

Effect of different concentrations of Phytostim® biostimulant application on growth, yield, and postharvest attributes of cherry tomato

By

Phuti Lucius Mokwena

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Faculty of Science and Agriculture

University of Limpopo

South Africa

Supervisor

Dr A.R Ndhkala

Co-supervisor

Dr S. Mpai

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Dedication

I dedicate this work to my family, church members, friends and GBRCE team.

Declaration

I, Phuti Lucius Mokwena declare that this dissertation submitted for the degree of Master of Science (Agricultural management), department of plant production, University of Limpopo has not been submitted previously by me or anybody for a degree at this or any other University. Also, this is my work in design and execution, and related materials contained herein had been duly acknowledged.

Candidate: Lucius Mokwena

Signature

Date

Supervisor: Dr A.R. Ndhala

Signature

Date

Co-Supervisors: Dr S. Mpai

Signature

Date

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Glossary

%= percentage

U.S= United States

°C= Degrees Celsius

GBRCE= Green Biotechnologies Research Centre of Excellence

FAO= Food and Agricultural Organization

LATS= Limpopo Agro-Food Technology Station

DAFF= Department of Agriculture, Fishery and Forestry

EU= European Union market

pH= Potential of Hydrogen

FAOSTAT= Food and Agriculture Organization Statistics

MLE= Moringa Oleifera Extracts

TSS= Total Soluble Solids

CIELAB= Commission Internationale de l'Eclairage

ANOVA= Analysis of Variance

SAS= Statistical Analysis of Software

DMRT=Duncan's Multiple Range Test

Se= Standard error

WL= Weight Loss

ICPE= Inductively Coupled Plasma Optical Emission Spectrometry

Ca= Calcium

K= Potassium

P= Phosphorus

Mg= Magnesium

Na= Sodium

N= Newtons

H₂SO₄= Sulphuric Acid

HNO₃= Nitric Acid

MT= Million Tons

AOAC= Association of Official Analytical Chemist

NRF= National Research Funding

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ABSTRACT

Tomatoes can be regarded as important economic crops worldwide. They can enhance food security and alleviate poverty due to its numerous health benefits possess. The production of tomatoes in South Africa is finite, it has been mainly sustained through the usage of hydroponics, plastic channels, fertilizers and biostimulants. However, the usage of moringa based biostimulants on tomatoes remains underexplored. There is no scientific literature on the effects of different concentrations of Phytostim® biostimulant on growth, yield, and postharvest attributes. Phytostim® biostimulant is an agro agricultural input that is organic and safe technology. It is a moringa-based biostimulant constituted from 22 amino acids. Phytostim® is a newly commercially available product that can enhance crop vigour and nutrient use efficiency. Therefore, the aim of this research was to investigate scientific information on the effects of different concentrations of Phytostim® biostimulant on growth, yield, and post-harvest attributes of tomatoes '*Roma*'. The specific objectives of the study were to: (1) To evaluate the effect Phytostim® biostimulant different concentrations on '*Roma*' tomato cultivar growth and yield. (2) to assess whether the application of Phytostim® biostimulant at different concentrations affect postharvest attributes in tomatoes cultivar '*Roma*'. Therefore, to achieve the above objectives the following procedures were taken:

To achieve objective 1: four-week-old seedlings of tomato were transplanted in the microplot in an open field for a period of 12 weeks. The experiment was laid out in completely block randomised design consisting of eight treatments which were different concentrations of Phytostim® biostimulant (0, 0.1, 0.5, 1, 2, 2.5, 3 and 4%, while 0% was control) replicated three times. Irrigation with 250 mL of tap water was performed whenever there was a status of 'dryness. Data was subjected to one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) was used for separating means using the Statistix 10.0 software package. Different concentrations of Phytostim® biostimulant were foliar applied in an interval of 14 days during the entire growing period. The growth attributes which included plant height, leaf length, and stem diameter were taken on a weekly basis. At harvest, yield components including the number of fruits per plant, diameter, size, and fresh weight of the fruits, were recorded. The effect of different concentrations of Phytostim® biostimulant significantly influenced ($p < 0.05$) the growth and yield attributes of tomato.

The study revealed that on the growth attributes Phytostim® biostimulant increased the stem diameter by 12 mm, plant height was increased by 95 cm, leaf length by 3.5 cm at the concentration of 0.1 %. On the yield attributes, Phytostim® biostimulant increased size of the fruits by 60 mm, diameter of the fruits by 56 mm, number of fruits per plant by 26 and fresh weight of the fruits by 51 g at the concentration of 0.1%.

To achieve objective 2: fresh tomato fruits were harvested at pink stage and each treatment combination consisted of twenty-six fruits from green biotechnology of research centre and excellent (GBRCE). The treatments consisted of 8 pre harvest different concentrations of Phytostim® biostimulant (0, 0.1, 0.5, 1, 2, 2.5, 3 and 4%, while 0% was control) laid in complete randomised design (CRD). Samples of six tomato fruits per treatment (Phytostim® biostimulant concentrations) were stored for 0, 5, 10, and 15 days) in a retailer box at ambient condition. The fruits were stored for 0,5,10 and 15 days for the assessment of percentage weight loss, firmness, pH, colour and total soluble solids (TSS). The parameters were assessed at an interval of 5 days and oven dried at 40 ° C for 48 hours for analysis of nutritional composition which were protein and mineral elements. The results revealed that the effects of different concentrations of Phytostim® biostimulant significantly influenced ($p < 0.05$) postharvest attributes and nutritional composition. Percentage weight loss gradually increased as days of storage increased. Percentage weight loss in 0.1 % concentration was 3.38 % at day 0 and 14.00 % at day 15. Firmness had a decline throughout the storage period. Firmness in 0.1% concentration was 5.5 N at day 0 and 1.6 N at day 15. TSS increased as days increased, whereby in 0.1 % it was 4.2 % at day 0 and 7.7 % at day 15. pH values were acidic for all the treatments throughout the storage, whereby in 0.1 % concentration it was 4.0 at day 0 and 4.53 at day 15. At the end of storage period, the fruits maintained a good colour quality, in 0.1 % concentration it was ($h^{\circ} = 24.58$ and $a^* = 18.46$). The results obtained revealed that percentage weight loss, firmness, TSS, pH and colour of tomatoes cultivar '*Roma*' were significantly ($p < 0.05$) influenced by different concentrations of Phytostim® biostimulant. However, the results showed that all the postharvest attributes were enhanced at 0.1 % concentration Phytostim® biostimulant compared to control and other Phytostim® concentration. The concentration of 0.1% can extend shelf-life of tomato fruits with 15 days. Furthermore, the study showed that different concentrations of Phytostim® biostimulant significantly ($p < 0.05$) influenced the nutritional composition

including protein and minerals (Mg, Ca, Fe, K, Zn, Cu, P and Na). The nutritional compositions were enhanced at 0.1% concentrations of Phytostim® biostimulant as compared to the control and other concentrations of Phytostim® biostimulant. In this regard it can be concluded that the concentration of 0.1% can be effectively used by farmers as an eco-friendly and cost-effective method to prolong shelf life of tomato and enhance its nutritional composition. Therefore Phytostim® biostimulant can be adopted by small- and large-scale farmers as a safe method to prolong shelf life and produce quality fruits without harming the environment.

CHAPTER 1: GENERAL INTRODUCTION

1.1 Background

Tomatoes are native to the region between Peru and Ecuador, and they are probably the ancestors of those grown now. The Aztecs used it for cooking and called it "*tomatl*" or "*xtomatl*," and this is how it came to be known today after a protracted domestication history that was most likely started in Central Mexico (González *et al.*, 2011; Liang *et al.*, 2017; Li *et al.*, 2022). Along with other crops including potatoes, peppers, chillies, and eggplant, it is a significant crop that is a member of the Solanaceae family (Gebhardt, 2016; Souiri *et al.*, 2020). Tomatoes have variety of cultivars including 'Roma'. The Roma cultivars are the most popular species cultivated in Brazil and worldwide. They have desirable characteristics such as oblong, sweet taste, high pulp yield and intense red colour (Monteiro *et al.*, 2008; Rosa *et al.*, 2011) as shown in figure 1.1. According to (Costa *et al.*, 2017) and (Brasil, 2018) oblong shape fruits are determined by longitudinal diameter higher than the transversal diameter; they are attractive for consumers for fresh consumption. The fruits of "*Roma*" cultivar are drought tolerant and susceptible to disease. While their ability to withstand external factors is genetically dependant on environmental factors such as water availability, temperature, management, and post-harvest storage (Monteiro *et al.*, 2008; Beckles, 2018).

Smallholder farmers in South Africa frequently choose to grow tomatoes due to the increased need for fresh goods and the potential they offer for industrial processing (Baloyi, 2010). According to DAFF (2015) there approximately 695 producers in both commercial and emerging sector. The commercial produces 95%, while emerging sector produces only 5%. Tomatoes are consumed widely throughout the world and their consumption has recently been demonstrated to possess health benefits because of its rich content of phytonutrients (Vlaisavljevic *et al.*, 2019). Fresh-market tomatoes are a popular and versatile fruit, making significant contributions to human nutrition throughout the world for their content of sugars, acids, vitamins, minerals, lycopene, and other carotenoids (Navarro-González *et al.*, 2011)

Global food security is the main issue for humanity, reports estimated that by 2050 the human population size will be at 9.5 billion (Fao,2011). One of the major obstacles facing the agricultural industry is ensuring and maintaining crop productivity under the

threat of climate change. farmers of tomatoes in South Africa are challenged to produce high yield and good quality of tomato to meet the requirements of local demand (Bogo *et al.*, 2019). According, to (Daff, 2018) consumption of tomatoes in South Africa is estimated to be 12kg per annum as compared to 32kg in Europe. Whereas the household consumes between five to ten tomatoes per week.. Recently there is an increase in the importation and manufacturing of biostimulants particularly plant biostimulants (Magnabosco *et al.*, 2023). Therefore, plant biostimulant in agriculture refers to substances that are from plant origin to enhance soil fertility, tolerance to abiotic stress, yield and deliver quality crops (Drobek *et al.*, 2019; Chrysargyris *et al.*, 2020; Rouphael *et al.*, 2020). The application of the amino acid and protein hydrolysed biostimulants has gained momentum in vegetable production (Mpai *et al.*, 2022). There are varieties of plant biostimulant biologically extracted from plant extract such as Phytostim®. It is a moringa based biostimulant constituted of 22 amino acids and extracted from the leaves through enzymatic hydrolysis (Mpai *et al.*, 2022). Phytostim® contains several bioactive compounds, that improve various physiological processes.. It is a newly commercially available product (Figure 1.2) that can enhance plant yield, crop vigour, nutrient use efficiency and can act as elicitors for mitigation of adaptation mechanisms during abiotic stress (Mpai *et al.*, 2022). Phytostim® can be a safe and cost-effective method to enhance the productivity of fruits and vegetables.



Figure 1.1: Tomato “Roma” cultivar (Source www.Malhi farm)



Figure 1.2: Phytostim® biostimulant (Source: Moringa products South Africa)

1.2 Problem statement

Production of tomatoes in South Africa is finite, mostly through protected environments, such as hydroponics and plastic tunnels which cause ailments such as waterborne disease (Maatjie, 2015). However, growing tomatoes within the hydroponic system is associated with improved yield and fruit quality. On the other hand, it can enhance nutrients use efficiency, ensure productivity throughout the year, and minimized environmental effects (Maboko *et al.*, 2011). Furthermore, tomatoes are highly susceptible to perishability at postharvest due to the fact that they are climacteric fruits. An accelerated rate of respiration and a subsequent burst of ethylene production are characteristics of climatic fruit ripening (Luengwilai *et al.*, 2013; Quinet *et al.*, 2019). Studies have been conducted on the application of inorganic fertilizers to improve the growth and productivity of tomatoes (Van der Berge *et al.*, 2000; Maboko *et al.*, 2013; Mooy, *et al.*, 2019). According to (Maboko *et al.*, 2013) it has been reported that the pre-harvest application of inorganic fertilizers is currently the main input for improving yields and the quality of tomatoes. However, consumer preference for organically grown produce has been rising, mainly due to the developed awareness

regarding the accumulation of non-nutritive secondary metabolites such as nitrates and nitrites resulting from the excess application of inorganic nitrogen (N) fertilizers (Buzby *et al.*, 2014; Mampholo *et al.*, 2018). Consequently, the application of more than 120 kg N/ha was associated with post-harvest losses because of product spoilage or shortened shelf life (Watanda, 1999). Scholarly studies report the effects of biostimulants on postharvest attributes of tomatoes (Zodape *et al.*, 2011; Møller *et al.*, 2015; Caruso *et al.*, 2019; Mzibra *et al.*, 2021). Although biostimulants have been extensively employed to prolong fruit storage life and retain quality, usage of Phytostim® to prolong shelf life of tomatoes remains underexplored. Information regarding the effects of different concentrations of Phytostim® biostimulant on growth, yield and postharvest attributes is limited. Moringa based biostimulant has already been produced and it is called Phytostim®. In response to the smart and sustainable agricultural practices, going green with Phytostim® biostimulant applications to improve growth, yield and post-harvest quality is a necessity. The application of Protein hydrolyses (PHs) as biostimulants on a wide range of horticultural and agronomic crops has been acclaimed (Szajewska *et al.*, 2001). However, information related to different concentrations of Phytostim® biostimulant on pre-harvest and post-harvest attributes in tomatoes is limited (Thanana *et al.*, 2017).

1.3 Motivation of the study

Tomatoes are regarded as important economic crops worldwide (Tonhati *et al.*, 2020). According to DAFF (2017), the average household in Republic of South Africa (RSA) eats between five to ten tomato fruit per week. According to the statistical data from Food and Agriculture Organization (FAO, 2020), tomatoes are consumable fruit worldwide. Tomatoes play a fundamental role in enhancement of food security, alleviating poverty and possess more health benefits. However as climacteric fruits they are highly susceptible to perishability at postharvest. Therefore, protein hydrolysed enhance tolerance to abiotic stresses during postharvest and extend the shelf life, notably through the stimulation of protective processes such as cell antioxidant activity and osmotic adjustment (Marthe *et al.*, 2023). The use of biostimulants in vegetable production is ideal for organic production (Khan *et al.*, 2018). The Phytostim® biostimulant is a commercially available moringa based, which is ecofriendly, cost effective and affordable technology to be adopted. Phytostim® constituent of 22 amino acids as the bioactive compounds and make up to 70% of

active ingredients (Mpai *et al.*, 2022). The mechanism of action for Phytostim® biostimulant is quite unclear but however it was found that it has the potential of regulating the plant metabolism and enzymatic activities involved in the fruits ripening and senescence by detecting physiological process. Moringa based biostimulants has been associated with enhanced crop yield, plant quality and can act as elicitors for mitigation of adaptation mechanisms during abiotic stress such as salinity or water stress (Abdelgawad *et al.*, 2018; Paul *et al.*, 2019).

The application of biostimulants of plant origin has gained momentum in vegetable production in particular the amino acid and protein hydrolysed categories (Mpai *et al.*, 2022). This is mainly due to their potential to influence growth, yields, post-harvest quality, nutrition and the secondary metabolites linked with health benefits (Calvo *et al.*, 2014; Zhang and Schmidt, 2000). There are various biostimulant used in agriculture however, this study was centred on *Moringa* based biostimulant known as Phytostim®. It is produced as part of a larger Department of Science and Innovation (DSI) flagship program on commercialisation of *Moringa* in South Africa. The product is envisaged to improve tolerance to abiotic stress, improves plant yield and postharvest quality. Therefore, it is important to assess the impact of different concentrations of Phytostim® biostimulant to obtain an optimum level to attain both pre- and post-harvest benefits. The information from this research will promotes adoption and contributes to the optimization of Phytostim® biostimulant.

1.4 Purpose of the study

1.4.1 Aim

Development of scientific information on the effects of different concentrations of Phytostim® biostimulant on growth, yield, and post-harvest attributes of tomatoes.

1.4.2 Specific Objectives

- i. To assess whether the application of Phytostim® biostimulant at different concentrations affect growth and yield in tomatoes cultivar 'Roma'.
- ii. To assess whether the application of Phytostim® biostimulant at different concentrations affect postharvest attributes in tomatoes cultivar 'Roma'.

1.4 Reliability, validity, and objectivity

In this study, reliability of data is based on statistical analysis of data using Statistix 10.0 software at the probability level of 5%, validity is achieved through replicating the

treatment as well as control and repeating the experiments in time, while objectivity is achieved through by ensuring that the findings are discussed based on empirical evidence, to eliminate all forms of subjectivity (Leedy and Ormrod, 2005)

1.5 Bias

In this study, bias was minimized by ensuring that the experimental error in each experiment and trial was reduced through increased replications and randomization (Leedy and Ormrod, 2005)

1.6 Scientific significance of the study

The study intends to provide essential information on the optimum concentration of the Phytostim® biostimulant on pre-harvest and post-harvest attributes of tomatoes. Furthermore, information obtained from this research can assist small and large-scale farmers to utilize the Phytostim® biostimulant to produce higher yield on the production of tomatoes and maintain good fruit quality. This research can stand in the knowledge gap regarding the application of Phytostim® biostimulant to enhance shelf life of tomato that have been subjected to preharvest application. This study has the potential to assist the agricultural industry by demonstrating the use of an eco-friendly method to increase the shelf life of tomatoes and reduce postharvest losses as an alternative to synthetic chemicals. Phytostim® resulted in chemical-free fruits for consumers, a higher profit margin for farmers, and a longer shelf life of fruit. It is hoped that reduction of tomato fruit spoilage and storage disorder can ensure supply throughout the year and reduce food insecurity since fruit contains various important nutrients.

1.7 Structure of the dissertation

The structure of this dissertation is like research papers outline. Whereby the chapters are categorised into:

Chapter 1: Provides a detailed and precise introduction on tomato fruit. Further elaborates the problem statement, rationale and the study aim and objectives.

Chapter 2: Consists of a literature review which furnishes an overview of the published, available data on the issues relevant to this topic. These include the origin, distribution, production of tomato. The subtopics are further flushed into the nutritional, phytochemicals, economic importance, uses, species and cultivar of tomato. Production areas of tomato in South Africa. The biostimulants that have been used in

accordance with their categories. An overview on postharvest strategies that has been used to reduce the postharvest losses. Role of biostimulants and how they can be a solution in reducing the adverse effects of climate change. It reviews how they can produce more qualitative fruits within a short period of time that are consumer marketable and enhance food insecurity.

Chapter 3: Addresses one of the objectives done in this research. It further gives in details the material and methods employed to carry out the growth and yield attributes and statistical analysis. The results, discussion, recommendation and conclusion regarding the growth and yield attributes are all recorded in this chapter.

Chapter 4: Comprise of the detailed materials and methods employed to carry out the second objective of this study. The results, discussions, recommendations, and conclusion for primary metabolites are all recorded in this chapter.

Chapter 6: Consists of summary of findings, significance, recommendations, and conclusions. Each chapter was enclosed the bibliographic references and the abstract. In text reference cited in every chapter are acknowledged at the end of that chapter, following the Harvard style of author-alphabet as approved by the Senate of UL.

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CHAPTER 2: Literature review

2.1 Work done on the problem statement

2.1.1 Brief description, origin, and distribution of tomatoes

Tomato (*Solanum lycopersicum* L.) is currently the most consumed vegetable in the world largely due to its versatility in cooking, processing, and raw usage. It belongs to the family Solanaceae, which also includes several other commercially species such as tobacco, chili peppers, potatoes, and eggplant, (Geisenberg and Stewart, 1986) modified by (Kimura and Sinha, 2008). Although often referred to as *S. pomiferum*, early botanists identified a tendency of tomato cultivars with the genus *Solanum* (Kimura *et al.*, 2008; Hoenig *et al.*, 2017).

Hoenig *et al.*, (2017) when citing Anguillara (1561), described the newly introduced tomato as a plant known as *Lycopersicon*, meaning wolf-peach. According to Tournefort (1694), cultivated tomatoes fall within the distinct genus under the name *Lycopersicon*. The name *Solanum lycopersicum* was given to tomatoes by Linnaeus (1753), who also identified them to fall under the *Solanum* genus. Tomato (*Solanum lycopersicum*) has two wild ancestor species namely, *Solanum pimpinellifolium* and *Solanum cerasiforme*. Other wild tomato species have been used to breed for disease resistance, colour improvement, and other desirable quality traits (Ranc *et al.*, 2008). The two botanical variations of the cultivated tomato, *Solanum lycopersicum*, are *Solanum lycopersicum* var. *cerasiforme*, or cherry tomato, and *Solanum lycopersicum* var. *lycopersicum*.

The world produces millions of tons of tomatoes and the major producers include China, USA, India, Turkey, Egypt, Italy, Iran, Spain, Brazil, and Mexico (see Table

2.1). The food crop ranks first among that are grown widely across the world, and accounts for over 14% of the world’s fruit production (FAO, 2021). The tonnage of tomato produced in Africa exported to the rest of the world is estimated to be 11.1% (FAO, 2021). In South Africa, tomato is the second most important and economical crop after potato from the Solanaceae family and it is one of the main plants sold on both local and export markets (DAFF, 2015).

Table 2.1: Average million tons (MT) of top 10 producers of tomatoes around the world (1994-2018).

Rank	Country	Production (MT)
1	China	33 352 918.78
2	USA	13 011 761.61
3	India	10 653 317.83
4	Turkey	9 744 947.35
5	Egypt	7 410 736.17
6	Italy	6 277 585.57
7	Iran	4 477 189.13
8	Spain	4 002 324.57
9	Brazil	3 544 036.96
10	Mexico	2 880 385.09

Source: (FAOSTAT, 2018)

2.1.2 Secondary metabolites compound present in tomatoes

Tomatoes play an important role in human nutrition because of they possess enhanced nutritional value which have well-established health benefits (Salehi *et al.*, 2019). For example, the seeds of tomato reportedly promote well-being and avert various illnesses given that they contain minerals and phytochemicals. Generally, the seeds of tomato are good sources of antioxidants and often exhibit a high concentration of phytochemical components (Toor and Savage, 2005; Peng *et al.*, 2008; González *et al.*, 2011). There exist growing evidence which support the hypothesis that several of the phenolic compounds found in tomato seeds may have some beneficial impacts on human health by acting as antioxidants. According to Persia *et al.* (2003), dietary fibre (35% DW), fat (20–30%) and polyunsaturated Fatty

Acids (FAs), proteins (25–30% DW) and essential amino acids, and minerals are all found in abundance in the seeds. Epidemiological research indicates that carotenoids are crucial in preventing cardiovascular diseases such as cataracts, and cancer (González, 2011). Tomatoes are commonly known to contain fibre protein, vitamins, minerals, essential amino acids, monounsaturated fatty acids, carotenoids, and phytosterols (Elbadrawy, 2016, Abdullahi, 2016 Ramos-Bueno, 2017 and Chaudhary, 2018). Also, studies show that they contain vitamins A, B, C, and E, carbohydrates like fructose and glucose, essential nutrient elements including phosphorus, sodium, potassium, calcium, magnesium, iron, copper, and zinc, as well as dietary fibre (Ayandiji and Adeniyi, 2011; John *et al.*, 2016; Yadav *et al.*, 2017). Furthermore, Vitamins A and C have been determined in tomato and these essential in that they prevent muscle deterioration and enhance vision while some studies show that they are effective blood cleansers and treat Urinary tract Infection UTIs. Given their high fiber content, tomatoes can help with weight loss and facilitate simple digestion (Arah *et al.*, 2015). As a result, when eaten, tomatoes supply vital nutrients (Reboloso-Fuentes, 2009 and Arah *et al.*, 2015; Guil-Guerrero). The rich concentration of lycopene, β -carotene pigment, and a potent antioxidant can help lower the risk of some chronic illnesses such as prostate cancer, heart disease, and type 2 diabetes (Tanksley, 2010 and Ram *et al.*, 2014). Additionally, tomatoes are an excellent source of flavonoids and folates (Freeman *et al.*, 2011; and USAID, 2018).

2.1.3 The economic importance and uses of tomato

Due to its many benefits, tomatoes rank among food crops that are significant in the commercial sector (Naika *et al.*, 2005) and are sold as raw produce or processed into various products. When processed, raw tomatoes can produce salads, soups, and pastes. The fresh fruit can be eaten in a variety of ways, including fresh in salads and sandwiches, cooked or processed in soups, chutneys, pickles, pastes, purees, juices, dried powder (Ayandiji and Adeniyi, 2011; Chaudhary, 2014; Yadav *et al.*, 2017). Instead of consuming 27,22 kg of commercially processed vegetables annually, the average American today consumes more than 11.34 kg of processed tomatoes (Wu *et al.*, 2022). In Europe, the fruit has been used as food since 1853 especially when seasoned with oil, salt, and pepper. These 'love apples' were eaten abroad, remarked Gerard, who in 1596 had tomatoes in his garden in England Gerard (1596). Miller noted in 1752 that tomatoes were used in England to enhance soups Miller (1752).


Currently, tomatoes are the most popular vegetable in canned goods, and they are utilized in large amounts when they are still fresh (Wu *et al.*, 2022). Tomato seeds make up around 60% of the waste and about 10% of the fruit. The global tomato-processing industry is expected to create 1.7 million tonnes of seeds annually (FAOSTAT, 2008). Dried tomatoes, tomato-based dishes, tomato preserves, and fresh fruit are some examples of processed tomato products (Górecka *et al.* 2020). The fruit's seeds and peel are among the many bio-wastes produced by the tomato processing industry. In addition to being used by humans, by-products of tomato are also widely used as animal feed (Persia *et al.*, 2003; da Silva *et al.*, 2009). They serve as a source of protein for making bread, mayonnaise, margarine, and tomato paste, tomato seeds as well as an edible oil. Tomato is highly adaptable, including to marginal growth conditions and this could explain its wide adoption and cultivation across the world. Currently, it is the fruit that is largely consumed by humans and is marked for direct consumption or processing into by-products. Food that is ready to eat, like tomatoes, requires little processing (John *et al.*, 2016).

2.1.4 Species and cultivar of tomatoes

Various cultivars of tomato that are sold and readily available are distinguished by having less noticeable nutritional and sensory qualities when compared to out-of-date genotypes (Tieman *et al.*, 2017).

The 'Roma' group tomato is one of the most popular in Brazil and around the world. Consumers prefer it for its oblong shape, vivid red colour, sweet flavour, and high pulp yield. Furthermore, they appeal to even the pickiest eaters and the gourmet market (Monteiro *et al.*, 2008, Rosa *et al.*, 2011) (see Table 2.2) shows different types of cultivars and species of tomato as well as other characteristics.

Table 2.2: Different cultivars and species of tomato

Name of cultivar	Species variety	Image	Characteristics
Garden tomato	<i>Lycopersium</i>		Vines that have a weak stem that sprawls and typically needs support (Chaerani <i>et al.</i> , 2006).

Potato-leafed tomato	<i>Grandifolium</i>		They look akin to, well, potato leaves (Busch <i>et al.</i> , 2011).
Cherry tomato	<i>Cerasiforme</i>		They are type of small round tomato believed to be an intermediate genetic mixture between wild currant-type tomatoes (Pinho <i>et al.</i> , 2011).
Dwarf type	<i>Validus</i>		They are very distinctive in having a thick central stem, stout compact growth, and dark green, crinkly looking (Zabel <i>et al.</i> , 2019).
Pear tomato	<i>Pyriforme</i>		This variety produce mid sized fruits with a characteristic small pear shape, yellow skin and really sweet (Sabatini <i>et al.</i> , 2005).
Tiny wild tomato	<i>Pimpinellifolium</i>		They are like wild currant tomatoes, are extremely adaptable and can survive in some of the driest, harshest desert regions to humid (Sun <i>et al.</i> , 2010)
Orange tomato	<i>Cheesmaiae</i>		They are sweet and tangy flavor, with a pleasant acidity that adds a refreshing zing to dishes and responsible for beta-Carotene (Vaughan and Geissler, 1997).
Roma	<i>Pyriforme</i>		They are egg- or pear-shaped and red when fully ripe. They have few seeds and are a good canning and sauce tomato. While Roma is an variety, in general it is not considered an heirloom tomato (Chaerani <i>et al.</i> , 2006).

Source :(Berrueto, 2017)

2.1.5 Production of tomato in South Africa

Tomatoes are grown in South Africa for commercial and subsistence purposes by farmers who cultivate in household gardeners, tunnels, and cropping fields. So far, the crop is cultivated across all the provinces of South Africa. However, the Limpopo province contributes the lion's share of tomatoes with an area covering 3590 hectares with 2700 hectares in the Northern Lowveld and 890 hectares in the far north. For other provinces, the Border area of the Eastern Cape province boasts most hectares while the Overberg area in the Mpumalanga province has the largest area with 770 hectares. In South Africa, tomato is better adapted to cultivation during the winter season especially in places that are frost-free or under protection (DAFF, 2013). Reports show that the main producing areas are found in the Mpumalanga, KwaZulu-Natal, Western Cape, and Limpopo provinces (Tshiala and Olwoch, 2010). Most tomato growers in South Africa work in open fields, but a minority grow tomatoes in protected soilless systems (Maboko *et al*, 2009). Furthermore, DAFF (2015) reported that 75% of the tomatoes in South Africa are produced in the northern areas of Limpopo province while the remaining 25% are produced Mpumalanga province and the border area of the Eastern Cape Province.

2.2 Overview of plant biostimulants, categories and uses

2.2.1 A brief definition on biostimulant

According to du Jardin (2015), biostimulants are compounds recommended for application at low concentrations to stimulate a plant's endogenous metabolism to alter its physiological processes, rather than directly protecting it from external stressors or providing nutrients. Furthermore, du Jardin (2012), referred to biostimulants as a wide class of compounds that can be introduced to a plant's surroundings to promote not just nutrition and development but also the plant's ability to withstand biotic and abiotic stress. Other definitions of biostimulants by Zhang and Schmidt, (1997) from the Department of Crop and Soil Environmental Sciences of the Virginia Polytechnic Institute and State University defined Biostimulants as "substances that, in minute amounts, stimulate plant growth". According to Kauffman *et al.*, (2007), biostimulants are substances that, when used in small quantities, encourage plant growth instead of fertilizers. The European Biostimulant Industry Council (www.biostimulants.eu) defined "A plant's ability to absorb nutrients, use nutrients efficiently, withstand abiotic stress, and produce high-quality crops". Generally, a plant

biostimulant is any material or microbe that is administered to plants to improve crop quality attributes, abiotic stress tolerance, and/or nutrition efficiency Irrespective of the substance's nutritional composition. They contain biologically active compounds such as amino acids, nutrient elements, hormones, proteins, vitamins and other compounds applied in small quantities (Yakhin et al., 2017; Kunicki et al., 2010; Takacs-Hajos, 2022). According to Brown (2015), biostimulants are not nutrients rather, they aid in the absorption of nutrients or advantageously support growth promotion or stress resistance. According to a recently developed paradigm, plants are not isolated organisms in their surroundings, but rather are both a host and a partner to various bacterial and fungal microorganisms. Because of these associations, both inside and outside of their tissues, plants can respond and adapt to both biotic and abiotic stressors (Vandenkoornhuyse *et al.*, 2015).

2.2.2 Types of biostimulant

Biostimulants can be divided into various types according to the active ingredient including humic and fulvic acids, beneficial bacteria and fungi, protein hydrolysates, seaweed and other botanical extracts, biopolymers such as chitosan, and inorganic compounds (du Jardin 2012; Gupta *et al.*, 2021). Table 2.2.2 and figure 2.1.5 represent various types of biostimulants and their active ingredients.

2.2.2.1 Humic and fulvic acids

Humic substances (HS) occur naturally and are components made up of organic matter found in soil that are produced by the breakdown of plant, animal, and microbial wastes, as well as from the metabolic processes that these substrates utilise. Given that they comprised heterogeneous molecules, humic substances were initially divided into fulvic acids, humins, and humic acids based on their solubility and molecular weights. Additionally, these compounds exhibit complicated dynamics of association into supramolecular colloids, which are impacted by plant roots through exudate and proton release. Thus, the interaction between the organic matter, microorganisms, and plant roots leads to the formation of humic compounds and their complexes in the soil (Nardi *et al.*, 2018).

2.2.2.2 Protein hydrolysates and other N-containing compounds

There exist commercial goods that are made from plant and animal protein hydrolysates. The safety of organisms that comprise hydrolysed protein and bioassays

utilizing yeasts and plants was recently evaluated, however, no genotoxicity, ecotoxicity, or phytotoxicity was reported (Corte *et al.*, 2014). However, the use of protein hydrolysates made from animal byproducts in the food chain is raising concerns about safety. Combinations of amino acids and peptides are produced by chemically and enzymatically hydrolyzing agroindustrial byproducts derived from both plant (crop residues) and animal wastes (e.g. collagen, epithelial tissues) (du Jardin, 2012; Calvo *et al.*, 2014, Halpern *et al.*, 2015).

These substances have demonstrated ability to function as as plant growth biostimulants (Calvo *et al.*, 2014; du Jardin, 2012, Halpern *et al.*, 2015). Modulation of nitrogen (N) absorption and assimilation, through controlling structural genes and the enzymes involved in N assimilation, as well as through influencing the signalling route of N acquisition in roots, are examples of direct effects on plants. Additionally, they contribute to the interaction between the carbon (C) and nitrogen (N) metabolisms by controlling tricarboxylic acid (TCA) cycle enzymes. It has also been observed that complex proteins and tissue hydrolysates include hormonal activity (Colla *et al.*, 2014). When protein hydrolysates are administered to plants and soils, agricultural practices also have important indirect impacts on plant nutrition and growth. Protein hydrolysates have been shown to improve soil respiration, microbial biomass and activity, and overall soil fertility.

The Emerging Landscape of Products – Broad and (Potentially) Confusing

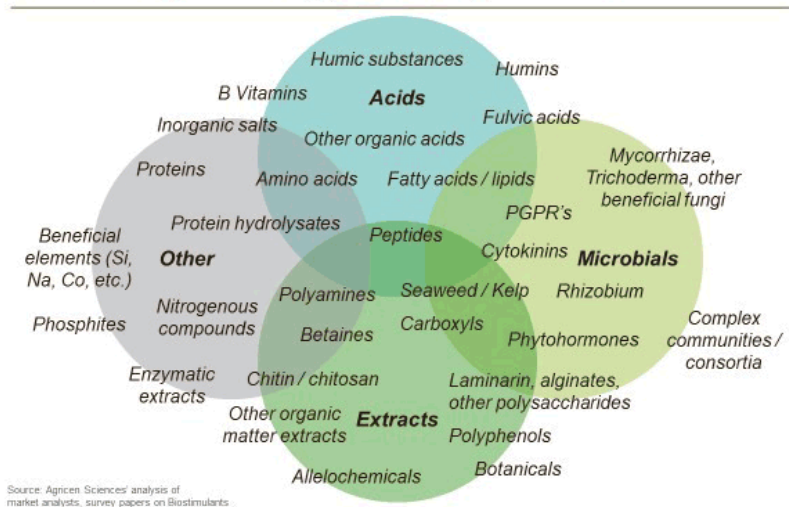


Figure 2.1: Types of biostimulants in Agriculture

Source : (Patric du Jardin *et al.*, 2015)

2.2 2.3 Effects of Protein hydrolysate on tomato

Reports by Bona *et al.*, (2018) report the effects of all biostimulants based on microorganisms. For example, the authors showed that the application of biostimulants that comprise microorganisms (arbuscular mycorrhizal fungi + *Pseudomonas* sp. Strain 19Fv1T, arbuscular mycorrhizal fungi + *Pseudomonas fluorescens* C7, arbuscular mycorrhizal fungi + *Pseudomonas* sp. 19 Fv1T and *Pseudomonas fluorescens* C7) caused an increase in tomato fruit mass, but the most effective result was demonstrated by biostimulants including arbuscular mycorrhizal fungi and *P. fluorescent* C7 caused an increase in tomato mass to 71.3 g (weight of control tomatoes = 64.4 g. Additional research by (Bona *et al.*, 2018) revealed that the microbes employed also induced tomatoes to slightly elongate. Fruit length ranged from 5.49 to 5.81 cm in the control group. However, fruit length ranged between 5.88 cm and 6.05 cm after the biostimulants were added. The fruit diameter also increased somewhat and ranged from 4.24 cm to 4.62 cm as well as between 4.64 cm and 4.78 cm, according to (Bona *et al.*, 2018). Results of an experiment conducted by (Caruso *et al.*,2019) showed that protein hydrolysate increased the number of marketable fruits of tomato by 26.0% compared to control (Table 2.3). Findings by Møller *et al.*, (2015) reported that protein hydrolysate increased lycopene content by 34.9% and 18% compared to control. Additionally, the same reports showed an increase by 27.3% in ascorbic acid content compared to control. (Caruso *et al.*, 2019) further reported an increase in mean weight of marketable fruits with 16.0% through the usage of tropical plant extract. Therefore, according to (Mzibra *et al.*,2021), the usage of Moroccan seaweed-based extracts increased the mean fruit diameter of tomato by 33.90% compared to control. (Zodape *et al.*, 2011) reported that the effects of *K. alvarezii* sap seaweed on height of tomato, increased by 34.44%, while increased root length with 45.05% compared to control.

Table 2.3: The effects of biostimulants on tomato growth, yield and postharvest quality

Biostimulants	Beneficial effects	Parameters	References
Protein Hydrolysed	Increased by 26.0 % compared to control.	Number of marketable fruits.	(Caruso <i>et al.</i> , 2019)

Protein hydrolysed	Increased by 34.9% and 18% compared to control.	Lycopene content.	(Møller <i>et al.</i> , 2015)
Protein hydrolysed	Increased by 27.3% compared to control.	Ascorbic acid content.	(Møller <i>et al.</i> , 2015)
Tropical plant extract	Increased by 16.0% compared to control.	Mean weight of marketable fruits.	(Caruso <i>et al.</i> , 2019)
Moroccan Seaweed-Based	Increased by 33.90% compared to control.	Mean fruit diameter.	(Mzibra <i>et al.</i> , 2021)
<i>K. alvarezii</i> sap Seaweed	Increased by 34.44% and 45.05% compared to control.	Height and root length.	(Zodape <i>et al.</i> , 2011)
<i>Arbuscular mycorrhizal fungi and P. fluorescent C7</i>	Increase in by 6.9 g compared to control.	Weight of the fruit.	(Bona <i>et al.</i> , 2018)
<i>Arbuscular mycorrhizal fungi and P. fluorescent C7</i>	Increased by 5.81cm	Fruit length	(Bona <i>et al.</i> , 2018)
<i>Arbuscular mycorrhizal fungi and P. fluorescent C7</i>	Increased by 4.62 cm	Fruit diameter	(Bona <i>et al.</i> , 2018)

2.4 Overview on postharvest losses in agriculture

2.4.1 Postharvest losses

Post-harvest losses are that reported and experienced by producers, processors, distributors, retailers as well as exporters especially during handling of agricultural

food produce after harvesting until it gets to the consumer (Kumar *et al.*, 2017). In general, postharvest losses contribute to food insecurity and are the main drawbacks in the production of food crops, including tomato. It is estimated that about 10 million tons of food produced in South Africa is lost through postharvest-related losses. In fact, the figure accounts for third of the 31 million tons of food produced annually. Also, it comprises 44% fruit and vegetables, 26% grains, 15% meat, and 13% roots, tubers, and oilseeds (Hlomendlini, 2019). Subsequently, food wastage and loss happen early in the food supply chain, with 50% occurring during the post-harvest stage, 25% during processing and packaging, 20% during distribution and retail, and 5% at the consumer level (Hlomendlini, 2019). The world human population is expected to grow up to 9.1 billion by the year 2050, and about 70% extra food ought to be produced in order to feed the growing population (Godfray *et al.*, 2010, Hodges *et al.*, 2011, Parfitt *et al.*, 2010). The growth in human population is not the only factor that is affected by postharvest losses but the agricultural and mechanical inputs as well (Kumar *et al.*, 2017). The agricultural inputs include the environment, the land, water, and energy used to produce the food lost during postharvest stage (Kumar *et al.*, 2017), whereas mechanical inputs include usage of tractors, ploughing, irrigation methods and pruning methods. According to FAO, (2013), unused food also results in extra carbon dioxide (CO₂) emissions, eventually affecting the environment which may led to global warming and climate change.

2.4.2 The causes of postharvest losses.

Postharvest losses are caused by factors such as poor handling and/or handling of on-farm and off-farm activities and often lead to loss of weight in agricultural goods due to spoilage, nutrients loss, commercial loss, and seed viability loss. Furthermore, such losses reduce the economic value of crops or may them unsuitable for human consumption (Boxall, 2001). On-farm losses are caused largely by small-scale farmers and attributed to their lack of knowledge on harvesting stages, excessive field heat, improper harvesting containers, poor farm sanitation and improper packaging materials. Small-scale famers also contribute to off-farm losses through lack of access to better road infrastructure, inappropriate transportation system, lack of processing factories, and lack of reliable market information (Kader *et al.*, 2002; Parfitt *et al.*, 2010; Hodges *et al.*, 2011). On the other hand, commercial crop farmers contribute to postharvest losses due to lack infrastructure needed for short-term storage of their

food produce, particularly at the farm level, as well as the lack of intermediate processing in the production catchments (Chegere, 2018). Also, on-farm losses from commercial settings include poor timing of harvest, poor methods, and equipment choice for harvesting and initial handling (Hlomendlini *et al.*, 2019). On the other hand, off-farm losses include transportation which relate to lack of temperature control and can cause chilling injury and multiple handling during loading and off-loading (Hlomendlini *et al.*, 2019). Report shows that postharvest losses are high are developing countries compared to developed countries (Arah *et al.*, 2015). The postharvest losses in developing countries are largely due to the lack of knowledge, inadequate technology and/or poor storage infrastructure. However, in developed countries, postharvest losses are mainly due to availability of advanced technologies and efficient crop handling and storage systems (Arah *et al.*, 2015).

2.4.3 Strategies to reduce postharvest losses

Postharvest losses are then regarded as a critical issue in South Africa and contribute to food insecurity. According to Tripathi *et al.* (2022), the latter is estimated to account for more than 40% of total losses in fruits and vegetables. Reducing postharvest losses helps to extend the shelf life of fruits and vegetables. Several reports have been conducted on strategies and innovation to reduce postharvest losses (Fagundes *et al.*, 2015; Sopanrao *et al.*, 2017; Miranda *et al.*, 2022; Ali *et al.*, 2024). Scholarly studies between 1990-1999, assessed whether strategies such as chemical and biological control can minimize postharvest losses and diseases through using fludioxonil and benzimidazole fungicides on tomato and grapes (Allah *et al.*, 2011; Abeer *et al.*, 2013). Years later, new strategies such as 1-methylcyclopropene, (1-MCP), edible coatings such as chitosan chitosan, chia mucilage and levan, gum Arabic, carnauba wax, plants extract, essential oils and controlled atmosphere packaging were introduced and researchers reported their efficacy (Fagundes *et al.*, 2015; Sopanrao *et al.*, 2017; Miranda *et al.*, 2022; Ali *et al.*, 2024). Furthermore, research demonstrated that fungicides and chemical compounds such as sodium bicarbonate, calcium chloride, and nitric oxide had little residual effect on feed crops and that their usage extend the shelf life of fruits and vegetables (Shehata *et al.*, 2021). In the main, postharvest losses are exacerbated by exposure of food products to high temperatures which elevate their ripening, leading to the rapid deterioration of their quality.

2.4.3.1 The effects of MAP (Modified Atmospheric Packaging) on the postharvest attribute of tomato.

Table 2.4. The effects of modified atmospheric packaging (MAP) and cold storage on the postharvest quality of cherry tomatoes.

The species/variety of tomato	Attributes assessed	Postharvest conditions	Outcome/findings	Sources/references
Cherry tomato	-Weight loss -Firmness -Sugar content -Lycopene -Color -Respiration rate	-Cold storage -At 5°C -For 25 days	Active MAP enhanced all the studied parameters during postharvest period.	(Fagundes <i>et al.</i> , 2015)

Lately, researchers and some crop farmers use MAP Modified atmosphere packaging to reduce postharvest losses, including on different varieties of tomato. Modified atmosphere packaging (MAP) and cold storage are marketed for use in order to increase the shelf life of fruits and vegetables. Findings by Fagundes *et al.* (2015) are presented above in table 2.3.4.1, and in the main, demonstrate that MAP and different storage conditions can enhance postharvest parameters of tomato. Reports further

show that MAP could extend the shelf life of cherry tomatoes to 25 days, largely due to the accumulation of CO₂ gas concentration that reduce the crop's transpiration. Therefore, MAP has a direct and positive effect on weight loss, colour, firmness, sugar, organic acids, colour, lycopene, respiration rate, and ethylene biosynthesis of cherry tomatoes.

2.3.4.2 The effects of UV-C on postharvest quality of tomato.

Recently, researchers have shown that UV-radiation can extend the shelf life of some food crops. UV radiation refers to the type of non-ionizing radiation that is emitted by sun and artificial sources and can be divided into three different UV-radiation which are UV-C (200–280 nm), UV-B (280–320 nm) and UV-A (320–400 nm). According to Pinheiro *et al.* (2015), UV-C can be used to extend the shelf life of tomato fruit during postharvest. The results showed that UV-C radiation affected colour, texture, total phenolic content (TPC), and weight loss (WL) when combined with low storage temperature (10 °C).

2.3.4.3 The effects of use of 1-methylcyclopropene on postharvest attributes of tomato

Lately, researchers have assessed the effects of supplying a concentration 1-Methylcyclopropene (1-MCP) on storage time and temperature on postharvest quality and shelf life of tomato. 1-Methylcyclopropene (1-MCP) refers to cyclopropene compound that is utilized as synthetic plant growth regulator (Sopanrao *et al.*, 2017). It shares structural similarities with the naturally occurring plant hormone ethylene and is used in industry to delay fruit ripening and preserve the cut of flowers (Sopanrao *et al.*, 2017). According to Sopanrao *et al.*, (2017), adding 1-MCP on harvested tomato improve postharvest quality attributes including decreasing percent physiological loss in weight, increasing total soluble solids, decreasing surface colour development, as well as decreasing Percent titrable acidity and ascorbic acid content. Findings by Sopanrao *et al.*, (2017) showed that exposure to gaseous 1-MCP at different concentrations (0.5, 1.0, 1.5 and 2.0) delayed the ripening of tomato fruits while the shelf life was increased as the concentration of 1-MCP was increased (Sopanrao *et al.*, 2017). The rate of weight loss of fruit was decreased with increase in 1-MCP concentration, total soluble solids increased with an increase in 1-MCP concentration, the rate of change in colour in terms of hue angle (°h) of tomato fruit was improved with increase in the exposure time (Sopanrao *et al.*, 2017).

2.5 The effects of edible coatings on postharvest attributes of tomato

Types of edible coatings	Postharvest attributes assessed	Tomato Species	Source
Chitosan, chia mucilage and levan	Weight loss, firmness, TA, Total soluble solids concentration, ascorbic acid content.	Tomato cocktail (cv "Seyit F1")	(Ali <i>et al.</i> , 2024)
Gum Arabic	Weight loss, firmness, titratable acids, soluble solid concentration, ascorbic acid content, decay percentage and colour.	Tomato ("Money Maker")	(cv (Miranda <i>et al.</i> , 2022)
Carnauba wax	Sugar acids, pH, water vapor loss, firmness, gloss, colour, ethylene, respiratory activity.	Tomatoes ("Debora")	(cv (Ali <i>et al.</i> , 2010)

Researchers studied the effects of chitosan, chia mucilage, and levan on the shelf life of cocktail tomatoes. Chitosan, some sugar from shellfish, has film-forming properties and antibacterial properties. Chai mucilage, a complex carbohydrate, includes uronic acids, protein, and lipids. Levan, a fructan, has high adhesion properties. The study assessed postharvest attributes like weight loss, TSS, flesh firmness, and antioxidant activity (Koide, 1998; Dick *et al.*, 2015; Narasimhamurthy *et al.*, 2022; Mujtaba *et al.*, 2023). According to (Ali *et al.*, 2024), weight loss was recorded in all the studied tomatoes in cold storage and shelf life. Ali *et al.* (2024) found that extending storage time increased TSS contents compared to harvest value while edible coating treatments did not significantly affect L*Brightness values, however, they increased C* value and decreased Hue angle. Additionally, extension in storage increased lycopene contents compared to harvest value. The study found no significant differences in total phenolic, flavonoid, chlorophyll, and antioxidant activity among edible coating treatments on tomatoes. However, these treatments prolonged storage duration and shelf life by minimizing weight losses and maintaining fruit quality (Ali *et al.*, 2024).

Gum arabic, a water-soluble dietary fiber from *Acacia senegal* trees, is a popular, inexpensive, and biocompatible polysaccharide with excellent emulsification, film forming, and encapsulation properties, and is used widely in the industrial sector (Motlagh *et al.*, 2006; Patel and Goyal, 2015; Williams and Phillips, 2021). A study by Ali *et al.* (2010) evaluated whether gum arabis can serve as an edible coating to extend the shelf life and improve postharvest quality of tomato fruits, particularly whether it

affected weight loss, firmness, and sensory evaluation. Ali *et al.* (2010) found that fruits coated with 10 and 15% gum arabic had decreased weight loss during storage, increased firmness, and enhanced colour change. The concentration of gum arabic significantly impacted soluble solids, titratable acidity, and ascorbic acid. No visible decay was observed in coated or control fruit until day 4. Ali *et al.* (2010) found that coating fruits of tomato stored at 20°C with 10% gum arabic significantly delayed the weight, improved firmness, titratable acidity, soluble solids concentration, and enhanced colour.

Carnauba wax refers to a wax that is produced from Brazilian palm tree (*Copernicia cerifera*). It is a natural, plant-based product that can shine on painted surfaces and often hard and used for synthesis of polish, lipsticks, and carbon paper (Miranda *et al.*, 2022; Oliveira *et al.*, 2022). Carnauba wax nanoemulsion has been used as an edible coating treatment for postharvest attributes quality of tomatoes. Miranda *et al.* (2022) evaluated the postharvest quality (sugar, acids, pH, water vapor loss, firmness, gloss, colour, ethylene, and respiratory activity) of fresh tomato that had been treated with carnauba wax nanoemulsion. The study by Miranda *et al.* (2022) found that carnauba wax emulsion had higher surface roughness and hydrophobic properties than nanoemulsion. Gloss decreased with 18% carnauba wax emulsion, and weight loss increased during storage. Treatments did not affect acid concentration, soluble solids, ratio, or pH. The study found that fruit firmness and colour parameters a* increased over storage, while ethylene production decreased. However, respiratory rate, including CO₂ production and O₂ consumption, was not affected by storage time or treatments.

2.5 Work not done on the effects of biostimulants on tomatoes

Literature that has reviewed different types of biostimulants and their impact on tomato is abundant. However, there is rarely enough literature on the usage of moringa based biostimulants (Phytostim®). Phytostim® is a *moringa*- based biostimulant composed of several number of bioactive compounds has which is 22 essential amino acids. The product is sold in retail some shops in South Africa and is gaining popularity among consumers as it is tolerant to abiotic stress and environmental conditions. It contains various compounds that can enhance physiological processes that contribute to the growth and development of plant. Currently, there is no published research on the effects of supplying different concentrations of Phytostim® on the growth, yield, and

postharvest attributes of tomato. Therefore, such information can provide suitable solution for small- and large-scale crop farmers because it can optimise the quality of fruits of tomato. The information can also help to extend the shelf life of tomato and reduce the postharvest losses.

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CHAPTER 3: Effect of different concentration of Phytostim® biostimulant on growth and yield attributes of tomato.

Abstract

Tomatoes are fruits cultivated worldwide however to meet the demand for the increasing human population, challenges related to postharvest ought to be addressed. There is a need for research that assess how the growth and yield of tomatoes can be enhanced through the usage of biostimulants particularly Phytostim®. Therefore, this study aimed at investigating the effects of different concentrations of Phytostim® biostimulant on growth and yield of tomato. Four weeks

old tomato seedlings replicated 8 times, were transplanted into microplots, arranged in a randomised complete block design in an open field, for a period of 12 weeks. Treatments involved different concentrations of Phytostim® biostimulant (0, 0.1, 0.5, 1, 2, 2.5, 3 and 4%, while 0% was control). Plants were irrigated using 250 mL of tap water whenever there was a status of 'dryness'. Data was subjected to one-way analysis of variance (ANOVA) and the Duncan's Multiple Range Test (DMRT) was used for separating means using the Statistical 10.0 software package. Different concentrations of Phytostim® biostimulant were foliar applied in an interval of 14 days during the entire growing period. The growth attributes which included plant height, leaf length, and stem diameter were taken on a weekly basis. At harvest, yield components including the number of fruits per plant, diameter, size, and fresh weight of the fruits, were recorded. The effect of different concentrations of Phytostim® biostimulant significantly influenced ($p < 0.05$) the growth and yield attributes of tomato. The study revealed that of growth attributes, Phytostim®-supplied plants exhibited increased the stem diameter by 12 mm, plant height was increased by 95 cm, and leaf length improved 3.5 cm at the concentration of 0.1 %. On the yield attributes, adding 0.1% Phytostim® markedly enhanced size of the fruits by 60 mm, diameter of the fruits by 56 mm, number of fruits per plant by 26, and fresh weight of the fruits by 51 g.

3.1 Introduction

Agricultural fruits and vegetables are severely threatened by especially the frequent weather events that are associated with harsh environmental conditions; thus, their production is decreased (Gedeon *et al.*, 2022). Securing yield that is sufficient while minimizing environmental harm is agriculture's primary challenge. Agricultural production is compromised due to lack of proper food in the industry (Duhamel and Vandenkoornhuysen, 2013). Climate change and rapid population growth pose a threat to the production and economy of agriculture (Egea *et al.*, 2022). Hence, cultivating fruits and vegetables through using strategies that are sustainable and ecofriendly can be an alternative way. The main goal of agriculture is to produce fruits with improved resistance to abiotic stress, tolerance, and increased nutritional value (Egea *et al.*, 2022). Tomato can alleviate malnutrition and food insecurity. The production of tomato may contribute to food security due to nutritional, production, and health benefits. Food security is attained when everyone has access to sufficient, available, safe, and

nutritious food all the time (FAO, 2010). Reports have shown that tomato is in high demand due to the recent increase in population (Adu-Dapaah *et al.*, 2022). Therefore, growth and yield of tomato must be the goal for agricultural production through sustainable strategies. Sustainability of fruits such as tomatoes in South Africa has been pinned, mostly through the usage of fertilizers, protected environments such as hydroponics and plastic tunnels. According to (Maboko *et al.*, 2011), soilless production systems can enhance the growth and yield of tomato which in turn could reduce the impact of climate change. Pre-harvest applications of various plant biostimulants has contributed to improved growth and yield of tomato recently (Saraswathi and Praneetha, 2013; Sani *et al.*, 2020; Mzibra *et al.*, 2021; Turan *et al.*, 2021; Gedeon *et al.*, 2022). It can be argued that the production of tomato is enhanced by the addition of biostimulants produced from seaweeds (Ahmed *et al.*, 2022) and yield positive results. Therefore, using biostimulants such as amino acids (Protein Hydrolysed) can be an alternative way. Phytostim® is a *moringa*-based biostimulant composed of several number of bioactive compounds. It contains such several number of natural antioxidants that can enhance growth and yield attributes. Phytostim® is effective when applied at a lower concentration optimally 3% which is the recommended dose on the labelling for leafy vegetables as mentioned on the packaging. Phytostim® can increase nutrient use efficiency, reduce the use of chemical fertilizers without adverse effects on yield and quality as mentioned on the packaging. It is a commercial product that is gaining momentum in agricultural production as it is tolerant to abiotic stress and environmental conditions. It contains various compounds that can enhance physiological processes that contribute to the growth and development of plants. Therefore, this study assessed the effect of supplying different concentrations of Phytostim® biostimulant on growth and yield attributes of tomato.

Objective of the study: To assess whether the application of Phytostim® biostimulant at different concentrations affect growth and yield in tomatoes cultivar '*Roma*'.

3.2 Materials and methods

3.2.1 Study location

The study was carried out in a microplots in an open field during summer season (December-February) at Green Biotechnologies Research Centre of Excellence, in Limpopo Province, South Africa (23°53'10"S, 29°44'15"). Day/night temperatures of

the area ranged between 21 and 28°C (Me-Ead, and McNeil, 2019). The average precipitation was about 500 mm in the soil in the area was of Hutton form.

3.2.2 Treatments and design

Each treatment involved eight different concentrations of Phytostim® biostimulant including: (0, 0.1, 0.5, 1, 2, 2.5, 3 and 4%, while 0% was control). The treatments were chosen from the background of research carried out by Mpai *et al.*, (2022). The treatments were laid out in a randomised complete block design (RCBD). Blocking was to ensure equal exposure to sunlight, temperature, and winds in the field.

3.2.3 Research procedure

Seeds of tomato (*cv "Roma"*) were obtained from the Agricultural Research Council (ARC) Pretoria, Roodeplaat. The seeds were sown in a disinfected 200 polystyrene seedling trays filled with Hygromix® (Hygrotech seed company, South Africa) growing medium up to 4 weeks of post-emergence and seedlings were transplanted into a 20 cm plastic pots with row spacing of 40 cm × 40 cm between plants in the field. The pots were filled with a mixture of Hygromix growth medium and pasteurized (300°C for 45 minutes) loam and sandy soil at a ratio of 2:1:1. Irrigation involved adding 250 mL of non-chlorinated tap water and was performed whenever there was a status of 'dryness' from the moisture probes (T10 Bodentester, South Africa) (Mpai *et al.*, 2022). Phytostim® biostimulant was applied and no fertilisers were added prior and during the growth of tomato in this study. Different concentrations of Phytostim® biostimulant were applied on the leaf (foliar) once after 14 days. The application rate was linear to the number of leaves such that 120 mL of a single Phytostim® biostimulant concentration covered eight plants (Mpai *et al.*, 2022).

3.2.3.1 Phytostim® biostimulant properties

Phytostim® biostimulant is a product that is available and has gained immense popularity. It is a biostimulant derived from plants that is taken from crude extracts of *M. oleifera* through enzymatic hydrolysis of proteins. The method used by Paul *et al.* (2019), with modifications, was used. The enzymatic hydrolysis approach was also used to separate the insoluble residues of amino acid complexes. Valine (323.8 mg/L), isoleucine (246.6 mg/L), leucine (437.4 mg/L), phenylalanine (259.2 mg/L), glutamic acid (507.6 mg/L), aspartic acid (315.0 mg/L), glycine (244.6 mg/L), serine (269.2 mg/L), threonine (249.9 mg/L), alanine (365.2 mg/L), and proline (222.1 mg/L)

comprised the final product of Phytostim® biostimulant, which makes up 70% of the active ingredients (Mpai *et al.*, 2022).

3.2.4 Data collection

Growth parameters including plant height, stem diameter, number of leaves, and leaf length were collected every week from week 1 up to week 12 during the growing period of tomato. Plant height was measured using a 30 cm ruler, from the base of the stem to the tip of the plant. Also, leaf length was measured from petiole to the lengthened tip. Stem diameter was measured at 30 cm from the soil, using a digital Vernier Calliper (KTV150-major Tech) and expressed in mm.

Harvesting was done after 12 weeks and the following yield components were determined: size of fruits was measured using a digital Vernier Calliper while the number of fruits was determined through physical count, and the weight (g) was measured through using an electronical weighing balance. At harvest, the number of fruits were taken from the experimental plot for all the replications.

3.2.5 Statistical analysis

Data was subjected to one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) was used for separating means using the Statistica 10.0 software package.

3.3 Results and discussion

3.3.1 The effect of different concentrations of Phytostim® biostimulant on growth attributes of tomato.

The chapter is intended to achieve objective 1: To assess whether the different concentrations of Phytostim® biostimulant affect growth and yield in tomato cultivar 'Roma'. The content below postulates the findings obtained from objective 1. Results obtained revealed that the different concentrations of Phytostim® biostimulant significantly ($p < 0.05$) influenced the assessed growth attributes. Plant growth were observed throughout the experiment on a weekly basis (from week 1 till week 12). The results obtained are presented in Figure 3.1 up to 3.4.

Figure 3.1: The effect of different concentrations of Phytostim® biostimulant significantly ($p < 0.05$) influenced the stem diameter of tomato (figure 3.1). The results revealed that the application of 0.1% resulted in thicker stems compared to all the other studied concentrations. Therefore, the results show that the using 0.1% Phytostim® can enhance the stem diameter of tomato, while 0% inhibited stem diameter. However, according to the labelling packaging, 3% is the recommended concentration to enhance growth and yield of maize, beans, and lettuce (Web 1). While the recommendation by the supplier could have been based on results from other vegetables other than tomatoes, in this study, the results show that the 3% cannot be regarded as the effective concentration to increase stem diameter of tomato. The variation in stem diameter among the treatments might be due to what was reported by Altaf Romaisa *et al.*, (2015) that a thicker plant stem can be attributed to the maximum plant height which captures maximum sunlight for photosynthesis which results in the formation of maximum photosynthesis that accumulate in the stem. Phytostim® is an amino acid that contain the auxins and gibberellin which contribute in a positive way to the growth and development of tomato. Stem elongation is caused by the auxins in this product; this finding is consistent with results reported by Pierik (1990), who observed that a high auxin ratio causes formation and elongation.

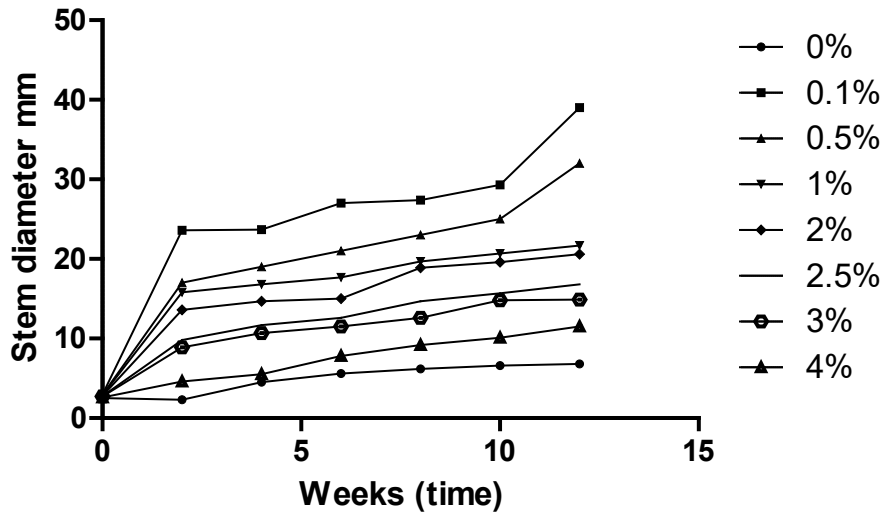


Figure 3.1: Effect of the different concentrations of Phytostim® biostimulant on stem diameter of tomato (cv "Roma").

Figure 3.2: The effects of different concentrations of Phytostim® biostimulant significantly ($p < 0.05$) influenced leaf length (figure 3.2). Of the selected and tested concentrations, 0.1% was found to be the most effective that enhanced the leaf length with five-fold higher than the benchmark (control), these treatments outperformed all the other studied treatments. In fact, plants supplied 0.1% outperformed that supplied with 3%, thus indicating that the lower concentration enlarged the leave size of tomato. However, according to the packaging of Phytostim® the 3% is the recommended concentration in terms of growth and yield for maize, beans, and lettuce. Therefore, the results show that the 3% cannot be regarded as the effective concentration to enlarge the leave size of tomato. The enlarged leaf length can be attributed to adding Phytostim® directly on leaves through foliar application where the absorption of nutrients on the leaves could have been quicker. Furthermore, it can stimulate various processes including photosynthesis and enhanced the chlorophyll (Wright *et al.*, 2017). As it has been said that biostimulant can enhance efficient nutrient uptake, assimilation, and translocation. Plants supplied with agricultural inputs through foliar application reportedly reveal quicker responses compared to that supplied through drench, that is, on the soil (Matt *et al.*, 2018). According to Matt *et al.*, (2018), certain nutrients are absorbed quicker when added through foliar spray. This can be explained by that the stomata of the leaves allow nutrients to pass through and water can enter through the stomata while they are open, whereby nutrients that have been dissolved will also enter with the water. Komosa (1990) reported that under conditions of low

nutritional status of tomato plants, absorption rates of nutrients applied through leaves were higher as compared to those of plants supplied with nutrients via the roots. There is a strong correlation between nutrient concentration on a leaf surface and the rate of its uptake by the epidermal cells. However, authors reported that, high concentrations cause leaf injury leading to the reduction in nutrient absorption. The results obtained showed that tomato has lower leaf surface which was stimulated by the application of foliar Phytostim® on the leaves. These results are in alignment with authors who reported that lower leaf surface takes up mineral nutrients more rapidly than the upper side (Whitman and Aarssen, 2010; Kleiman and Aarssen, 2007; Yang *et al.*, 2008).

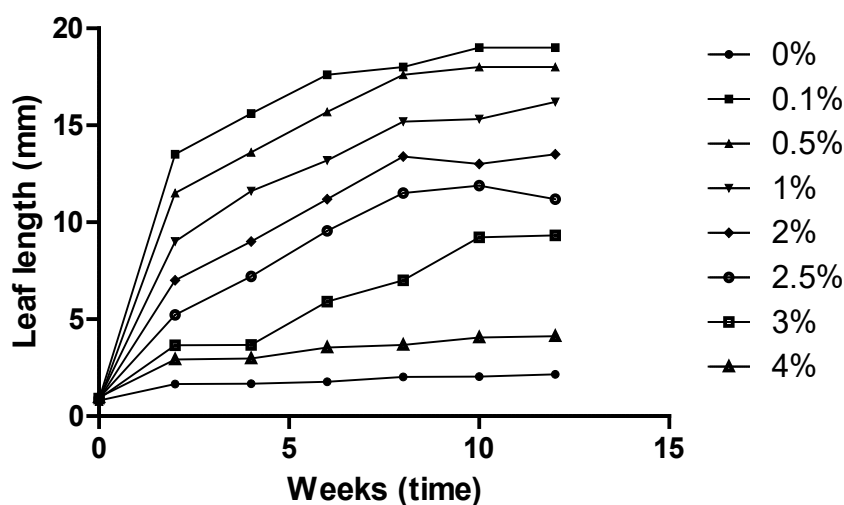


Figure 3.2 Effect of the different concentrations of Phytostim® biostimulant on stem diameter of tomato (cv "Roma").

Figure 3.3: The effects of different concentrations of Phytostim® biostimulant significantly ($p < 0.05$) influenced plant height (figure 3.3). The application concentration at 0.1% increased the plant height with nineteen-fold taller than benchmark (control) and outperformed all the other studied treatments. By contrast, the application of 0% concentration inhibited the plant height compared to all the other studied concentrations and the control. The performance of tomatoes supplied at 0.1% performed better than that of their counterparts supplied at 3%, thus indicating that for some species of tomato, foliar-spray of Phytostim® is effective at low concentration, and that the 3% cannot be the optimum concentration for all crops (fruits and vegetables) but for mostly leafy vegetables and others. The 0.1% can increase height of tomatoes as compared to 0%. While it can be said that the 3% is the recommended concentration to enhance growth and yield for crops such as maize, beans, and

lettuce, results of this study show that the 3% cannot be regarded as the recommended concentration to increase the height of tomato cultivar. The increase in the height of the tomato plants supplemented with biostimulants may be a synergy effect between its compounds and the resulting increase in endogenous levels of cytokinins and auxin, which can stimulate plant growth by affecting photosynthesis, respiration, and activation of plant defense systems, as was found in Kentucky bluegrass (Zhang *et al.* 2003). Amino acid-based biostimulants have been linked to increased plant development (Barneix and Causin, 1996) due to their role in controlling plant nitrogen biosynthesis, ammonium uptake and fusion, nitrate accumulation, and protein biosynthesis.

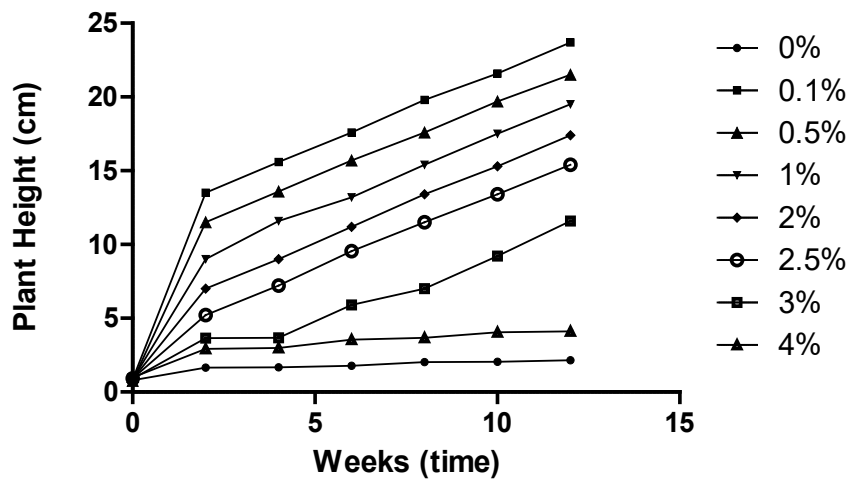


Figure 3.3 Effect of the different concentrations of Phytostim® biostimulant on stem diameter of tomato (cv "Roma").

3.1 The effect of the different concentrations of Phytostim® biostimulant on yield attributes of tomato (cv “Roma”).

Table 3.1 show a summary on the effects of different concentrations of Phytostim® biostimulant on attributes determined at the harvest. The effects of different concentrations of Phytostim® biostimulant significantly ($p < 0.05$) influenced the yield attributes such as number of fruits, diameter of fruits, size of fruits and fresh weight of fruits.

Treatment Phytostim® Concentrations	Number of Fruits	Size of the fruits (mm)	Diameter of the fruits(mm)	Fresh weight of the fruits (g)
0%	4±0.29 ^e	43.4±4.56 ^d	22±0.8 ^a	18±1.22 ^a
0.1%	26±1.23 ^a	67.1±2.24 ^a	43.2±0.02 ^a	51±5.46 ^a
0.5%	19±1.08 ^a	65.2±1.14 ^b	40.6±0.01 ^b	47±6.2 ^a
1%	17±0.03 ^b	60.5±2.25 ^b	38.8±0.02 ^c	43±0.8 ^a
2%	15±0.21 ^b	57.8±2.28 ^c	36.0±0.02 ^c	40±0.4 ^a
2.5%	10±0.02 ^d	50.7±6.8 ^d	30.3±2.25 ^d	36±2.35 ^a
3%	8±0.01 ^d	47.0±2.31 ^d	29.8±2.02 ^e	34±0.24 ^a
4%	6±0.02 ^d	40.7±2.02 ^e	26.2±0.87 ^e	25±0.17 ^a
SE ±	2.25	5.58	2.22	3.24
P-value	0.038	0.025	0.083	0.04

Value (Mean± SE) followed by different letters on each column based on treatments were significantly different at ($p < 0.05$), according to DMRT.

The effects of different concentrations of Phytostim® biostimulant significantly ($p < 0.05$) influenced the number of fruits, size, diameter, and fresh weight of the tomato fruits, at the harvest stage. Growth attributes are the main factors that determine the kind of yield attributes of the studied crop. The growth parameters such as plant height and number and/or size of leaves, play a critical role in a plant’s metabolic functions such as photosynthesis, which is a determinant of the overall yield (Evans, 2013). Therefore, it was expected that the lower concentration of Phytostim® that enhanced growth parameters would also attain superior yield attributes. Therefore, Phytostim® at 0.1% enhanced the yield parameters (number of fruits by 26, size of the fruits by 67.1 mm, diameter of the fruits by 40 mm, and fresh weight of the fruits with 47 g) at harvest, compared to control and other concentrations (see Table 3.1). Results obtained showed that Phytostim® can be effective at low concentration, and that the 3% cannot be regarded as the optimum concentration for increasing yield in tomato fruits. Therefore, it can be argued that the 3% is the recommended concentration that

can enhance the yield for crops such as maize, beans, and lettuce (Web1; Mpai *et al.*, 2022). On the other hand, concentration of 0.1% accelerated ripening and resulted in higher percentage of fresh weight during storage compared to control. The observed response could be attributed to the effects of phytohormones such as auxins and gibberellins, which are widely reported as present in most plant biostimulants. Furthermore, it may relate to the participation of these growth-stimulating hormones in different growth and development stages: pollen germination, fruit set, cell division, and elongation after pollination. Generally, the final yield (kg/ plant) showed an increase of values in all the parameters in treatments with biostimulator applications. Most of other research has revealed significant impacts of biostimulant concentrations on fruit yield components (Abdelkader *et al.*, 2019; Francesca *et al.*, 2020; Majkowska-Gadomska *et al.*, 2021). According to Bulgari *et al.*, (2014) as well as Van Oosten *et al.*, (2017), applying biostimulants can act directly on plant physiology and metabolism or indirectly by improving the absorption of nutrients and other beneficial molecules from the soil (e.g., mycorrhiza). Furthermore, the approach can result in the modification of some molecular processes that improve water and nutrient use efficiency in crops, consequently these enhances the growth and yield component of tomato plant. Results by Mpai *et al.* (2022) are in agreement with results obtained from this objective. For example, Mpai *et al.* (2022) reported that Phytostim® biostimulant improved the growth and yield of lettuce. Several researchers indicated that extracts containing *Moringa oleifera* have the plant hormone zeatin, which is involved in the assistance of plant physiological processes (Ngcobo, and Bertling, 2019; Zulfiqar *et al.*, 2020). Further, studies by Zulfiqar *et al.*, (2020) reported that the various physiological processes involved in plant growth, development, and stress response are coordinated by endogenous plant hormones such as auxins and gibberellins as the major growth promoting phytohormones. However the results of this study are in agreement with that by Abdalla (2013) who showed that plants sprayed with lower concentrations, including 2% and 3% of moringa leaf extracts improved endogeneous auxins, gibberellins and cytokinins levels compared to control of rocket (*Eruca vesicaria subsp. sativa*). Similarly, Elzaawely *et al.*, (2017) reported that *Moringa oleifera* extracts (MLE) applied at different concentrations increased auxins and gibberellins of beans (*Phaseolus vulgaris*). Therefore, it is intensively not surprising that Phytostim® biostimulants, derived from extracts of *Moringa oleifera* which contains amino acids, produced similar results to that of aforementioned biostimulants.

It is evident that the effects of different concentrations of Phytostim® biostimulants can stimulate the yield parameters at large.

3.5 Conclusion

The results obtained from this research indicate that the effects of different concentrations of Phytostim® biostimulant has significant positive influence on the growth and yield attributes of the tomato cultivar 'Roma' including that of stem diameter, plant height, leaf length, size, diameter, number of fruits and fresh weight of fruits. Phytostim® biostimulant at 0.1% enhanced the growth and yield parameters compared to control and other concentrations. It can be concluded that Phytostim® biostimulant is effective at lower concentration. The results obtained add new knowledge because they do not concur with what was recommended on the labelling packaging that 3% can enhance growth and yield, but 0.1% can be recommended for fruits such as tomatoes. Phytostim® biostimulant at the concentration of 0.1% produced good quality fruits with more yield that control and other concentration.

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CHAPTER 4: Effect of different concentrations of Phytostim® biostimulant application on postharvest attributes of tomato.

Abstract

Tomatoes contain several numbers of phytonutrients that confer many health advantages. However, being climacteric fruits makes them susceptible to postharvest losses. Phytostim® biostimulant can be employed to enhance their postharvest attributes. Therefore, this study aimed at investigating whether the different preharvest concentrations of Phytostim® biostimulant affect postharvest attributes in tomatoes. Fresh fruits of tomato were harvested at pink stage and each treatment combination consisted of twenty-six fruits. The treatments consisted of 8 pre-harvest different concentrations of Phytostim® biostimulant (0, 0.1, 0.5, 1, 2, 2.5, 3, and 4%, while 0% was control) laid in CRD. The fruits were stored for 0, 5, 10, and 15 days at ambient conditions and later assessed for percentage weight loss, firmness, pH, colour, and TSS. The parameters were assessed at an interval of 5 days and oven-dried at 40 °C for 48 hours for analysis of nutritional composition (protein and mineral elements). The results obtained revealed that the effects of different concentrations of Phytostim® biostimulant significantly ($p < 0.05$) influenced postharvest attributes (Percentage weight loss, Firmness, TSS, pH and Colour). All the above-mentioned postharvest attributes were enhanced at 0.1 % concentration of Phytostim® biostimulant compared to control and other concentrations. Furthermore, the study further revealed that different concentrations of Phytostim® biostimulant significantly ($p < 0.05$) influenced the mineral elements (Mg, Ca, Fe, K, Zn, Cu, P and Na) and the protein content. The nutritional composition was enhanced at 0.1 % concentration of Phytostim® biostimulant in comparison to the control and other concentrations. Hence, it can be concluded that Phytostim® biostimulant at the concentrations of 0.1% can be used to enhance the postharvest parameters and to extend the shelf life of tomatoes in ambient condition. Lower concentration of Phytostim® can be used to manipulate and improve nutritional composition regardless of the environmental conditions. Furthermore Phytostim® biostimulant can be recommended to be used effectively at low concentrations.

4.1 Introduction

Organic crop farming has gained immense popularity among farmers and researchers lately, in particular, farmers show interest on the approach, more than the conventional inorganic farming. The yearn to adopt organic farming by farmers, is as a result of the gained awareness about the impact of non-edible pre-harvest treatments aimed at improving post-harvest shelf life. This is with no exception to the South African tomato growers. It is known that addition of chemicals such as pesticides (herbicides and insecticides) can improve the quality of tomatoes (Kumar *et al.*, 2023). Yet, the costly and largely scanty inorganic fertilisers are still the major agricultural inputs used by most farmers for improved yields, and without a doubt, have improved postharvest quality of food crops. Post-harvest losses rank among significant drawbacks to tomato marketing. Losses are largely associated with shorter shelf life that goes up to 2–3 weeks before fruits can no longer be marketable or consumed (Hossain and Rashid, 2021). In response to the negative effects of postharvest losses of food products, different postharvest strategies have been implemented to mitigate them especially in tomatoes. Strategies include the use of modified atmospheric packaging, organic and inorganic coatings, cold storage, and UV irradiation (Moneruzzaman *et al.*, 2009; Gharezi *et al.*, 2012; Peralta-Ruiz *et al.*, 2020). Usage of biostimulants in crop agriculture has also gained popularity. However, there is still a gap on information, especially on whether adding biostimulants to food crops at the pre-harvest stage can enhance postharvest quality of food crops. One such biostimulant that has been assessed is *Moringa* based Phytostim® and was used in this study as a pre-harvest treatment to minimize postharvest losses in tomatoes. Phytostim® biostimulant is an organic biostimulant made from *Moringa oleifera* tree extracts. Phytostim® is incorporated with 22 essential amino acids that contribute to the growth, development, and extend the shelf life. This biostimulant is composed of Glutamic acid followed by leucine, alanine, valine, aspartic acid, serine, threonine, phenylalanine, isoleucine, glycine, and proline. These amino acids have the ability to improve tolerance to abiotic stress after postharvest in fruits stored under ambient conditions and has been reported to increase growth and yield in lettuce vegetables (Mpai *et al.*, 2022). However, its impact is still lacking on the postharvest shelf life of tomatoes. Some work performed on the application of biostimulants as elicitors for improving postharvest

includes: a poly-saccharide-enriched seaweed extract and *Ecklonia maxima* (auxins, cytokins, poliamides, gibberellins, and brassinosteroids) that were used to improve the tomatoes and lettuce postharvest quality (Moncada *et al.*, 2022). Their work has envisioned the thought of investigating amino acid-based biostimulant on tomato postharvest quality. Thus far, it can be argued that the effectiveness of Phytostim® biostimulant may depend on the concentration used. According to (Mpai *et al.*, 2022), the lower concentration tend to be more effective than the higher concentrations. Safe and low-cost methodologies that can extend the shelf life while at the same time retain the quality under ambient conditions need to be accessed. In this study, foliar application of different concentration of Phytostim® biostimulant has been employed to observe its impact on postharvest attributes on tomato.

Objective of the study: To access whether the different preharvest concentrations of Phytostim® biostimulant affect postharvest attributes in tomatoes.

4.2 Materials and methods

4.2.1 Study location

The experiment was performed at the green biotechnology research centre of excellence (GBRCE) at University of Limpopo Campus. This experiment was conducted at the laboratory setting and at ambient temperature during the month of March whereby dry bulb air temperature and relative humidity of the experimental area varied from 23.8 to 28.6°C and 59.2–63.5°C. After 15 days of postharvest storage the samples for all treatments were oven dried for 48 hours. Then ground unto powder for nutritional analysis. Mineral composition experiment took place at the University of Limpopo, Limpopo Agro-Food Technology Station (LATS).

4.2.2 Treatments and design

The experiment was portioned into CRD, with eight preharvest Phytostim® concentrations stored at ambient condition for 15 days. Days were selected according to (Shahi *et al.*, 2012). The treatments were arranged in completely randomised design.

4.2.3 Experimental procedures

Fresh tomato fruits were randomly harvested at pink stage and each treatment combination consisted of twenty-four fruits. Samples of six tomato fruits per treatment (Phytostim® biostimulant concentrations) were stored for 0, 5, 10, and 15 days) in a

retailer box at ambient condition. On each sampling date, the postharvest parameters were determined and measured. After 15 days of postharvest storage, the samples for all treatments were oven-dried for 48 hours at 40 °C. The samples were thereafter ground unto powder, for later determination of nutritional analysis.

4.2.4 Data collection

Colour

Color was measured non-destructively using a portable colorimeter (Chroma Meter, Konica Minolta Sensing Inc., Japan) which was calibrated with a white calibration card. The CIELAB color space; a^* (-value mean greenness and + value means redness, and h° (colour intensity $>180^\circ$; means yellowness; $<180^\circ$ means green) were recorded on the three readings on the equatorial region of the leafy vegetables. In the CIE color system, negative a^* values describe the intensity of green color. Whereas hue angle (h°) represents the changes in aging as explained by McGuire (1992).

Total Soluble Solids (TSS):

TSS was measured using digital refractometer (Model: Atago PR-1, Atago Co, Ltd., Tokyo, Japan) and recorded as °Brix. The procedures described by Mazumdar and Majumder (2003) were used. Three fruits from each treatment were blended and 2–3 drops of filtered juice was used. Average of two readings were used with handheld refractometer (model: ERB-32, India) ranging from 0 to 32%.

Percentage Weight loss:

It was determined as percent loss from initial weight as described by (Kumar *et al.*, 2018). The weights of freshly harvested fruits were recorded at the time of harvesting. On each day of observation, the stored fruits were weighed and the weight losses on each sampling date were calculated using the following formula.

$$\text{Formula: PWL (\%)} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100$$

Firmness:

Firmness was measured non-destructively using a hand-held firmness tester on the whole fruit. Fruit firmness measurement was taken at two equidistant points in the equatorial regions and the results expressed as Newton (N). Three readings were

taken around the circumference of each fruit and the average reading recorded in newtons (N).

pH:

It was measured using AOAC (2005) method. Three fruits from each treatment were chopped and homogenized using blender. The homogenates were filtered through cloth and a pH meter (Model: AC-3118M, Abron Exports, India) that had been previously calibrated to pH 4 and pH 7 was used for the determination of the pH value.

Determination of protein:

The micro Kjeldahl method, as described by the Association of Official Analytical Chemists (AOAC) (1990), was used. Two grams (2 g) of each sample was mixed with 10 mL of concentrated sulphuric acid (H_2SO_4), in a heating tube. One tablet of selenium catalyst was added to the tube and the mixture was heated inside a fume cupboard. The digest was transferred into a 100 mL volumetric flask made up with distilled water. Ten millilitre portion of the digest was mixed with an equal volume of 45% NaOH solution and poured into a Kjeldahl distillation apparatus. The mixture was distilled, and the distillate was collected into a 4% boric acid solution, containing 3 drops of indicator. A total of 50 mL of distillate was collected and titrated as well. The sample was duplicated, and the average value was taken. The nitrogen content was calculated and converted to percentage protein by using a protein conversion factor of 6.25.

Determination of minerals composition:

The 10 g of dried samples of tomato materials were digested in 40 mL of 4% nitric acid (HNO_3), followed by placing the container on a vortex to allow for complete wetting of the mixture. The materials were magnetically stirred, thereafter incubated in a 95°C water bath for 90 minutes, allowed to cool down at room temperature, filtered, and decanted into 50 mL tubes which were covered with a foil and then selected nutrient elements were analysed using the inductively coupled plasma optical emission spectrometry (ICPE-9000).

4.2.5 Statistical analysis

Data was subjected to one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) was used for separating means using the Statistix 10.0 software package

4.3 Results and discussion

The second trial was undertaken to address the second objective of the study which was to assess whether the different preharvest concentrations of Phytostim® biostimulant affect postharvest attributes in tomatoes. The results are postulated below:

The effects of different concentrations of Phytostim® biostimulant significantly ($p < 0.05$) influenced the following postharvest parameters: percentage weight loss, firmness, TSS, pH, colour, and nutritional composition.

4.3.1 Percentage weight loss

The preharvest Phytostim® concentrations and post-harvest storage time had a significant ($p < 0.05$) influence on the percentage weight loss of tomatoes during 15 days of storage at ambient condition on day 0, 5, 10, and 15 of storage period (Table 4.1.) respectively. Loss in weight gradually increased for all the treatments at ambient condition as days increased from 0 to 15 day. The total weight loss in tomato for (0 %) control remained higher during the entire period of storage. At the fifth day of storage, it was 8.93 %, thereafter, it was raised to 11.45 % on day 10 (table 4.1), followed by an increase of up to 14 % at day 15. The concentration of 0.1% induced lowest weight loss compared to all the other studied treatments, with 3.88 % at day 0, then migrated to 5.72 % at day 5, migrated to 10.75 % on day 15. The results showed a similar pattern of an increase of percentage weight loss with all the treatments. The trends shown in these results are similar to that by Gharezi *et al.*, (2012) that percentage weight loss progressively increases as days of storage is increased. On the other hand, results obtained are in contradiction with the findings by Kazemi *et al.*, (2011) who reported that percentage weight loss decreased as days increased. Low percentage in weight loss means that the quality of tomato fruits is still maintained. In this case, low concentration (0.1%) of Phytostim® reduced the percentage weight loss which led to good quality of tomato fruits. According to the Phytostim® labelling packaging, regard 3 % concentration as the recommended dose for beans, maize,

and lettuce while the results showed that it cannot be recommended for tomato, but 0.1 % it can be the effective concentration in this case. The continuous increase in the percentage weight loss could be attributed to the maintenance of proline and glutamic acid contained in the Phytostim®, as when they are applied in low concentrations, they are able to mitigate the effect of inconstant temperatures. The amount of water present at harvest determines whether a fruit of tomato would continue to lose water during postharvest storage. According to Yin *et al.*, (2022), glutamic acid has the molecular mechanism in mitigating stress such as low or high temperature. Transpiration and respiration are the main cause of weight loss in tomatoes (Gharezi *et al.*, 2012). However, reports by (Rab *et al.*, 2015) reported that after storage, fruit continue to respire and loss water to the surroundings environment due to transpiration.

Table 4.1: The effects of preharvest foliar application of Phytostim® biostimulant and storage condition on the Percentage weight loss (%) of tomatoes on day 0, 5, 10 and 15 of storage.

Treatments	Storage period (days)			
	0	5	10	15
Biostimulant concentrations				
(0%)	7.55±0.24 ^a	8.93±0.22 ^a	11.45±0.12 ^a	14.00±0.14 ^a
0.1%	3.38±0.23 ^f	5.72±0.23 ^e	9.92±0.02 ^b	10.75±0.13 ^d
0.5%	4.13±0.22 ^e	6.25±0.22 ^d	7.86±0.02 ^e	9.45±0.12 ^e
1%	4.72±0.21 ^e	5.60±0.23 ^e	7.00±0.01 ^e	8.24±0.23 ^f
2%	5.22±0.22 ^d	6.15±0.21 ^d	8.26±0.21 ^d	10.64±0.22 ^d
2.5%	5.65±0.21 ^c	8.11±0.12 ^b	9.22±0.21 ^c	10.82±0.21 ^c
3%	6.32±0.32 ^b	8.60±0.21 ^c	9.43±0.21 ^c	11.59±0.12 ^c
4%	6.11±0.22 ^b	7.23±0.21 ^a	8.50±0.21 ^b	10.89±0.22 ^b
SE ±	1.32	24.53	1.37	1,18
P value	0.03	0.05	0.002	0.04

Value (Mean± SE) followed by different letters on each column based on treatments were significantly different at ($p<0.05$), according to DMRT.

4.3.2 The effects of different concentrations of Phytostim® biostimulant on firmness of tomato during storage at ambient condition for 15 days.

The effects of preharvest Phytostim® concentrations showed significant ($p<0.05$) influence on the firmness of tomato. Table 4.2 shows the effects of different preharvest concentrations of Phytostim® biostimulant on the firmness of tomatoes on day 0, 5, 10, and 15 of storage. There was a decline in firmness, for all the studied treatments during the storage period at ambient condition. The concentration of 0% (control) experienced a decline in firmness as days of storage increased with 1.4N at day 0, then 0.3 N at day 10 then decreased to 0.2 N at day 15. On the other hand, the concentration of 0.1% showed an increase in firmness but when days increased, it started to decline with 5.5 N at day 0, then 4.2 N at day 5, then 3.6 N at day 10 and at day 15 it was 1.6 N. Reports by Gharezi *et al.*, (2012) showed that firmness is one of the most important parameters in visuality aspect because it determines the consumer preference and marketability of fruits. Further reports by Javanmardi and Akbari (2016) reported that firmness is an important physical parameter for postharvest storage, transportation and monitoring the fruit ripening process. On the other hand, Khedr *et al.*, (2023) reported that firmness is one of the most key factors in the visual quality of postharvest products because it is associated with fruit senescence and ripening once fruit shows signs of softening. Firmness and weight loss have a direct relationship, in this case, firmness decreased for all the studied treatments is due to the decrease in weight loss. Report is in agreement with findings by Ali *et al.*, (2004), who reported that a decline in fruit firmness after storage was due to high weight loss, which was enhanced by high water loss and cell wall degradation. Research by Silva *et al.*, (2009) revealed that firmness declined due to pectin depolymerization in the cell wall, which culminates in dissolution of pectin. In this study, the decline in firmness for all the studied treatments was probably due to bioactive compounds contained in Phytostim® as they are responsible for the delay of senescence which affects texture. In this sense, it has been reported that the extracts obtained from *Moringa* extract (leaves) contain phytochemicals such as glucosinolates, flavonoids, phenols, and antioxidant compound, that contribute in different ways to the delay of senescence and may have effects on respiration, transpiration rates, and on the texture and therefore contribute

to these positive effects in tomato fruits treated with the extracts. The results of this study are similar to that by (Zahra *et al.*, 2022) since they indicate that the application of antioxidant treatments increases the possibility of firmer fruits regardless of the concentration, because antioxidant treatments delay or slow down the ripening process. Furthermore, (Estiaque *et al.*, 2021) observed the highest firmness in cherry tomato fruits treated with garlic extracts compared to other organic treatments such as chitosan. However, (Aloui *et al.*, 2021) observed a decrease in weight loss and firmness, when using coatings generated from tomato residues in cherry tomato postharvest conservation, attributing this effect to the cuticle components and bioactive compounds present in the coatings generated.

Table 4.2: The effects of preharvest foliar application of Phytostim® biostimulant and storage condition on the Firmness (N) of tomatoes on day 0, 5, 10 and 15 of storage.

Treatments	Storage period days			
	0	5	10	15
Biostimulant				
Concentrations				
Control 0%	1.4±0.01f	1.2±0.11e	0.3±0.21g	0.2±0.01d
0.1%	5.5±0.02a	4.2±0.3a	3.6±0.23a	1.6±0.21a
0.5%	4.2±0.1b	3.5±0.3b	2.8±0.21b	1.3±0.21a
1%	3.6±0.2c	3.0±0.21c	2.5±0.11c	1.2±0.11b
2%	3.2±0.1d	2.9±0.22c	2.2±0.10d	0.4±0.01c
2.5%	2.1±0.21e	1.9±0.12d	1.2±0.02e	0.1±0.01c
3%	2.1±0.21e	1.8±0.22d	1.1±0.03f	0.3±0.01c
4%	1.5±0.01f	1.3±0.19e	1.2±0.1e	0.2±0.01d
SE%	1.50	1,08	1.09	0,61
P value	0.02	0.06	0.04	0.02

Value (Mean± SE) followed by different letters on each column based on treatments were significantly different at ($p < 0.05$), according to DMRT.

4.3.3 The effects of different concentrations of Phytostim® biostimulant on TSS of tomato during storage of ambient condition for 15 days.

The effects of preharvest Phytostim® concentrations showed significant ($p < 0.05$) influence on the TSS of tomato (Table 4.3). There was a gradual increase in TSS for all the studied concentrations as days increased. The concentration of 0 % had the least TSS with 1.9 % at day 0, increased to 2.5 % at day 5, then 3.8 % at day 10, and at day 15 migrated to 4.0 %. On the other hand, the concentration of 0.1% exhibited the highest TSS value compared to all the studied concentrations and control, with an increase of 4.2% at day 0, then 5.9% at day 5, then 6.9% at day 10 and then migrated to 7.7 % at day 15. Total soluble solids (TSS) reflect the tasting quality of produce and it is considered as an index of the ripening, an indicator of the number of soluble minerals and sugar present in fresh produce (Abiso *et al.*, 2015). The findings in this study are contrary to that by Tolasa *et al.* (2021) who reported that the TSS progressively increased as days increased. However, the results shown in this study are similar to that reported by Tietel *et al.*, (2012) who stated that TSS was high during the storage period attributed by a high temperature. Moringa can increase the glucose level in crops (Frederic *et al.*, 2020) therefore the high TSS in this regard can be attributed to the mechanism of action of Phytostim® that involves the increase of sugar content incorporated in moringa leaves and seeds. The polysaccharide fraction of the moringa seeds contains total sugar of 45% in which arabinose (44.7%) and galactose (13.1%) were present in the ratio of 3.4: 1. The findings in this study could be also due to the role of auxins in wide range of physiological and metabolic responses including delaying of the ripening process and decrease of fruit decay (Zeng *et al.*, 2006). *Moringa* is also rich in calcium which engages in the reduction of the activities of enzymes responsible for starch hydrolysis to soluble sugars. Furthermore, it could be attributed to the presence of higher temperature in ambient storage leading to higher rate of respiration and transpiration.

Table 4.3: The effects of preharvest foliar application of Phytostim® biostimulant and storage condition on the TSS (%) of tomatoes on day 0, 5, 10 and 15 of storage.

Treatments	Storage day			
	0	5	10	15
Biostimulant Concentrations				
Control 0%	1.9±0.01f	2.5±0.23f	3.8±0.33e	4.0±0.31f
0.1%	4.2±0.22a	5.9±0.34a	6.9±0.33b	7.7±0.21a
0.5%	3.8±0.23a	5.7±0.21b	6.5±0.12a	7.3±0.12b
1%	3.2±0.21b	4.9±0.22c	5.6±0.21d	6.9±0.22c
2%	3.0±0.23bc	4.4±0.22bc	5.7±0.21bc	6.6±0.32bc
2.5%	2.9±0.22c	3.4±0.21e	5.5±0.21c	6.3±0.43d
3%	2.2±0.02d	3.6±0.02d	4.7±0.23d	5.9±0.33e
4%	2.0±0.01e	3.4±0.01e	3.7±0.22e	4.9±0.32f
SE±	0.78	1.20	1.16	1.24
P value	0.02	0.03	0.04	0.01

Value (Mean± SE) followed by different letters on each column based on treatments were significantly different at ($p < 0.05$), according to DMRT.

4.3.4 The effects of different concentrations Phytostim® biostimulant on pH of tomato during storage of ambient condition for 15 days.

The pH values of tomatoes fruits were not significantly ($p > 0.05$) influenced by preharvest treatments of Phytostim® on day 0, 5, 10 and 15 storage periods in ambient condition (Table 4.4). The pH values of control tomatoes from day 0 to day 15 were acidic. The lowest pH values were recorded on tomatoes sprayed with concentrations of 0 %. With pH 2.0 at day 0, it increased to 4.50 at day 15. Whereas the highest was recorded on the control and tomatoes sprayed with concentrations of 0.1%, with the

pH of 4.0 at day 0 to 4.53 at day 15 during ambient condition. pH measures the acidity or alkalinity level of fruits. In this regard, all treatments were acidic during the storage period at ambient condition. pH is a good index of ripening indicating the degradation or respiration of organic acids (Mujtaba and Masud, 2014). The results of this study showed that pH values increased with the advancement of ripening and this is similar to results by Al-Dairi *et al.*, (2021). The reduction of pH values with Phytostim® treatments could probably be due to the availability of more water inside the cells through production of osmotic pressure and enzymatic activities (Guan *et al.*, 2009). Respiration and metabolic activities of tomatoes are directly related to the temperatures of the storage (Arah *et al.*, 2015), which might have influenced the pH to be acidic.

Table 4.4: The effects of preharvest foliar application of Phytostim® biostimulant and storage condition on the pH of tomatoes on day 0, 5, 10 and 15 of storage

Treatments	Storage days			
	0	5	10	15
Biostimulant Concentrations				
Control 0%	2.0±0.02	2.98±0.02bc	4.32±0.34b	4.50±0.3c
0.1%	4.0±0.01a	4.11±0.01a	4.41±0.34a	4.53±0.31b
0.5%	3.88±0.04b	4.06±0.05b	4.27±0.44b	4.46±0.03d
1%	2.98±0.03c	3.08±0.22d	4.31±0.32bc	4.55±0.04a
2%	2.66±0.03c	3.11±0.23d	4.28±0.32b	4.55±0.04a
2.5%	2.45±0.03c	3.09±0.24d	4.31±0.34bc	4.47±0.03e
3%	2.32±0.03c	3.00±0.22d	4.35±0.34b	4.49±0.01d
4%	2.00±0.03c	2.39±0.34d	4.32±0.45b	4.52±0.23bc
SE±	0.79	0.55	0,04	0.03
P value	0.43	0.24	0.45	0.32

Value (Mean± SE) followed by same letters on each column based on treatments were not significantly different at ($p < 0.05$), according to DMRT.

4.3.5 The effects of different concentrations Phytostim® biostimulant on colour change of tomato during storage of ambient condition for 15 days.

Colour change in tomatoes allows the monitoring of visual quality during storage as well as the selection of new varieties with unique colour characteristics (Thole *et al.*, 2020). Colour development in tomatoes as an indicator of fruit ripeness, is important,

particularly in cherry tomato fruits (Gharezi *et al.*, 2012). In this study, the colour of the tomato fruits showed differences between treatments, with significant differences ($p < 0.05$) observed for parameter b^* (Table 4.5). Fruits treated with 1% concentration showed the lowest b^* value at day 0 for concentration of 0, 2 and 2.5%, indicating red fruits (dark red). In the hue ($^{\circ}h$) results, significant differences ($p < 0.05$) were also found among all the studied treatments. The results further demonstrated day 0, hue angle (h°) of tomato fruits treated with different concentrations of Phytostim® biostimulant between 2.5, 3 and 4% had higher values ($130^{\circ} < 150^{\circ}$) meaning there were light red in color as compared to control. Also, fruits treated with different concentrations of Phytostim® biostimulant between (0.1 to 2%) ranged from ($90^{\circ} < 130^{\circ}$) meaning that they were red. Therefore, fruit colour was not modified except for fruits treated with 1% concentration. Colour is also one of the most important parameters of fruit visuality and indicate whether tomatoes are still in good condition. Colour determines the fruit maturity stages of tomatoes. Colour of tomato fruits during storage at ambient condition was attributed to tissue softening and skin darkening (Aloui *et al.*, 2021). Tomato fruit changes its colour from green to red during ripening, because of chlorophyll degradation simultaneously to carotenoid biosynthesis as it has been observed in our results similarly to the reported by Hobson and Davies (1971) and (Yahia *et al.*, 2017).

Table 4.5: The effects of preharvest foliar application of Phytostim® biostimulant and storage condition on the colour of tomatoes on day 0, 5, 10 and 15 of storage.

Treatments	Storage Days	L	a^*	b^*	C	$^{\circ}h$
Control (0%)	0	64.89±0.32 ^a	2.91±0.32 ^f	13.77±0.21 ^c	14.08±0.01 ^d	76.95±0.33 ^a
	5	59.98± 0.2 ^b	16.73±0.12 ^d	15.70±0.23 ^c	23.08±0.02 ^b	43.55±0.32 ^d
	10	53.06±0.3 ^a	16.74±0.13 ^d	6.39±0.01 ^e	17.92±0.22 ^d	20.88±0.33 ^f
	15	52.46±0.4 ^a	20.29±0.15 ^a	14.08±0.32 ^b	24.70±0.23 ^b	34.75±0.22 ^e
(0.1%)	0	62.86±0.4 ^a	15.32±0.62	17.81±0.32 ^c	23.49±0.32 ^b	89.30±0.34 ^d
	5	51.75±0.3 ^b	20.10±0.13 ^a	15.35±0.22 ^d	25.29±0.14 ^b	37.37±0.23 ^e
	10	56.16±0.3 ^c	21.48±0.13 ^a	10.89±0.21 ^d	24.08±0.15 ^{bc}	26.87±0.23 ^d
	15	49.08±0.2 ^d	18.46±0.14 ^b	8.44±0.01 ^d	20.29±0.33 ^{bc}	24.58±0.22 ^d
(0.5%)	0	60.34±0.2 ^a	12.58±0.02 ^f	20.06±0.34 ^a	23.68±0.23 ^{bc}	98.90±0.31 ^a
	5	57.48±0.2 ^b	22.14±0.13 ^g	12.14±0.35 ^b	12.16±0.23 ^e	25.26±0.21 ^f
	10	56.96±0.31 ^b	24.41±0.13 ^f	11.63±0.37 ^b	27.04±0.12 ^a	25.47±0.12 ^f
	15	43.47±0.32 ^d	11.91±0.10 ^g	3.25±0.35 ^f	12.35±0.11 ^e	15.28±0.21 ^g
(1%)	0	65.04±0.22 ^a	1.03±0.46 ^f	22.73±0.36 ^a	22.75± 0.4 ^{ab}	98.61±0.01 ^b

	5	50.34±0.21 ^c	11.13±0.22 ^b	6.79±0.36 ^h	13.04±0.32 ^e	31.36±0.02 ^e	
	10	49.51±0.21 ^c	15.78±0.41 ^c	13.43±0.33 ^{bc}	20.72±0.42 ^a	40.41±0.04 ^c	
	15	43.81±0.21 ^d	12.96±0.01 ^b	3.37±0.34 ^{fg}	13.39±0.44 ^e	14.56±0.03 ^f	
(2%)	0	62.88±0.31 ^a	0.55±0.022 ^g	18.35±0.35 ^a	18.35±0.42 ^f	109.73±0.02 ^a	
	5	56.39±0.2 ^b	14.04±0.22 ^d	12.72±0.37 ^e	18.94±0.40 ^f	42.17±0.01 ^e	
	10	53.91±0.21 ^c	19.98±0.31 ^c	10.44±0.38 ^d	22.54±0.39 ^a	27.58±0.22 ^f	
	15	44.43±0.22 ^d	9.91±0.01 ^e	1.48±0.24 ^{ef}	10.02±0.38 ^g	8.50±0.23 ^g	
(2.5%)	0	61.36±0.21 ^a	2.42±0.04 ^e	21.48±0.22 ^a	22.61±0.37 ^a	108.85±0.32 ^a	
	5	60.62±0.22 ^a	14.42±0.05 ^c	16.71±0.27 ^e	22.07±0.36 ^a	49.22±0.31 ^f	
	10	54.80±0.22 ^c	22.69±0.01 ^b	9.11±0.28 ^d	24.45±0.23 ^{bc}	21.86±0.22 ^g	
	15	42.17±0.23 ^e	9.79±0.04 ^e	4.46±0.29 ^g	10.76±0.23 ^e	24.50±0.41 ^g	
(3%)	0	60.24±0.21 ^a	14.71±0.21 ^d	21.64±0.30 ^a	26.16±0.32 ^b	103.79±0.12 ^d	
	5	54.31±0.31 ^c	15.97±0.22 ^d	10.04±0.31 ^c	18.86±0.43 ^d	32.16±0.12 ^{ef}	
	10	52.48±0.31 ^c	13.30±0.21 ^d	9.48±0.32 ^d	16.33±0.45 ^d	35.48±0.21 ^{fg}	
	15	46.02±0.31 ^d	14.26±0.31 ^e	6.35±0.33 ^d	15.61±0.44 ^d	23.99±0.11 ^h	
(4%)	0	55.38±0.21 ^b	24.48±0.21 ^a	15.30±0.34 ^b	28.87±0.41 ^{ab}	102.02±0.12 ^{ab}	
	5	54.23±0.21 ^c	24.36±0.23 ^a	18.06±0.35 ^b	26.55±0.42 ^a	21.03±0.22 ^{bc}	
	10	54.73±0.22 ^c	24.07±0.21 ^a	8.59±0.34 ^c	25.56±0.41 ^a	19.65±0.23 ^c	
	15	46.81±0.24 ^d	16.48±0.12 ^c	6.84±0.35 ^c	17.86±0.22 ^d	22.56±0.02 ^d	
P value		P<0.04	P<0.437	P<0.422	P<0.001	P<0.007	P<0.002

Value (Mean± SE) followed by same letters on each column based on treatments were not significantly different at ($p<0.05$), according to DMRT.

4.3.6 The effect of different concentrations of Phytostim® biostimulant on protein.

The effects of different concentrations of Phytostim® biostimulants significantly ($p<0.05$) influenced the protein content of tomatoes. The treated plants showed to be higher in comparison to the untreated plants. The high protein content found was 79% at 0.1% concentrations of Phytostim® biostimulants. The lowest protein content found was at 22% at 0% compared to all the other studied concentrations. The results obtained showed that Phytostim® is an amino acid biostimulant that can enhance the protein content as shown in the (Figure 4.1). According to (Vergara-Jimenez *et al.*, 2017) stated that Moringa extracts might encompass bioactive elements capable of influencing metabolic processes within plants. These substances have the potential to impact the pathways associated with the synthesis of proteins. Therefore, Phytostim® which is a moringa based biostimulant contains several number of bioactive compounds that may indirectly contribute to protein synthesis by maintaining optimal conditions for growth. This supports that biostimulants contain the natural antioxidants that make the various crops overcome environmental stresses (Ghebreslassie, 2003).

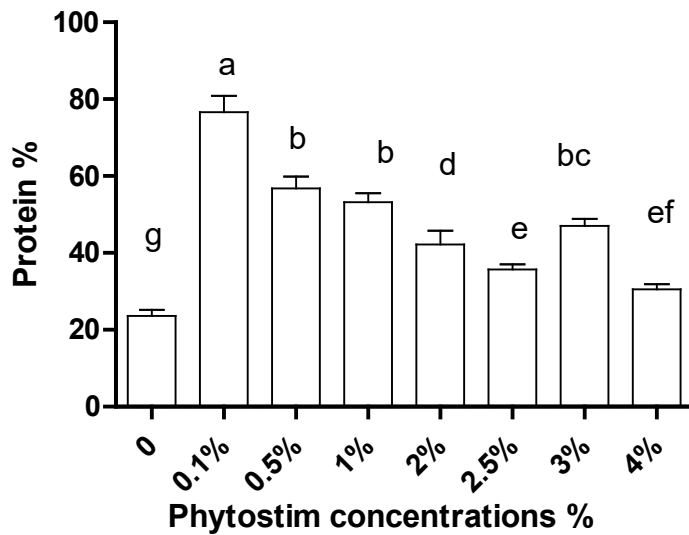


Figure 4.1: The effect of different concentrations of Phytostim® biostimulant on protein.

4.3.7 The effect of different concentrations of Phytostim® biostimulant on micro and macro elements of tomato.

The effects of different preharvest Phytostim® biostimulants on the mineral elements of tomatoes were assessed. The results revealed a significant ($p < 0.05$) influence on mineral elements including that of calcium (Ca), magnesium (Mg), potassium (K), phosphorus (P), nitrogen (N), iron (Fe), copper (Cu) and zinc (Zn) increase was shown in Phytostim® biostimulant treatments fruits in comparison to the untreated plants.

Among the macro-elements studied, potassium was by far the most abundant mineral components in the fruit of tomato, followed by calcium, phosphorus, magnesium and finally sodium (see Table 4.6). The results indicate that the increase in the concentration of macronutrients (K and Mg) in fruits of the cultivar of tomato could have been induced by the application of the moringa based biostimulant 0.5% and could have been mediated through multiple mechanisms involving: (1) the presence

of bioactive compounds in Phytostim® biostimulant (soluble peptides, carbohydrates and free amino acids) which may have increased sink strength and influenced the movement of nutrient substrates, including minerals, within the plant (Calvo *et al.*, 2014), (2) the greater uptake of minerals through a stimulation of root growth (Billard *et al.*, 2014; Colla *et al.*, 2014), and (3) the higher expression of nutrient transporters in cell membranes (Ertani *et al.*, 2017).

Most studies which reviewed secondary metabolites in tomato, there is a gap in the concentration of essential nutrient elements found in tomato despite the fact that it is a nutritive fruit that is rich in micro-and macronutrients. According to Ayomide *et al.* (2019), tomato is susceptible to nutritional and organoleptic degradation, and this brings about the need for various postharvest storage methods applied in retaining its quality, nutrients, colour, and texture. Normally, once fruits are harvested, they exhibit a gradual loss in quality. There is a myriad of processes and conventional methods of storage that can be used to slow down deterioration and bring about an increase in shelf or storage life. Evaluating the effect of different concentration of Phytostim® biostimulant showed a significant impact on the mineral composition of tomato ($p < 0.05$). Results showed that the concentration of essential nutrient elements including calcium (Ca), potassium (K), magnesium (Mg) and phosphorus (P) were predominantly the highest in the test cultivar of tomato (table 4.6). A clear trend demonstrated higher contents of the nutrient element in the concentration of 0.1% except for K content which demonstrated higher content in the 0.5%. Furthermore, 0.1% contained higher nutrients than the control. Calcium, potassium, and magnesium are found in tomato, providing about 8%, 9% and 14%, respectively, of the daily value (Sacks *et al.*, 2001). Calcium is well known for its function in maintaining bone and teeth health but is also critical to cell signalling, blood clotting, muscle contraction, and nerve function. Dietary potassium intake has been demonstrated to significantly lower blood pressure in a dose-responsive manner in both hypertensive 87 and normotensive individuals in observational studies (Appel *et al.*, 1997; Sacks *et al.*, 2001; Appel, 2010; Sacks and Campos, 2010).

Table 4.6: The effects of different concentrations of Phytostim® biostimulant on the macronutrients of tomato.

Macro elements	Ca	Mg	K	P	Na
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Phyostim® concentrations					
Control 0%	26.1 ^e	27.7 ^d	607 ^g	3.79 ^f	8.57 ^c
0.1%	40.2 ^a	44.7 ^a	870 ^a	48.8 ^a	11.2 ^a
0.5%	38.7 ^a	38.9 ^b	864 ^b	36.8 ^b	11.2 ^a
1%	37.1 ^b	35.5 ^c	788 ^c	9.78 ^e	10.7 ^b
2%	33.7 ^c	33.4 ^b	740 ^d	8.67 ^f	10.5 ^b
2.5%	32.8 ^c	31.4 ^c	676 ^e	8.65 ^d	9.89 ^c
3%	28.2 ^d	28.4 ^d	646 ^f	6.54 ^d	9.43 ^d
4%	27.3 ^d	27.9 ^b	623.3 ^d	4.47 ^e	8.54 ^e
SE±	1.85	2.25	1.25	2.65	2.55
P-Value	0.02	0.03	0.04	0.04	0.01

Macro minerals: Magnesium (Mg), Calcium (Ca). Nitrogen (N), Sodium (Na) Potassium (K). Value (Mean± SE) followed by different letters on each column based on treatments were significantly different at ($p < 0.05$), according to DMRT.

Table 4.7: The effects of different concentrations of Phyostim® biostimulant on the micronutrients of tomato.

Micro elements	Fe	Cu	Zn
Phyostim® concentrations			
Control 0%	10.7 ^b	1.01 ^d	1.24 ^d
0.1%	18.5 ^a	1.62 ^a	1.92 ^c
0.5%	11.1 ^b	1.07 ^c	2.79 ^b
1%	8.55 ^{bc}	0.946 ^f	0.038 ^e

2%	9.57 ^c	1.12 ^b	0.35 ^d
2.5%	8.19 ^d	0.941 ^e	1.71 ^c
3%	8.22 ^{bc}	0.97 ^e	1.0753 ^{bc}
4%	8.65 ^{bc}	0.994 ^d	3.93 ^a
SE±	1.89	1.88	1.87
P-Value	0.02	0.05	0.02

Micro minerals: Copper (Cu), Zinc (Zn), Iron (Fe). Value (Mean± SE) followed by different letters on each column based on treatments were significantly different at ($p < 0.05$), according to DMRT.

4.4 Conclusion

The results shown in this chapter of the study reveal that the postharvest attributes and nutritional composition can be enhanced at the concentration of 0.1% of Phytostim®. Furthermore, the results showed that foliar-spraying fruits of the tomato cultivar with 0.1 can extend its shelf life at ambient condition until day 15, compared to control. Phytostim® is an eco-friendly and cost-effective method to preserve tomato fruits without deteriorating their quality. Thus, the current findings demonstrate that employing Phytostim® as a promising and effective natural compound can be an alternative technology to synthetic fertilizer and provide desirable tomato fruits to consumers. Therefore, Phytostim® can be adopted by farmers such as ZZ2 companies to preserve tomato fruits.

5.6 References

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Chapter 6

6.1 Summary of findings

The increase in human population, coupled with an increase in food insecurity, motivate for a need to conduct research on the sustainability of fruits and vegetables partly through using biostimulants as agricultural inputs. The approach has gained immense popularity among researchers and crop farmers. The results of this study provide new empirical information on the effectiveness of a plant biostimulant named Phytostim®. This research showed enhanced growth and yield attributes, improved shelf life of tomato fruits as well as greater nutritional composition of tomato. The primary objective of this study to evaluate effects of supplying different concentrations of Phytostim® biostimulant on growth and yield attributes of tomatoes. Results revealed that Phytostim® biostimulant enhanced the growth attributes stem diameter (mm), plant height (cm), and leaf length (cm). Yield attributes included size (mm), diameter (mm), number of fruits, and fresh weight of the fruits (g) in the main, the results obtained concluded that of the test concentrations of Phytostim, the most effective optimum concentration for enhancing the growth and yield attributes of tomatoes was at 0.1%. The secondary objective of this study was to assess effects of different concentrations of Phytostim® biostimulant on postharvest quality attributes and nutritional composition of tomatoes. The results showed that Phytostim® biostimulant enhanced the postharvest quality attributes, prolonged shelf life with six days higher than the retailers' shelf life and further improved nutritional composition of tomatoes at the concentration of 0.1%. It can further be concluded that Phytostim® biostimulant is very effective at low concentrations.

6.2 Significance

The results documented in this study will be used in the future to improve the production of agricultural crops (fruits and vegetables) in the family of tomatoes. As shown in other scholarly studies, the use of biostimulants can reduce the impact of climate change, global warming, and greenhouse gas emissions given that they are eco-friendly and less harmful. Therefore, Phytostim® is one of biostimulants that can be introduced to farmers to improve their productivity through enhancing the growth of food crops while extending their shelf life at ambient condition without compromising the nutritional composition.

6.3 Recommendations

Based on the findings of this study, it is recommended to use the 0.1% concentration of Phytostim® biostimulant in tomato, can enhance growth, yield, and postharvest attributes in settings of small-scale and commercial cropping systems. Furthermore, the 0.1% concentrations of Phytostim biostimulant has proven to be effective when applied on leaves, that is, as foliar-spray as it can further enhance the nutritional composition of 'Roma' tomato. When applied at 0.1%, Phytostim® can be effectively increase plant performance, however, the efficacy is yet to be assessed along that of synthetic fertilizers. Whatever the case, it is an approach that is economically reasonable. It is anticipated that the results of this study will encourage further studies on whether the application of 0.1% concentration of Phytostim® on tomato fruit and other agricultural food crops does enhance growth, yield, and postharvest attributes under other settings. Ensuring the effectiveness and successful commercialization of moringa-based biostimulants during storage is a necessity. Due to the demand for high-quality fruit in the market, the use of moringa-based biostimulants such as Phytostim® is significant in these markets.

6.4 General Conclusions

In conclusion, determining a concentration of a biostimulant that is most effective is very important and in the case of this study, that of Phytostim was assessed in fruits of tomatoes, treated at pre- and post-harvest stages. The results of this research work shows the potential nutritional importance of tomato as well as its role in health and nutrition improvement. Incorporation of tomato into a diet may help in improving its quality and subsequently reduce the risk for the development of chronic non-communicable diseases. The study revealed that using Phytostim® biostimulant as a moringa-based biostimulant at a low concentration can be an affordable source of nutrients. As a result of complex in the nature of disease and different factors linked to their occurrence.