plant Height

Source	DF	SS	MS	F	P
Treatment	3	297.896	99.2986	4.65	0.0365
Error	8	170.833	21.3542		
Total	11	468.729			

Table A3 Completely Randomized ANOVA for Effectiveness of Soil Moisture and NCF-MSA on Number of Leaves

Source	DF	SS	MS	F	P
Treatment	3	73.17	24.389	3.9	0.0175
Moisture	3	1675.5	558.5	89.36	0
Treatment*Moisture	9	225	25	4	0.0017
Error	32	200	6.25		
Total	47	2173.67			

Table A4 Completely Randomized ANOVA for Effectiveness of Nanocellulose on Number of Leaves

Source	DF	SS	MS	F	P
Treatment	3	154.25	51.4167	10.82	0.0034
Error	8	38	4.75		
Total	11	192.25			

Table A5 Completely Randomized ANOVA for Effectiveness of Soil Moisture and NCF-MSA on Leaf Area

Source	DF	SS	MS	F	P
Treatment	3	19232	6410.7	5.06	0.0056
Moisture	3	252211	84070.4	66.3	0
Treatment*Moisture	9	35017	3890.8	3.07	0.0091
Error	32	40576	1268		
Total	47	347037			

Table A6 Completely Randomized ANOVA for Effectiveness of Nanocellulose on Leaf Area

Source	DF	SS	MS	F	P
Treatment	3	5099.03	1699.68	3.65	0.0635
Error	8	3724.17	465.52		
Total	11	8823.2			

Table A7 Completely Randomized ANOVA for Effectiveness of Soil Moisture and NCF-MSA on Shoot Biomass

Source	DF	SS	MS	F	P
Treatment	3	141.6	47.19	0.85	0.4775
Moisture	3	15295.4	5098.45	91.71	0
Treatment*Moisture	9	321.7	35.75	0.64	0.752
Error	32	1779	55.59		
Total	47	17537.6			

Table A8 Completely Randomized ANOVA for Effectiveness of Nanocellulose on Shoot Biomass

Source	DF	SS	MS	F	P
Treatment	3	81.514	27.1714	1.51	0.2856
Error	8	144.398	18.0498		
Total	11	225.912			

Table A9 Completely Randomized ANOVA for Effectiveness of Soil Moisture and NCF-MSA on Root Biomass

Source	DF	SS	MS	F	P
Treatment	3	15.702	5.2339	0.88	0.4603
Moisture	3	59.057	19.6858	3.32	0.032
Treatment*Moisture	9	95.461	10.6068	1.79	0.1092
Error	32	189.678	5.9274		
Total	47	359.898			

Table A10 Completely Randomized ANOVA for Effectiveness of Nanocellulose on Root Biomass

Source	DF	SS	MS	F	P
Treatment	3	16.092	5.364	4.62	0.0371
Error	8	9.2934	1.16168		
Total	11	25.3854			

Table A11 Completely Randomized ANOVA for Effectiveness of Soil Moisture and NCF-MSA on Length of Longest Root

Source	DF	SS	MS	F	P
Treatment	3	986.3	328.755	1.33	0.2818
Moisture	3	259.2	86.394	0.35	0.7897
Treatment*Moisture	9	2396.2	266.246	1.08	0.4056
Error	32	7909.8	247.182		
Total	47	11551.5			

Table A12 Completely Randomized ANOVA for Effectiveness of Nanocellulose on Length of Longest Root

Source	DF	SS	MS	F	P
Treatment	3	408	136	0.98	0.4478
Error	8	1106.67	138.333		
Total	11	1514.67			

Table A13 Completely Randomized ANOVA for Effectiveness of Soil Moisture and NCF-MSA on Root Volume

Source	DF	SS	MS	F	P
Treatment	3	273.06	91.0191	1.25	0.3084
Moisture	3	103.56	34.5191	0.47	0.7028
Treatment*Moisture	9	522.67	58.0747	0.8	0.6217
Error	32	2332.17	72.8802		
Total	47	3231.45			

Table A14 Completely Randomized ANOVA for Effectiveness of Nanocellulose on Root Volume

Source	DF	SS	MS	F	P
Treatment	3	278	92.6667	0.94	0.4664
Error	8	790.67	98.8333		
Total	11	1068.67			

Table A15 Completely Randomized ANOVA for Effectiveness of Soil Moisture and NCF-MSA on Number of Flowers

Source	DF	SS	MS	F	P
Treatment	3	390.8	130.3	0.89	0.4582
Moisture	3	30131.8	10043.9	68.41	0
Treatment*Moisture	9	2923.4	324.8	2.21	0.0477
Error	32	4698	146.8		
Total	47	38143.9			

Table A16 Completely Randomized ANOVA for Effectiveness of Nanocellulose on Number of Flowers

Source	DF	SS	MS	F	P
Treatment	3	562.25	187.417	1.77	0.2303
Error	8	846.67	105.833		
Total	11	1408.92			

Table A17 Completely Randomized ANOVA for Effectiveness of Soil Moisture and NCF-MSA on Fruit Yield

Source	DF	SS	MS	F	P
Treatment	3	21142	7047	1.09	0.3676
Moisture	3	1349520	449840	69.56	0
Treatment*Moisture	9	99723	11080	1.71	0.1266
Error	32	206951	6467		
Total	47	1677335			

Table A18 Completely Randomized ANOVA for Effectiveness of Nanocellulose on Fruit Yield

Source	DF	SS	MS	F	P
Treatment	3	18677.8	6225.94	0.97	0.4544
Error	8	51524.6	6440.58		
Total	11	70202.5			

Table A19 Completely Randomized ANOVA for Effectiveness of Soil Moisture and NCF-MSA on Number of Fruits

Source	DF	SS	MS	F	P
Treatment	3	97.4	32.465	0.5	0.6832
Moisture	3	415.23	138.41	2.14	0.1143
Treatment*Moisture	9	454.02	50.447	0.78	0.6352
Error	32	2067.33	64.604		
Total	47	3033.98			

Table A20 Completely Randomized ANOVA for Effectiveness of Nanocellulose on Number of Fruits

Source	DF	SS	MS	F	P
Treatment	3	162	54	1.83	0.2206
Error	8	236.667	29.5833		
Total	11	398.667			

Table A21 Completely Randomized ANOVA for Effectiveness of Soil Moisture and NCF-MSA on Irrigation Water Productivity

Source	DF	SS	MS	F	P
Treatment	3	9.267	3.0891	0.75	0.5309
Moisture	3	97.811	32.6037	7.91	0.0004
Treatment*Moisture	9	17.313	1.9237	0.47	0.8861
Error	32	131.955	4.1236		
Total	47	256.347			

Table A22 Completely Randomized ANOVA for Effectiveness of Nanocellulose on Irrigation Water Productivity

Source	DF	SS	MS	F	P
Treatment	3	5.3933	1.79778	0.81	0.5239
Error	8	17.794	2.22424		
Total	11	23.1873			

Table A23 Completely Randomized ANOVA for Effectiveness of Soil Moisture and NCF-MSA on Fruit pH

Source	DF	SS	MS	F	P
Treatment	3	0.03228	0.01076	0.66	0.5798
Moisture	3	0.19849	0.06616	4.09	0.0145
Treatment*Moisture	9	0.26497	0.02944	1.82	0.1031
Error	32	0.51796	0.01619		
Total	47	1.01371			

Table A24 Completely Randomized ANOVA for Effectiveness of Nanocellulose on Fruit pH

Source	DF	SS	MS	F	P
Treatment	3	0.18401	0.06134	41.09	0
Error	8	0.01194	0.00149		
Total	11	0.19595			

Table A25 Completely Randomized ANOVA for Effectiveness of Soil Moisture and NCF-MSA on Total Soluble Solids

Source	DF	SS	MS	F	P
Treatment	3	5.0107	1.67022	4.38	0.0108
Moisture	3	3.6294	1.20979	3.17	0.0375
Treatment*Moisture	9	4.1129	0.45699	1.2	0.3302
Error	32	12.2096	0.38155		
Total	47	24.9626			

Table A26 Completely Randomized ANOVA for Effectiveness of Nanocellulose on Total Soluble Solids

Source	DF	SS	MS	F	P
Treatment	3	0.44102	0.14701	0.23	0.8717
Error	8	5.07037	0.6338		
Total	11	5.51139			

Table A27 Completely Randomized ANOVA for Effectiveness of Soil Moisture and NCF-MSA on Fruit Length

Source	DF	SS	MS	F	P
Treatment	3	19.41	6.47	0.47	0.7042
Moisture	3	2276.12	758.706	55.31	0
Treatment*Moisture	9	371.88	41.32	3.01	0.0102
Error	32	438.95	13.717		
Total	47	3106.36			

Table A28 Completely Randomized ANOVA for Effectiveness of Nanocellulose on Fruit Length

Source	DF	SS	MS	F	P
Treatment	3	139.331	46.4438	7.84	0.0091
Error	8	47.372	5.9216		
Total	11	186.704			

Table A29 Completely Randomized ANOVA for Effectiveness of Soil Moisture and NCF-MSA on Leaf Greenness (Flowering)

Source	DF	SS	MS	F	P
Treatment	3	20.052	6.6839	0.58	0.6302
Moisture	3	32.632	10.8772	0.95	0.4284
Treatment*Moisture	9	160.683	17.8537	1.56	0.1704
Error	32	366.6	11.4563		
Total	47	579.967			

Table A30 Completely Randomized ANOVA for Effectiveness of Soil Moisture and NCF-MSA on Leaf Greenness (Fruiting)

Source	DF	SS	MS	F	P
Treatment	3	19.65	6.5497	0.17	0.9161
Moisture	3	99.07	33.0236	0.86	0.4742
Treatment*Moisture	9	399.52	44.391	1.15	0.3589
Error	32	1235.6	38.6125		
Total	47	1753.84			

Table A31 Completely Randomized ANOVA for Effectiveness of Nanocellulose on Leaf Greenness (Flowering)

Source	DF	SS	MS	F	P
Treatment	3	23.833	7.9444	0.64	0.6112
Error	8	99.553	12.4442		
Total	11	123.387			

Table A32 Completely Randomized ANOVA for Effectiveness of Nanocellulose on Leaf Greenness (Fruiting)

Source	DF	SS	MS	F	P
Treatment	3	20.3267	6.77556	0.85	0.5055
Error	8	63.9333	7.99167		
Total	11	84.26			

Table A33 Completely Randomized ANOVA for Effectiveness of Soil Moisture and NCF-MSA on Membrane Stability Index (Flowering)

Source	DF	SS	MS	F	P
Treatment	3	6	2	0.35	0.7918
Moisture	3	54.833	18.2778	3.17	0.0376
Treatment*Moisture	9	84.167	9.3519	1.62	0.1513
Error	32	184.667	5.7708		
Total	47	329.667			

Table A34 Completely Randomized ANOVA for Effectiveness of Soil Moisture and NCF-MSA on Membrane Stability Index (Fruiting)

Source	DF	SS	MS	F	P
Treatment	3	39.22	13.0726	0.47	0.7073
Moisture	3	124.51	41.5046	1.48	0.2378
Treatment*Moisture	9	79.83	8.8705	0.32	0.9634
Error	32	895.59	27.9873		
Total	47	1139.16			

Table A35 Completely Randomized ANOVA for Effectiveness of Nanocellulose on Membrane Stability Index (Flowering)

Source	DF	SS	MS	F	P
Treatment	3	37.6667	12.5556	2.32	0.152
Error	8	43.3333	5.4167		
Total	11	81			

Table A36 Completely Randomized ANOVA for Effectiveness of Nanocellulose on Membrane Stability Index (Fruiting)

Source	DF	SS	MS	F	P
Treatment	3	61.4629	20.4876	4.47	0.0402
Error	8	36.6836	4.5854		
Total	11	98.1465			

Table A37 Completely Randomized ANOVA for Effectiveness of Soil Moisture and NCF-MSA on Leaf Relative Water Content (Flowering)

Source	DF	SS	MS	F	P
Treatment	3	326.92	108.972	6.4	0.0016
Moisture	3	1350.42	450.141	26.42	0
Treatment*Moisture	9	830.28	92.254	5.42	0.0002
Error	32	545.15	17.036		
Total	47	3052.78			

Table A38 Completely Randomized ANOVA for Effectiveness of Soil Moisture and NCF-MSA on Leaf Relative Water Content (Fruiting)

Source	DF	SS	MS	F	P
Treatment	3	399.91	133.305	7.74	0.0005
Moisture	3	976.24	325.415	18.9	0
Treatment*Moisture	9	1130.45	125.605	7.29	0
Error	32	551.01	17.219		
Total	47	3057.61			

Table A39 Completely Randomized ANOVA for Effectiveness of Nanocellulose on Leaf Relative Water Content (Flowering)

Source	DF	SS	MS	F	P
Treatment	3	517.937	172.646	18.41	0.0006
Error	8	75.02	9.378		
Total	11	592.957			

Table A40 Completely Randomized ANOVA for Effectiveness of Nanocellulose on Leaf Relative Water Content (Fruiting)

Source	DF	SS	MS	F	P
Treatment	3	148.531	49.5105	3.27	0.0802
Error	8	121.213	15.1517		
Total	11	269.745			

Table A41 Completely Randomized ANOVA for Effectiveness of Soil Moisture and NCF-MSA on Crop Water Stress Index

Source	DF	SS	MS	F	P
Treatment	3	0.07996	0.02665	0.92	0.4438
Moisture	3	0.05076	0.01692	0.58	0.6311
Treatment*Moisture	9	0.22508	0.02501	0.86	0.5688
Error	32	0.9302	0.02907		
Total	47	1.28599			

Table A42 Completely Randomized ANOVA for Effectiveness of Nanocellulose on Crop Water Stress Index

Source	DF	SS	MS	F	P
Treatment	3	0.01057	3.52E-03	1.09	0.4086
Error	8	0.02593	3.24E-03		
Total	11	0.0365			

Table A43 Completely Randomized ANOVA for Effectiveness of Soil Moisture and NCF-MSA on Net Photosynthetic Rate

Source	DF	SS	MS	F	P
Treatment	3	64.244	21.4148	1.57	0.2148
Moisture	3	197.858	65.9526	4.85	0.0068
Treatment*Moisture	9	85.852	9.5391	0.70	0.7029

Table A44 Completely Randomized ANOVA for Effectiveness of Nanocellulose on Net Photosynthetic Rate

Source	DF	SS	MS	F	P
Treatment	3	2.5065	0.8355	0.07	0.9739
Error	8	94.2625	11.7828		
Total	11	96.769			

Table A45 Completely Randomized ANOVA for Effectiveness of Soil Moisture and NCF-MSA on Stomatal Conductance

Source	DF	SS	MS	F	P
Treatment	3	0.02744	0.00915	1.38	0.2658
Moisture	3	0.17878	0.05959	9.01	0.0002
Treatment*Moisture	9	0.07896	0.00877	1.33	0.2626
Error	32	0.21169	0.00662		
Total	47	0.49687			

Table A46 Completely Randomized ANOVA for Effectiveness of Nanocellulose on Stomatal Conductance

Source	DF	SS	MS	F	P
Treatment	3	0.00658	2.19E-03	0.26	0.8534
Error	8	0.0679	8.49E-03		
Total	11	0.07448			

Table A47 Completely Randomized ANOVA for Effectiveness of Soil Moisture and NCF-MSA on Transpiration Rate

Source	DF	SS	MS	F	P
Treatment	3	4.6811	1.5604	1.33	0.2817
Moisture	3	38.9824	12.9941	11.08	0
Treatment*Moisture	9	13.5031	1.5003	1.28	0.2857
Error	32	37.5249	1.1727		
Total	47	94.6914			

Table A48 Completely Randomized ANOVA for Effectiveness of Nanocellulose on Transpiration Rate

Source	DF	SS	MS	F	P
Treatment	3	1.0111	0.33702	0.24	0.8673
Error	8	11.3165	1.41456		
Total	11	12.3275			

Table A49 Completely Randomized ANOVA for Effectiveness of Soil Moisture and NCF-MSA on Maximum Quantum Yield of PS II

Source	DF	SS	MS	F	P
Treatment	3	4.19E-04	1.40E-04	1.36	0.2713
Moisture	3	9.54E-04	3.18E-04	3.1	0.0403
Treatment*Moisture	9	1.16E-03	1.29E-04	1.26	0.2944
Error	32	3.28E-03	1.02E-04		
Total	47	5.81E-03			

Table A50 Completely Randomized ANOVA for Effectiveness of Nanocellulose on Maximum Quantum Yield of PS II

Source	DF	SS	MS	F	P
Treatment	3	1.79E-03	5.95E-04	1.51	0.2848
Error	8	3.16E-03	3.94E-04		
Total	11	4.94E-03			

Table A51 Completely Randomized ANOVA for Effectiveness of Soil Moisture and NCF-MSA on Effective Quantum Yield of PS II

Source	DF	SS	MS	F	P
Treatment	3	0.00042	1.41E-04	0.4	0.7505
Moisture	3	0.00032	1.05E-04	0.3	0.823
Treatment*Moisture	9	0.00946	1.05E-03	3.03	0.0099
Error	32	0.0111	3.47E-04		
Total	47	0.0213			

Table A52 Completely Randomized ANOVA for Effectiveness of Nanocellulose on Effective Quantum Yield of PS II

Source	DF	SS	MS	F	P
Treatment	3	2.39E-03	7.98E-04	2.3	0.154
Error	8	2.78E-03	3.47E-04		
Total	11	5.17E-03			

VITA

The student completed her schooling in Assam, India with 92.67% in the HSLC board exams. She opted for science stream during her higher secondary education and completed the same in the year 2018 with a percentage of 89% in the AHSEC board exams. The student started her Bachelor's degree program in Agriculture and received her degree in B.Sc. (Honors) in Agriculture with a CGPA of 8.99 in the year 2022 from the College of Agriculture, Assam Agricultural University, India. Presently she is pursuing her Master's degree in Bio-Nano Material Science and Engineering in the department of Industrial Systems Engineering, School of Engineering and Technology, Asian Institute of Technology. She obtained a CGPA of 3.79 at the end of the first two semesters. This research work is performed as a requirement for the completion of her M.Sc. in Bio-Nano Material science and Engineering. The student has further research interests in nanotechnology related applications in agriculture for higher education. Her specific research areas are plant stress management with silicon and other elements, production and investigation of nano-based agrochemicals and agro-fertilizers for plants.