RESPONSE OF BIOZYME AND CHITOSAN ON GROWTH, YIELD AND QUALITY OF STRAWBERRY (*Fragaria x ananassa* Duch) Cv. CHANDLER

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A Thesis Submitted to the Graduate School in Partial Fulfillment of the Requirements for the Master of Science Degree in Horticulture of Egerton University

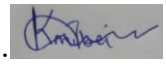
EGERTON UNIVERSITY

AUGUST, 2023

DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been presented in this University or any other for the award of Degree.

Signature …  Date...03/05/2023.

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Recommendation

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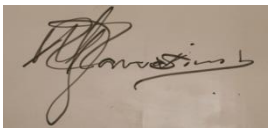
Signature ……  Date……5.6.2023…

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DEDICATION

I dedicate this thesis to my parents, siblings and relatives for their encouragement and prayers throughout my studies at Egerton University.

ACKNOWLEDGEMENTS

I thank God for His love, care, strength, guidance and good health that He has granted me throughout my studies at Egerton University. I also thank the African Development Bank for their financial support in paying for my tuition fee and the research project through Professor Joshua Ogweno and Dr. Isaac Maina Kariuki. I am also grateful to Egerton University for giving me a chance to pursue my masters degree programme. My profound gratitude goes to my supervisors, Prof. Robert Morwani Gesimba and Dr. Japhet Muthamia for their tireless guidance, encouragements and constructive comments throughout my study. Special thanks go to the staff of the Department of Crops, Horticulture and Soils of Egerton University for the moral support they accorded me during my studies. I am also grateful to Principal Technologist, Horticulture section, Mr. Jacob Ochieng and his team for their technical support and advice. I am also grateful to my family members, colleagues, relatives, Willy Tuimising, Rono Tuimising, Geoffrey Korir, Mercy Koech, Edinah Shikoli and all those who contributed to this work in one way or the other.

ABSTRACT

Biostimulants (chitosan and biozyme) are emerging category of products that target to increase the production of strawberry (*Fragaria x ananassa* Duch) and are used as alternative to agrochemicals. This is due to the growing concern of consumers on healthy and safe products. With the exclusion of agrochemicals in strawberry production, the yield has declined by 5-34% due to limited nutrition. The objective of this study was to investigate the response of biozyme and chitosan rates on the growth, yield and quality of strawberry fruit at Horticulture Research and Teaching Farm (Field 3) of Egerton University, Njoro, Kenya. The experimental design was randomized complete block design with nine treatments replicated three times. The treatments were; control (distilled water), 25ppm chitosan, 50ppm chitosan, 25ppm biozyme, 25ppm biozyme+25ppm chitosan, 25ppm biozyme+50ppm chitosan, 50ppm biozyme, 50ppm biozyme+25ppm chitosan, 50ppm biozyme+50ppm chitosan. The results obtained shows that the combination use of 25ppm biozyme and 25ppm chitosan significantly influenced the growth, yield and quality of strawberry plant. Maximum number of leaves, leaf length, leaf width, plant height, flower number, fruits number, fruit weight, fruit length, fruit diameter, total soluble solids and shelf-life was achieved with the use of 25ppm biozyme and 25ppm of chitosan in combination. Titratable acidity of strawberry fruits was lowest with the application of 25ppm biozyme and 25ppm of chitosan in combination. Thus, findings from the current study indicate that biozyme and chitosan at their lowest rates could be used as environment-friendly alternatives for sustainable production of high quality strawberry. Therefore, the recommendation was that the combination use of 25ppm biozyme and 25ppm chitosan can be used as foliar spray in the growth and development of strawberry plants thus high strawberry fruit yield.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
CM	Centimeter
CV	Cultivar
DAP	Days After Planting
FAOSTAT	Food and Agriculture Organization Statistics
HSD	Honest Significance Difference
KES	Kenya Shilling
MM	Millimeter
PPM	Parts Per Million
RCBD	Randomized Complete Block Design
USDA	United States Department of Agriculture

CHAPTER ONE
INTRODUCTION

1.1 Background information

Strawberry (*Fragaria×ananassa* Duch.) is one of the most popular and widely consumed berry due to its taste, health benefits and other desirable qualities (Parveen *et al*., 2012). The main characteristics associated with the quality of ripe strawberries are their texture, flavor (sugar to acid ratio and volatile compounds) and colour (Nadim *et al*., 2015). Strawberries are also rich in nutrients and antioxidants (e.g. polyphenols and anthocyanins, vitamins and amino acids) (Van *et al*., 2013). The largest strawberry (*Fragaria x ananassa* Duch) producers in the world are China, United States of America, Spain and Japan (FAOSTAT, 2017). China is the leading producer of strawberry with the annual production of 3.7 million tons while in Kenya its production is 942 tons annually (FAOSTAT, 2017).

In most strawberry farmlands in the world including Kenya, synthetic fertilizers and pest management products are applied during growth and development to maximize yield. The continuous use of synthetic chemicals (fertilizers and pesticides) in both crop production and protection has become a threat to both human health and environment. Furthermore, the use of the chemicals has been reported to affect the soil fertility, health and crop productivity adversely mainly due to their negative effects on soil fauna and flora (Seneviratne, 2009). Due to these problems, scientists are trying to come up with other production techniques to exclude synthetic chemicals in strawberry production. These techniques are of natural origin, environmentally friendly, cost effective and sustainable-based which can be used for promoting plant growth and nutritional quality (Saavedra *et al*., 2016).

Biostimulants can enhance the growth, yield, and quality of crops significantly. Several types of plant/algal biostimulant provide added benefit to plants when applied as foliar spray or drenching. Scientific studies in this field have been going on for close to 70 years. However, there are still unknowns and commercialization has been relatively minor.

Chitosan, a given name to a deacetylated form of chitin, is a natural biodegradable compound derived from crustaceous shells such as crabs and shrimps, whose main attributes corresponds to its polycationic nature (Bautista-Baños *et al*., 2006). It is considered environment-friendly for agricultural uses as it is easily degradable in the environment and is nontoxic to humans. Chitosan and its derivatives have been reported to elicit natural defense responses in plants and have been used as a natural compound to control pre- and post-harvest pathogens (Malerba *et al*., 2018). Antimicrobial activities of chitosan against various

phytopathogens have been reported (Rahman *et al*., 2014). Enhancement of storability and preservation of anthocyanin content in chitosan-coated strawberry fruit has been reported from multiple studies (Ghaouth *et al*., 1991). Chitosan has been widely used as a coating agent of various fruit mainly for protection from post-harvest losses due to microbial infections (El-Sawy *et al*., 2010; Sakif *et al*., 2016). However, many investigators have also reported that using chitosan as a foliar spray increased vegetative growth, yield and biochemical contents in vegetable plants (El-Miniawy *et al*., 2013; Mukta *et al*., 2017; Pirbalouti *et al*., 2017).

Biozyme, is extracted from *Ascophyllum nodosum* (L.) Le Jolis, a seaweed alga which is known to be rich in cytokinins and auxin precursors, enzymes, some chelating agents, minerals, betaines, polyamines, organic acids, oligosaccharides, amino acids, and hydrolyzed proteins (Khan *et al*., 2009). *Ascophyllum nodosum* is the most widely researched seaweed species for agricultural purposes (Verkleij, 1992). Seaweed now is recognized as an excellent source of natural plant growth regulators with demonstrated activity (Cardozo *et al*., 2007; Khan *et al*., 2009), which include cytokinins (Blunden, 1977) and gibberellins (Wildgoose *et al*., 1978). Some plant/algal residues such as chitosan and seaweed (*Ascophyllum nodosum*) are believed to be good sources of biostimulants (Khan *et al*., 2009).

Kumar *et al*. (2010) describe biozyme as an environmentally friendly growth stimulant which enhances the plant's physiological system by improving yield. It increases plant nutrient uptake by promoting solubilisation of nutrients (Freitas *et al.,* 1997) and symbiotic nitrogen fixation (Boddey & Dobereiner, 1995) leading to enhanced fruit set, quality and general crop performance. The purpose of study was to investigate the response of biozyme and chitosan biostimulants on growth, yield and quality of strawberry plants grown in a situation where no synthetic fertilizers are used and therefore indicate new potential solutions to improve nutrient availability to plants in an organic system thus enhancing productivity.

1.2 Statement of the problem

Strawberry farming is a multi-billion dollar business around the world. The strawberry fruit is a high value crop for export and local market, though the export market has not been exploited in Kenya due to limited supplies. The estimated total strawberry production in Kenya is 1487 tons while the demand is over 15,000 tons. It is worth noting that 90 percent of strawberry (fresh and pulp) is imported into Kenya. The enormous use of inorganic fertilizers and pesticides on the environment in the production of the strawberry in many parts of the world, including Kenya also has been a great challenge. This is due to the fact that these

inorganic fertilizers mainly affect nutrient availability (e.g., P, Fe, Mn, Zn and Cu) for plant uptake due to the formation insoluble complexes (e.g., tricalcium phosphate in alkaline soils or ferric phosphate/aluminum phosphate in acid soils). Also, the activity of the micro-organisms is affected and are expensive to small scale farmer leading to low production. This reduction in the supply of strawberry fruits affects the demand causing companies like Trufood, Zesta, Brookside and New Kenya Co-operative Creameries Companies among others which are in need of strawberry fruits to import them so as to meet their daily demands. On the same note, scientific-based information on biostimulants and their use on crop specificity is still incomplete especially in Kenya and thus they are not currently used. Several studies on biozyme and chitosan biostimulants have been done, but research pertaining to contribution of these biostimulants products to fruit tree species on the establishment, yield and fruit quality remains extremely minor. Therefore, this study was done to investigate the response of biozyme and chitosan on growth, yield and quality of strawberry Cv. chandler under field condition.

1.3 Objectives

1.3.1 General Objective

To contribute towards increased knowledge and production of strawberry fruit through enhancement of fruit yield and quality by using biozyme and chitosan as foliar spray under field conditions for increased nutritional security in Kenya

1.3.2 Specific Objectives

i. To determine the effect of biozyme and chitosan on growth and yield of strawberry (*Fragaria x ananassa* Duch) fruit
ii. To determine the effect of biozyme and chitosan on quality of strawberry (*Fragaria x ananassa* Duch) fruit
iii. To determine the interaction effect of biozyme and chitosan on growth, yield and quality of strawberry (*Fragaria x ananassa* Duch) fruit

1.4 Hypotheses

i. There is no significant effect of biozyme and chitosan on growth and yield of strawberry (*Fragaria x ananassa* Duch) fruit
ii. Biozyme and chitosan have no significant effect on quality of strawberry (*Fragaria x ananassa* Duch) fruit

iii. There is no significant interaction effect of biozyme and chitosan on growth, yield and quality of strawberry (*Fragaria x ananassa* Duch) fruit

1.5 Justification

Foliar application of biozyme and chitosan in strawberry production helps in enhancing the yield and nutritional properties of strawberry fruit crops and also reduces the health risks. According to Calvo *et al.* (2014), biostimulant effects on plants are amongst other, to foster plant growth and development throughout the crop life cycle from seed germination to plant maturity; to improve efficiency of the plant's metabolism to induce yield increases and enhancing crop quality; to increase plant tolerance to and recovery from abiotic stresses; to facilitate nutrient assimilation, translocation and use; to enhance quality attributes of produce, including sugar content, colour, fruit seeding, rendering water use more efficient; to enhance certain physicochemical properties of the soil and foster the development of complementary soil micro-organisms. In addition, biostimulants also enhance root and foliage development, improve soil texture and structure, increase availability of micro and macro nutrients, improve plant's ability to recover from disease and insect damage, enhance plant resistance to environmental stresses, improve the efficiency of any fertility program and reduce the effects of pH and soil imbalance (EBIC, 2012). They also have the ability to modify physiological processes of plants, elicit natural defense responses, promoting solubilization of nutrients (Freitas *et al.,* 1997) and symbiotic nitrogen fixation together with the increase in soil enzymatic and microbial activities. This will change the situation of low yields in organic strawberry production to high yields. They are also recognized as environment-friendly compounds with beneficial effects on plants. This is due to the fact that they decrease the use of mineral fertilizers by increasing the amount of macro- and micro-nutrients taken up by plants, positively influencing root morphology and plant growth. Biozyme and chitosan are also biodegradable, biocompatible and have been reported to have strong antimicrobial and antifungal activities. They also contain nutrients in a naturally chelated form which helps in improving cell division and cell enlargement resulting into increased production. Also, the use of biostimulants enhances plant resilience to the nutrient limitation. The applications of biostimulant substances promote nutrient uptake and assimilation. The increase of plant nutrient uptake is due to an increase in soil enzymatic and microbial activities, modifications in root architecture as well as an enhancement in micronutrient mobility and solubility.

CHAPTER TWO
LITERATURE REVIEW

2.1 Cultivated strawberry

The cultivated strawberry (Fragaria × ananassa) is an important fruit which originated from natural hybridization between *Fragaria virginiana* and *Fragaria chiloensis*. Due to good flavor, fragrance and nutritional value it is a very popular crop (Hossain *et al*., 2018; Qiao *et al*., 2016). The plant belongs to Rosaceae family. It is a low growing herbaceous plant with fibrous root system and its crown produces basal leaves. Typically, they have three leaflets, but the leaflets number may vary from one or five. The flowers are usually white, borne in small clusters on slender stalks arising from the leaf axils. The root system with aging becomes woody and the crown (of mother plant) sends out runners (stolons) that touch ground and root, thus enlarging the plant vegetatively (Hummer *et al.*, 2011; Poling, 2012; Trejo-Téllez *et al*, 2014).

Strawberry fruits are highly prized for their universal appeal to the human senses of sight, smell, taste and nutritional value. Common uses of fresh strawberries include sliced on cakes, breakfast cereal, and in salads (both mixed fruit and green) and dipped whole in melted chocolate. Processed strawberries are used in ice cream, jam, fruit leather, and mixed drinks. Fresh strawberries can be a valuable component of a healthy diet. Strawberries are low in calories, but high in fiber, folic acid, vitamin C, and several other antioxidants (Cao *et al.*, 1998).

2.2 Strawberry growth and reproduction

2.2.1 Vegetative Growth

The strawberry plant is an herbaceous perennial with short internodes forming a modified stem rosette (Savini *et al*., 2005). This modified stem is commonly known as a crown, where long-petiole trifoliate leaves and axillary meristems converge spirally around its axis, ending in a terminal inflorescence. Strawberry leaves present a typical dicotyledonous structure with long petioles and foliaceous basal stipules (Savini *et al*., 2005). Leaf lifespan can exceed 3 months in favorable conditions (Poling, 2012). Axillary meristems can differentiate into branch crowns, which stay near and are structurally identical to the original crown, or stolons (also called runners), which give rise to separate daughter plants (Demchak, 2011). Crowns typically produce one to two branch crowns in a season but have been known to produce more than five;

from a production standpoint, three to four total crowns per plant is desirable, as more can result in decreased fruit size (Poling, 2012).

2.2.2 Flower Structure

Inflorescences have two internodes and develop terminally on the crown or branch crown of the plant in a structure known as a dichasial cyme (Savini *et al*., 2005). Dichasial cymes have a terminal, primary flower branch with opposite secondary branches beneath the terminal bud, leading to secondary flowers. In strawberry, the inflorescence is commonly known as a flower cluster, and the primary flower, known as the "king lower", typically bears the largest fruit (Thomson, 2004). Secondary branches begin at the juncture of the first and second internodes; some inflorescences have tertiary and quaternary branches and flowers as well. Strawberry flowers have five sepals; fleshy green structures beneath the petals which enclose the flower at bud stage and eventually become the 'calyx', or cap of the berry. Stamens discharge pollen and fertilize the pistils, which are secured on a conical stem known as the receptacle. This receptacle becomes the full, fleshy "berry" at fruit maturity. Despite this plant's common name, the fruit itself is not botanically classified as a berry. The seed-like organs embedded on the epidermis of the receptacle are actually modified dry fruits known as achenes. Each achene is connected to the interior of the receptacle by fibrovascular strands, and hold the true seed within their pericarp (Fait *et al*., 2008). In Fragaria vesca auxin and gibberellin biosynthesis occurs in the endosperm and seed coat of the developing achenes, which in turn triggers the maturity of the surrounding receptacle (Kang *et al*., 2013). Because the strawberry fruit contains multiple achenes and is comprised of a receptacle in addition to its ovaries it can be classified as both an aggregate and accessory fruit.

2.2.3 Genetics of flower induction

The underlying genetics that promote or inhibit flowering is complex and the idea of florigen, a plant hormone (or family of hormones) responsible for flower initiation and development in all flowering plant species. A quest to isolate and identify the florigen hormone took place thereafter, spanning the rest of the 20th century (Zeevaart, 2006). The existence of florigen as a universal floral initiator is doubted after genetic research discovered multiple distinct flowering pathways in different species, but this dissonance was resolved after it was seen that each separate pathway converged to a shared set of flower promoting genes, the most well-known being FLOWERING LOCUS T (FT) (Putterill *et al*., 2004; Samach *et al*., 2000; Simpson & Dean, 2002). Thus, florigens are indeed understood to be universal flowering inducers but the productions of florigen hormones is regulated by single genes in certain

species and are polygenic in others. The protein produced by FT, now considered to be a florigen hormone, travels through the phloem to the shoot apical meristem and interacts with other proteins already present in the meristem to induce flower differentiation (Abe *et al*., 2008; Notaguchi *et al*., 2008).

2.3 Health benefits of strawberry

Strawberry is beneficial to human health because it provides a high amount of nutrients making it a good choice as part of a balanced diet. Antioxidant levels of strawberry vary between cultivars but phenolic compounds are not significantly different between cultivars (Meyers *et al*., 2003). Pajk *et al*. (2006) used pigs as a model to demonstrate that apple and strawberry contributed the most antioxidants to the diet. (Figure 1).

Strawberry contains high levels of antioxidants associated with the potential to reduce the risk of cardiovascular disease, DNA damage, several common cancers, and other oxidative stress related diseases (Cao *et al*., 1998; Meyers *et al*., 2003). In addition, strawberry, significantly increase the serum antioxidant capacity on elderly women that helps to decrease oxidative activity by decreasing malondialdehyde (MDA) formation and decrease cell damage during oxidation by protecting mononuclear blood cells, thus decreasing the free radical attack on cellular DNA (Cao *et al*., 1998). Vitamin C, the most abundant vitamin in strawberry is an antioxidant which enhanced the overall antioxidant status in the serum (Cao *et al*., 1998). Based on several studies, strawberries are superior compared to apples and tomatoes with respect to in vitro antioxidant capacity and result in a higher level of water-soluble antioxidant in blood plasma (Pajk *et al*., 2006).

Figure 1: Health benefits of strawberry (Meyers *et al*., 2003).

2.4 Nutritional value of strawberry

Strawberry fruits generally, are low in calorie carbohydrate but abundant in vitamin C and fibre (Basu *et al*., 2010).). The author further outlined its main constituents (per 100g FW) as vitamin C (64.0mg), water (91.75g), protein (0.61g), fat (0.37g), fibre (2.3g), calcium (14.0mg), carbohydrate (7.02g), potassium (166.0mg/160g), respectively, vitamin A 27 IU, pH range of 3.27-3.86 (Table 1), while University of Illinois Extension reported that strawberry has the highest vitamin C which makes it to reduce the risk of cancer of gastrointestinal track. Basu *et al.* 2010 also identified that the acidic constituent's range between 0.58-1.30% with citric and malic acid making up the primary organic acids that are responsible for flavour enhancement.

The soluble solid (^{0}Brix) composition ranges between 8.0-11.5% and are the main source of the juice concentrate of the fruit. The soluble solid/acid ratio ranges from 8.52-13.79g which is equal balance of sweet-tart flavour. Strawberry fruits also contain mineral elements such as phosphorus (P), potassium (K), magnesium (Mg), manganese (Mn), aluminium (Al), cupper (Cu), and zinc (Zn), but their concentrations decrease as the fruit maturity increases except potassium (Mahmood *et al*., 2012). It also contains folate and ellagic acid which has a great antioxidant and anti-carcinogen properties that are beneficial to the body (Hoover *et al*., 2008).

Rodas *et al*. (2013) explained that the attractive colour of strawberry fruit is from the anthocyanin content in the fruit which is the natural colour pigment from sugar. The author further reported that low molecular weight carbohydrate is the highest soluble solid of the strawberry and can related to aroma, flavour, texture and attractive colour of the fruit. Zhang *et al.* (2011) reported that ripening strawberry fruits contain 300 chemical classes of volatile compounds including their major component such as ketones, aldehydes, ester, alcohol and lactones, the contributing groups of sulphur compounds (including furans epoxide, acetals, alkanes and phenol) which are present in small amounts at the concentration of 10-100ppm of fresh fruit weight, these contributed to the overall strawberries flavour and plays an important roles in maintaining the fruit nutritive values and growth.

Table 1: Approximate nutritional value for raw portions of strawberry (100 grams) (USDA, 2009).

Nutrient	Value per 100 grams.	Nutrient	Value per 100 grams.
Protein	0.67 g	Selenium	0.4 mg
Total lipid (fat)	0.30 g	Vitamins C, (ascorbic acid)	58.8 mg
Carbohydrate	7.68 g	Thiamine	0.024 mg
Sugars	4.89 g	Riboflavin	0.022 mg
Calcium	16 mg	Niacin	0.386 mg
Iron	0.41 mg	Panthothenic acid	0.125 mg
Magnesium	13 mg	Vitamin B-6	0.047 mg
Phosphorus	24 mg	Folate, total	24 mg
Potassium	153 mg	Vitamin A, RAE	1.0 mg
Sodium	1.0 mg	Vitamin A, IU	12 mg
Zinc	0.14 mg	Vitamin K	2.2 mg
Copper	0.048 mg	Vitamin E	0.29 mg
Manganese	0.386 mg		

Source: USDA (2009)

2.5 Environmental Requirement of Strawberry

The plant thrives on loam, well-drained soils with a pH of 5.5–6.5. Temperature requirements are in the range of 10 to 30°C and an average rainfall of 900 to 1200 mm (Kasperbauer, 2010). However, in places where this is not attainable then 25mm per week or irrigation is necessary (Kasperbauer, 2010). Though production can take place in different areas, the ideal altitude is from 1500m-2200m above sea level (Martínez-Ferri, 2016)

2.6 Physico-chemical properties of strawberry fruit

Strawberries (*Fragaria* × *ananassa* Duch.) are classified as non-climacteric fruit, showing no increases in respiration rate and ethylene production during ripening (Biale & Young, 1981). The strawberry fruits are generally harvested at the fully matured stage since the ripening process does not normally continue after detachment (McGlasson, 1985). Several researchers revealed that strawberries can change red colour during storage, even when harvested at the early fruit ripening stages but they do not sufficiently accumulate sugars and acids to reach an acceptable level for fresh consumption (Forney *et al*., 1998; Kalt *et al*., 1993; Spayd & Morris, 1981).

Ripening is a biochemical process in which physical and chemical characteristics including dramatic bioactive compounds are produced. In general, strawberry fruits become softer, deep red and sweeter during the ripening process. Many scientists have reported that strawberry fruits have an initial phase of growth and enlargement followed by a maturation phase. Firmness, colour, and the sugar/acid ratio are important determinants of overall quality in ripe strawberry fruits (Shamaila *et al*., 1992). The loss of firmness during the ripening is a major factor determining the strawberry fruit quality and postharvest shelf-life. The profile changes in numerous bioactive compounds in strawberry fruits are observed during ripening (Manning, 1994). The sugars and ascorbic acid contents increase rapidly until the fruit is fully ripened (Moing *et al*., 2001; Montero *et al*., 1996). However, the citric and malic acids decline gradually during fruit ripening (Hancock, 1999). Additionally, anthocyanin content of strawberry fruits increases during the ripening progress (Kosar *et al*., 2004; Williner *et al*., 2003) as shown in figure 2.

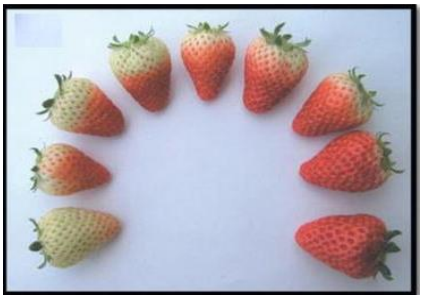

Figure 2: Stages of fruit development (Hayashi *et al*., 2010), from left to right color development from tip toward receptacle (anthocyanin development).

2.7 Shelf-life of strawberry

Strawberry fruit is a non-climacteric fruit which is characterized by a short postharvest life, often estimated for less than 5 days. It is very prone to rapid dehydration, physiological disorders, bruising and other mechanical injuries as well as to infections caused by several pathogens that can rapidly reduce quality of ripe fruits (Sallato, 2007). Every non-climacteric fruit has problem with storage because the fruit has to be picked fully ripened. The fully ripened fruit is more sensitive to storage and transfer conditions.

Climacteric fruits can be treated by ethylene and thus it is possible to modify the timing of ripening to a moderate extent. Climacteric and non-climacteric fruits have different ripening physiology which is genetically determined (Giovannoni, 2004). Optimum storage conditions for strawberries are 0°C and 90-95% relative humidity. In such conditions, strawberries can have 7-10 days of storage life. However, storage life is very dependent on handling of berries during and after harvest.

2.8 Plant biostimulants

Plant biostimulants are substances and materials, with the exception of nutrients and pesticides, which, when applied to plants, seeds or growing substrates in specific formulations have the capacity to modify physiological processes of plants in a way that provides potential benefits to growth, development, and/or stress response (Jardin, 2015). They contain amino acids, low molecular weight polypeptides, vitamins, enzymes, hormones (cytokinins, auxins, and gibberellins), sugars, betaines, and antioxidants (Thirumaran *et al*., 2009). Biotic (pests and diseases) and abiotic stresses (drought, salinity, extreme temperatures, ultraviolet radiation, and ozone exposure) make plants to divert energy reserves and photosynthates to control the stresses or reduce their impact on the plant thus reducing crop yields (Gawronska, 2008). Depending on their composition and expected results, biostimulants can be soil-or leaf-applied

(Kunicki *et al*., 2010). Their physiological effects occur after their entrance into plant tissues and cells, where these compounds are involved in the plant's metabolism, signaling, and hormonal regulation of growth and development.

2.9 Categories of plant biostimulants

According to Jardin (2012) major categories of plant biostimulants have been widely identified by scientists, regulators and stakeholders covering both substances and microorganisms. These are;

2.9.1 Humic substances

Humic substances (HS) are natural constituents of the soil organic matter resulting from the decomposition of plant, animal and microbial residues but also from the metabolic activity of soil microbes using these substrates (Canellas *et al*., 2015; Jardin, 2012). Most sources of humic substances used in agriculture are non-renewable and include natural humified organic matter, such as peat and organic soils, and mineral deposits, such as leonardite and soft coal. (Canellas *et al*., 2015). Humic substances have been recognized as essential contributors to the physico-chemical properties of soils. Moreover, most biostimulant effects of humic substances result in stimulation of root growth and improvement of plant nutrition resulting from the increase of soil nutrient availability (Canellas *et al*., 2015; Jardin, 2012).

Humic substances enhance soil nutrient availability by increasing cation exchange capacity and buffering (neutralize) soil pH (Canella *et al*., 2015; Jardin, 2015). Another important positive effect of HS on soil nutrient availability for plant uptake is the formation of soluble HS complexes with micronutrients (i.e., iron). The trace element-humic complex has been often considered as a strategy to improve plant nutrition of micronutrients by preventing leaching and making micronutrients more available to plants (Chen *et al*., 2004; Garcia-Mina *et al*., 2004).

2.9.2 Protein Hydrolysates

Protein hydrolysates are defined as 'mixtures of polypeptides, oligopeptides and amino acids that are manufactured from protein sources using partial hydrolysis (Schaafsma, 2009). Other nitrogenous molecules include betaines, polyamines and 'non-protein amino acids', which are diversified in higher plants but poorly characterized with regard to their physiological and ecological roles (Vranova *et al*., 2011). Glycine betaine is a special case of amino acid derivative with well-known anti-stress properties (Chen & Murata, 2011). They are

available as soluble granular or powder as well as liquid extracts and may be applied as foliar sprays (Colla *et al*., 2015a).

Protein hydrolysates improve soil respiration, the biomass and activity of microbial because microorganisms can easily use amino acids and peptides as C and N source (Farrell *et al*., 2014). They also influence plant nutrition by forming complexes and chelates between peptides/amino acids and soil micronutrients (i.e., Cu, Fe, Mn and Zn), thereby contributing to nutrients availability and acquisition by the root system (Colla *et al*., 2015a; Jardin, 2015). Moreover, the ability of peptides/amino acids to form complexes and chelates with some macro- (i.e., K, Ca and Mg) and micronutrients (i.e., Cu, Fe, Mn and Zn) are currently used by some industries to develop fertilizers having high nutrient use efficiency.

2.9.3 Seaweed Extracts

According to Khan *et al*. (2009) as cited by Craigie (2011), the use of organic matter being derived from fresh seaweeds and fertilizer dates back to ancient agriculture but the effects of biostimulants have been observed in recent years. *Ascophyllum nodosum, Ecklonia maxima, Macrocystis pyrifera* and *Durvillea potatorum* are the most frequently seaweed extracts commercially used by the extract industries (Khan *et al.,*2009). Seaweed extracts act on soils and on plants (Craigie *et al.,* 2008; Craigie, 2011; Khan *et al*., 2009). In soils, their polysaccharides contribute to gel formation, water retention and soil aeration. The poly-anionic compounds contribute to the fixation and exchange of cations, which is also of interest for the fixation of heavy metals and for soil remediation.

In plants, nutritional effects via the provision of micronutrients and macronutrients indicate that they act as fertilizers, beside their other roles. Although cytokinins, auxins, abscisic acid, gibberellins and other classes of hormone-like compounds, like sterols and polyamines, have been identified in seaweed extracts by bioassays and by immunological tools (Craigie, 2011), there is evidence that the hormonal effects of extracts of the brown seaweed *Ascophyllum nodosum* are explained to a large extent by the down and up regulation of hormone biosynthetic genes in plant tissues, and to a lesser extent to the hormonal contents of the seaweed extracts themselves (Wally *et al*., 2013).

2.9.4 Chitosan

Chitosan, a biopolymer chemically derived from crustaceans and soluble in organic acids is one of a range of natural compounds that has been used against pathogens in strawberries and other crops (Malerba *et al*., 2018). Chitosan is a low acetyl form of chitin

mainly composed of glucosamine, 2-amino-2deoxy-b-D-glucose (Freepons, 1991). Becker *et al*. (2000) reported that chitosan contains nitrogen in the basic unit of its formula. When the nitrogen contained in the chitosan is dissolved, it penetrates gradually and remains in the soil for a long period of time. It is considered environment-friendly for agricultural uses as it can be easily degraded in the environment, and nontoxic to humans. Chitosan and its derivatives have been reported to elicit natural defense responses in plants, and have been used as a natural compound to control pre- and post-harvest pathogenic organisms (Rahman *et al*., 2014).

Antimicrobial activities of chitosan against various phytopathogens have been reported (Rahman *et al*., 2014). Enhancement of storability and preservation of anthocyanin content in chitosan-coated strawberry fruit has been reported from several studies. Chitosan has been widely used as a coating agent of various fruit mainly for protection from post-harvest losses due to microbial infections (El-Sawy *et al*., 2010; Sakif *et al*., 2016). However, many investigators also have reported that using chitosan as a foliar spray increased vegetative growth, yield and biochemical contents in plants but have not been exploited completely especially on the fruits (Abdel-Mawgoud *et al*., 2010; El-Miniawy *et al*., 2013; Mondal *et al*., 2012, Mukta *et al*., 2017).

2.9.5 Biozyme

Biozyme is an eco-friendly nontoxic commercial growth stimulant which influences the plants physiological system at low concentrations and known to be rich in cytokinins and auxin precursor, enzymes and hydrolyzed protein is a storehouse of naturally occurring nutrients derived from Norwegian seaweed *Ascophyllum nodosum* (Kumar *et al.,* 2000). It contains nutrients in a naturally chelated form which helps to improve cell division and cell enlargement resulting into better chlorophyll content and increased production. According to Wu *et al.* (2008), enhanced plant yield and growth by biozyme could be attributable to nutrient absorption as well as improved status of nutrients in plants.

The use of agrochemicals especially formulations containing sulfur, copper, zinc or other synthetic compounds have the potential to exert toxic effects on non-target organisms and pollute the environment. Many of these chemicals are also too expensive for the resource-poor farmers of Africa (Vasudevan *et al*., 2010) and Kenya in particular. One potential approach to decrease negative environmental impacts resulting from the use of agrochemicals is to explore low cost environmentally friendly production options and determine their efficacy through empirical methods. Although natural products based alternative approaches have long been used for improved crop productivity, stress alleviation and disease suppression (Chung *et al*.,

2015; Flores-Félix *et al*., 2015; Islam *et al*., 2009; Sunar *et al*., 2015), their responses have been quite variable and reproducibility of results over years have been challenging. Therefore, this study was conducted to investigate the responses of various rates (0, 25 and 50ppm) of biozyme and chitosan spray on growth, yield and quality of strawberry so that farmers can use them in strawberry production.

2.10 Adverse effects of inorganic fertilizers and chemicals

The application of inorganic fertilizers and chemicals is an inexpensive and effective method of supplying crops with mineral nutrients (Chen, 2006). While N application can be sufficient to improve plant production, it also leads to a worldwide concern about environmental contamination resulting from excessive nitrate leaching (Dong *et al.*, 2005). In addition, fertilizers are often washed from the field in the runoff and can become unavailable to the crops through chemical, physical, or biological transformation (Halpern *et al.*, 2015). To compensate for these processes, farmers need to apply more chemical fertilizer than the plant actually needs and these large quantities of chemical fertilizers are used to replenish soil N and P, resulting in high costs and severe environmental contamination or the remainder is often released into the environment, polluting the air and water (Vance, 2016). Furthermore, the industrial production of chemical fertilizers is an energy-intensive process that is known to significantly contribute to global carbon (iv) oxide emissions (Vance, 2016).

Some of the major constraints in using chemical fertilizers include: (i) leaching and pollution of water resources, destruction of micro-organisms and beneficial insects, crop susceptibility to disease attack, acidification, alkalization of the soil or reduction in soil fertility, thus causing irreparable damage to the overall system; (ii) oversupply of N leads to softening of plant tissue resulting in plants that are more sensitive to diseases and pests; (iii) reduction of the colonization of plant roots with mycorrhizae and inhibit symbiotic N fixation by rhizobia due to high N fertilization; (iv) enhancing the decomposition of soil organic matter, which leads to degradation of soil structure and (v), easy loss of nutrients from soils through fixation, leaching or gas emission that can lead to reduced fertilizer efficiency (Chen, 2006; Kangueehi, 2008; Taiz *et al.*, 2015; Van Schoor, 2009).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Description of the study site

The study was carried out in the Horticulture Research and Teaching Farm (Field 3) of Egerton University, Njoro, Kenya. The site lies at a latitude of 0º 23' South, longitude 35º 35' East in the Lower Highland III Agro Ecological Zone (LH3) at an altitude of approximately 2,238 meters above sea level. The average maximum and minimum temperatures range from 19 ºC to 22 ºC and 5 ºC to 8 ºC, respectively, with a total annual rainfall ranging from 900 to 1100 mm. The soils are well-drained sandy-loam-vintric mollic Andosols (Jaetzold *et al.*, 2012). The beneficiaries of this research are the local farmers within Egerton, Nakuru, Gilgil, Naivasha, Meru and other parts of Kenya.

3.2 Materials used in the study

Disease free strawberry (*Fragaria x ananassa* Duch) cultivar chandler splits were obtained from Thika strawberry farm in Thika. Cultivar chandler was used due to its popularity, high yielding and has quality fruits. Biozyme was obtained from Arysta Lifescience (K) Ltd and Chitosan, on the other hand was sourced from Kobian Scientific Kenya Limited.

3.3 Treatments application

The treatments that were used in the study were biozyme and chitosan each at 0ppm, 25ppm and 50ppm. The treatment combinations were as shown in table 2;

Table 2: Treatment combinations used in the study

NOTATION	TREATMENTS
T_1	0ppm biozyme+0ppm chitosan
T_2	0ppm biozyme+25ppm chitosan
T_3	0ppm biozyme+50ppm chitosan
T_4	25ppm biozyme+0ppm chitosan
T_5	25ppm biozyme+25ppm chitosan
T_6	25ppm biozyme+50ppm chitosan
T_7	50ppm biozyme+0ppm chitosan
T_8	50ppm biozyme+25ppm chitosan
T_9	50ppm biozyme+50ppm chitosan

3.4 Experimental design and layout

The experimental design used in the study was a 2x3 factorial embedded in a Randomized Complete Block Design (RCBD) with 3 replications. The blocks were used to minimize the occurrence of experimental errors caused by soil fertility. The treatments were: control (0 ppm), 25ppm chitosan, 50ppm chitosan, 25ppm biozyme, 25ppm biozyme+25ppm chitosan, 25ppm biozyme+50ppm chitosan, 50ppm biozyme, 50ppm biozyme+25ppm chitosan and 50ppm biozyme+50ppm chitosan. Land was prepared by ploughing, harrowing and hand pulverized to a fine tilth to ensure uniform water distribution and root growth. Each plot measures 2m × 2.4m raised 15cm above the main field. A path of 60cm and 1m were made to separate the plot and block from the other respectively. The splits were planted in rows of 4 with the spacing of 45cm by 60cm. There were 16 splits per plot totaling to 144 splits per block giving 432 splits in the whole experiment. The treatments were applied three weeks after planting and at the intervals of two weeks throughout the growing period. No basal fertilizers were used.

3.5 Preparation of treatments used in the study

Biozyme and chitosan were used and the following procedure was used to prepare them before applying to the established strawberry splits.

3.5.1 Preparation and application of chitosan solution

Practical grade chitosan biopolymer (poly β-1,4-D-glucosamine) available in powder form was purchased from Kobian Scientific Kenya Limited. It is commercially prepared by the alkaline deacetylation of chitin obtained from shrimp shells (*Pandalus borealis*). The degree of de-acetylation is ≥85% with low viscosity. Three different concentrations, 0, 25, and 50 ppm of chitosan solution were prepared by measuring the required amount of product (25mg) followed by dissolving in 0.1 N HCl and diluting with 1litre of distilled water with pH adjusted at 6.5 by 0.1 NaOH to give 25ppm (Benhamou *et al.*, 2000). Freshly prepared chitosan solutions were applied onto strawberry canopy in each experimental unit starting from three weeks after planting up to run off, at flowering and one week before harvesting the fruits. Plants in the control plots were sprayed with equal volume of distilled water

3.5.2 Preparation and application of biozyme solution

Biozyme was obtained from Arysta Lifescience (K) Ltd which is located in Tulip House, 2nd Floor, Mombasa Road Nairobi. The three different concentrations (0ppm, 25ppm and 50ppm) required were prepared by measuring the needed amount of the biozyme

(25ml/litre) and diluted with distilled water before applying onto the strawberry canopy before flowering. Treatment application started three weeks after planting, at flowering and one week before harvesting of the fruits. Spraying was done in a clear and calm morning hour. The application was performed until run off with the use of hand sprayer.

3.6 Data collection

3.6.1 Data on growth and yield parameters

The data were collected from a total of 4 plants from each plot in each block. The outer rows and columns were used as the guard rows and column. Data collected on growth were plant height measured using a 30cm ruler from the base of the plant to the top apex of the leaf (figure 3), number of all the leaves from the four plants in the middle in each treatment by counting, leaf width using 30cm ruler (figure 4) and leaf length using a 30cm ruler. The width and length of the leaf was obtained from a single tagged leaf throughout the experiment. The data on yield components were obtained from; number of the flowers by counting, number of freshly harvested ripe fruits per plant per treatment and their average fruit weight measured using an electric balance and flower trusses.

Figure 3: Measurement of strawberry plant height

Figure 4: Measurement of strawberry leaf width

3.6.2 Determination of quality parameters

Fruit samples were harvested from the experiment at full maturity for quality analysis. Ten fruits were harvested randomly from the four plants in each treatment and taken to the laboratory for analysis. The data was collected on fruit size by measuring the length (figure 5) and diameter of the fruit at the broad basal end with the help of a digital vernier caliper. The average size was then expressed in centimeter (cm).

Figure 5: Measurement of strawberry fruit length

Fruit weight was measured using an electric balance and the average weight expressed in grams per fruit. A total soluble solid (TSS) of the fruit was determined by hand held refractometer (0 to 32º Brix) by putting drop of juice on the prism. The refractometer was calibrated with distilled water before each use. The total soluble solids in fruits was expressed in degree Brix (ºB) (AOAC, 2005).

Titratable acidity was obtained by taking twenty-five grams of fruits pulp thoroughly homogenized with distilled water in an electric blender and volume made to 250 ml in volumetric flask. The mixture was then be filter through Whatman No.1 filter paper. 5 ml sample was then titrated against 0.1 N NaOH solution using phenolphthalein as an indicator till it gave pink coloured end point according to (Association of Official Analytical Chemists, 2005). Total titrable acidity was then calculated using the formula:

$$\frac{\text{Grams}}{\text{litre acid}} = \frac{\text{normality of titrant} * \text{titre} * \text{equivalent weight of predominant acid}}{\text{volume of the sample} * 10}$$

Shelf-life was determined by randomly selecting 10 berries from each treatment and kept in a refrigerator maintained at 2 ± 1ºC. The number of days the fruit remain in healthy

state was recorded. The berries that remained firm and without rot for 7 to 10, 5 to 6, 3 to 4, and ≤ 2 days were considered to be of the best, better, good and poor storage life, respectively (Jackson and David, 1985).

3.7 Data analysis

Data were subjected to analysis of variance (ANOVA) using the GLM procedure of SAS version 9.2 and significant means separated using Tukey's honestly significant difference (Tukey's HSD) test at $P \leq 0.05$.

The basic model fitted for both experiments was:

$Y_{ijk} = \mu + B_k + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$ i=1, 2, 3; j=1, 2, 3; k=1, 2, 3

where;

$Y_{ijk=}$ strawberry plant response

μ= the overall mean

B_k-the k^{th} blocking effect

α_i= the i^{th} effect of biozyme treatment

β_j= the j^{th} effect of chitosan treatment

$(\alpha\beta)_{ij}$= the i^{th} and j^{th} interaction effect of biozyme and chitosan treatment at different levels

ε_{ijk}= Random error component which is assumed to be normally and independently distributed about mean zero and a common variance σ^2.

CHAPTER FOUR

RESULTS

Results obtained in this study are presented in this chapter following the order: growth variables, fruit yield variables and quality variables.

4.1 Response of biozyme and chitosan on growth and yield of 'Chandler' Strawberry (*Fragaria X Ananassa* Duch.)

4.1.1 Leaves number

The application of the biozyme and chitosan on the strawberry plants significantly ($p \leq 0.05$) influenced the number of the leaves throughout the growing period in the two trials (Table 3). Over the two growing trials, the number of the leaves was recorded highest in the treatment with both 25ppm biozyme and 25ppm chitosan compared to all the treated plots. The lowest number of the leaves was recorded from the untreated plot (control-distilled water). There was no significant difference in the number of the leaves from the treatment with 25ppm biozyme and the interaction in trial one. Treated plots had many leaves compared to the untreated plots in all sampling days. There was no significance difference in the number of the leaves from the interaction in the second trial.

Table 3: Effect of biozyme and chitosan rates on the number of leaves of 'Chandler' Strawberry (*Fragaria X Ananassa* Duch.)

Treatments	Trial	Days After Planting			
		28	42	56	70
Control (distilled water)	1	5.07d	5.92d	7.83d	9.25c
25ppm cht	1	8.58abc	9.67bc	14abc	15.25abc
50ppm cht	1	6.83bcd	8.83bcd	11.95bcd	14.17bc
25ppm bzy	1	8.75ab	12.08ab	14.92ab	17.33ab
25ppm cht+25ppm bzy	1	10a	13.17a	18.5a	20.75a
25ppm bzy+50ppm cht	1	6.25d	7.83cd	9.83cd	11.42bc
50ppm bzy	1	6.42cd	7.5cd	10cd	12.53bc
50ppm bzy+25ppm cht	1	5.8d	6.67cd	9.12d	11.03bc
50ppm bzy+50ppm cht	1	5.08d	6.42cd	8.5d	10.17c
Control (distilled water)	2	4.22c	5.5c	6.42b	8.67b
25ppm cht	2	5.67abc	6.67bc	7.83b	10.5ab
50ppm cht	2	6.42ab	7.17bc	8.67ab	10.67ab
25ppm bzy	2	6.08abc	7.5ab	8.42ab	11ab
25ppm cht+25ppm bzy	2	7.83a	9.25a	10.58a	12.08a
25ppm bzy+50ppm cht	2	5bc	6.5bc	7.5b	9.67ab
50ppm bzy	2	5.5bc	6.5bc	7.25b	9.33b
50ppm bzy+25ppm cht	2	5.58bc	6.25bc	6.83b	9b
50ppm bzy+50ppm cht	2	4.83bc	5.92bc	6.67b	8.75b

*****Means within a column followed by the same letter are not significantly different according to Tukey's Honestly Significant Difference test at $p \leq 0.05$.

Key: ppm-parts per million, cht-chitosan, bzy- biozyme.

4.1.2 Leaf length

The results clearly revealed that foliar application of biozyme and chitosan significantly ($p \leq 0.05$) had influence on the leaf length in both trials (Table 4). Treatment with both the lowest rates of biozyme and chitosan recorded the longest leaf length (21.48cm and 18.47cm) on 70 DAP compared with the rest of the treatments in trial 1 and 2 respectively. However, the strawberry plants treated with distilled water (control) recorded the shortest leaf length (12.74cm) in trial 1 and 13.64cm from the plot treated with the highest rates (50ppm) of both

biozyme and chitosan in trial 2. There was no significance difference in the length of the leaves from the plots treated with both 25ppm of biozyme and chitosan in both trials. There was no significance difference in the length of the leaves treated with 50 ppm chitosan in 42 DAP, 56 DAP and 70 DAP of trial two.

Table 4: Effect of biozyme and chitosan rates on leaf length of 'Chandler' Strawberry (*Fragaria X Ananassa* Duch.)

Treatments	Trial	Days After Planting			
		28	42	56	70
Control (distilled water)	1	8.86c	11.63d	12.3d	12.74d
25ppm cht	1	12.67ab	16abc	16.66bc	17.43abc
50ppm cht	1	11.66abc	16.02abc	16.72bc	17.33abc
25ppm bzy	1	14.88a	16.48ab	17.87ab	18.33ab
25ppm cht+25ppm bzy	1	15.03a	18.42a	20.48a	21.48a
25ppm bzy+50ppm cht	1	11.7abc	14.22bcd	15.43bcd	15.73bcd
50ppm bzy	1	9.84bc	15.01abcd	16.68bc	17.01bc
50ppm bzy+25ppm cht	1	9.72bc	12.83cd	13.76cd	14.36bcd
50ppm bzy+50ppm cht	1	9.27bc	12.37d	12.7d	13.75cd
Control (distilled water)	2	10.95c	11.67d	13.28b	14.03b
25ppm cht	2	14.38ab	15.79abc	16.33ab	16.84ab
50ppm cht	2	13.48abc	15.88ab	16.03ab	16.57ab
25ppm bzy	2	12.89bc	13.31bcd	14.68ab	16.31ab
25ppm cht+25ppm bzy	2	16.33a	17.73a	18.16a	18.47a
25ppm bzy+50ppm cht	2	13.13bc	13.28bcd	14.03b	15.15ab
50ppm bzy	2	12.82bc	13.29bcd	13.62b	14.78ab
50ppm bzy+25ppm cht	2	13.18bc	13.32bcd	13.56b	13.75b
50ppm bzy+50ppm cht	2	12.25bc	12.57cd	13.36b	13.64b

*****Means within a column followed by the same letter are not significantly different according to Tukey's Honestly Significant Difference test at $p \leq 0.05$.

Key: ppm-parts per million, cht-chitosan, bzy- biozyme.

4.1.3 Leaf width

Application of the biozyme and chitosan on the strawberry plants has marked influence on the leaf width (Table 5). The longest leaf width (9.31cm and 8.15cm) was recorded on the strawberry plants treated with 25ppm biozyme and 25ppm chitosan in trial 1 and 2 at 70 DAP respectively. However, the shortest leaf width (5.45cm and 5.49cm) was recorded on the strawberry treated with distilled water (control) in trial 1 and 2 at 70 DAP respectively. There was no significance difference on leaf width of strawberry plants treated with 25ppm biozyme

and 25ppm chitosan in trial 1 and 2 at 28DAP, 42DAP, 56DAP and 70DAP respectively (Table 5).

Table 5: Effect of biozyme and chitosan rates on leaf width of 'Chandler' Strawberry (*Fragaria X Ananassa* Duch.)

Treatments	Trial	Days After Planting			
		28	42	56	70
Control (distilled water)	1	4.41e	4.8e	5.19d	5.45d
25ppm cht	1	6.20bc	6.65bc	7.03abc	7.34bc
50ppm cht	1	5.14cde	5.77cde	6.43bcd	7.04bcd
25ppm bzy	1	6.64ab	7.35ab	7.70ab	8.16ab
25ppm cht+25ppm bzy	1	7.83a	8.17a	8.7a	9.31a
25ppm bzy+50ppm cht	1	5.65bcd	5.99cd	6.53bcd	6.88bcd
50ppm bzy	1	6.14bc	6.12cd	6.53bcd	6.73bcd
50ppm bzy+25ppm cht	1	5.01cde	5.78cde	5.93cd	6.43cd
50ppm bzy+50ppm cht	1	4.91de	5.18de	5.51cd	5.93cd
Control (distilled water)	2	4.30c	4.77c	5.22c	5.49bc
25ppm cht	2	5.31abc	5.89abc	6.09abc	6.40bc
50ppm cht	2	6.12ab	5.59ab	6.96ab	7.02abc
25ppm bzy	2	5.81abc	6.03abc	6.54abc	7.12ab
25ppm cht+25ppm bzy	2	6.66a	7.33a	7.43a	8.15a
25ppm bzy+50ppm cht	2	5.37abc	5.57bc	5.69bc	6.05bc
50ppm bzy	2	5.12bc	5.65abc	5.69bc	5.77bc
50ppm bzy+25ppm cht	2	5.18abc	5.45bc	5.48bc	5.53c
50ppm bzy+50ppm cht	2	5.36abc	5.92abc	6.01abc	6.08bc

*****Means within a column followed by the same letter are not significantly different according to Tukey's Honestly Significant Difference test at $p \leq 0.05$.

Key: ppm-parts per million, cht-chitosan, bzy- biozyme.

4.1.4 Plant height

The application of biozyme and chitosan significantly ($p \leq 0.05$) influenced plant height throughout the growing period (Table 6). The longest plant height (20.58cm for trial 1 and 18.83cm for trial 2 at 70 DAP) was recorded from the strawberry plants treated with 25ppm biozyme and 25ppm chitosan. However, the shortest plant height (14.14cm for trial 1 and

14.55cm for trial 2 at 70 DAP) was recorded from the strawberry plants treated with the distilled water (control) (Table 6). There was no significance difference in the plant height from the plants treated with 25ppm chitosan alone and also 25ppm biozyme alone in trial 1 together with both 25ppm biozyme and 25ppm chitosan in both trials at 28 DAP, 42 DAP, 56 DAP and 70 DAP respectively (Table 6).

Table 6: Effect of biozyme and chitosan rates on plant height of 'Chandler' Strawberry (*Fragaria X Ananassa* Duch.)

Treatments	Trial	Days After Planting			
		28	42	56	70
Control (distilled water)	1	11.43c	12.52c	13.25e	14.14d
25ppm cht	1	15.40abc	16.26abc	17.62abc	18.04abc
50ppm cht	1	13.63bc	15.01bc	16.50bcd	17.33abcd
25ppm bzy	1	15.87ab	17.89ab	18.36ab	19.17ab
25ppm cht+25ppm bzy	1	19.28a	19.64a	20.63a	20.58a
25ppm bzy+50ppm cht	1	12.76bc	14.24bc	14.85cde	16.58bcd
50ppm bzy	1	13.7bc	14.22bc	15.85bcde	16.42bcd
50ppm bzy+25ppm cht	1	12.28bc	13.59c	14.48de	15.48cd
50ppm bzy+50ppm cht	1	12.21bc	12.76c	13.86de	14.72d
Control (distilled water)	2	11.65d	12.36d	13.52c	14.55c
25ppm cht	2	16ab	16.33ab	16.17ab	17.09abc
50ppm cht	2	13.83c	15bc	16.11ab	17.48ab
25ppm bzy	2	14.41c	14.87bc	15.32abc	16.39abc
25ppm cht+25ppm bzy	2	16.48a	16.72a	17.2a	18.83a
25ppm bzy+50ppm cht	2	13.53c	14.55c	14.76bc	15.73bc
50ppm bzy	2	14.67bc	14.83bc	15.35abc	15.33bc
50ppm bzy+25ppm cht	2	13.86c	13.78cd	14.12bc	14.73c
50ppm bzy+50ppm cht	2	13.63c	14.28c	14.5bc	15.07bc

*****Means within a column followed by the same letter are not significantly different according to Tukey's Honestly Significant Difference test at $p \leq 0.05$.

Key: ppm-parts per million, cht-chitosan, bzy- biozyme.

4.1.5 Flower trusses number

The number of the flower trusses on the strawberry plants was significantly ($p \leq 0.05$) influenced by the application of biozyme and chitosan (Table 7). The highest number of the flower trusses (3.50) was recorded at 25ppm chitosan and at both 25ppm biozyme+25ppm chitosan (2.83) in trial 1 and 2 at 70 DAP respectively. However, the lowest number of the flower trusses (1.17 and 1.50) was recorded from the plots treated with the distilled water (control) in trial 1 and 2 respectively at 70 DAP.

In trial 1, there was no significant difference($p \geq 0.05$) in the number of the flower trusses at 50ppm chitosan, 25ppm biozyme+50ppm chitosan and 50ppm biozyme + 25ppm chitosan at 28 DAP, 42 DAP and 56 DAP respectively but at 25ppm biozyme+25ppm chitosan, there was no significance difference in all sampling dates (Table 7). In trial 2, there was no significant difference in the number of the flower trusses at 25ppm biozyme+25ppm chitosan in all sampling dates.

Table 7: Effect of biozyme and chitosan rates on flower trusses of 'Chandler' Strawberry (*Fragaria X Ananassa* Duch.)

Treatments	Trial	Days After Planting			
		28	42	56	70
Control (distilled water)	1	0.58c	1.00c	1.17c	1.17b
25ppm cht	1	2.00ab	2.33bc	2.58bc	3.50ab
50ppm cht	1	1.50bc	2.42bc	2.25bc	2.92ab
25ppm bzy	1	2.25ab	3.33b	3.40b	2.75b
25ppm cht+25ppm bzy	1	2.75a	5.67a	5.68a	5.17a
25ppm bzy+50ppm cht	1	1.33bc	2.17bc	2.33bc	2.83ab
50ppm bzy	1	1.42bc	2.17bc	2.02bc	2.17b
50ppm bzy+25ppm cht	1	1.08bc	2.33bc	2.42bc	1.58b
50ppm bzy+50ppm cht	1	0.62c	1.67c	1.83bc	1.25b

Control (distilled water)	2	1.50d	1.83c	2.22b	1.50b
25ppm cht	2	3.08bc	2.60bc	2.92ab	2.25ab
50ppm cht	2	3.00bc	2.67bc	2.42b	1.92ab
25ppm bzy	2	3.58b	3.17ab	3.08ab	2.12ab
25ppm cht+25ppm bzy	2	4.75a	4.17a	2.83a	
25ppm bzy+50ppm cht	2	2.92bc	2.75bc	3.08ab	2.22ab
50ppm bzy	2	3.58b	2.50bc	3.42ab	1.52b
50ppm bzy+25ppm cht	2	2.58bcd	2.08bc	2.75b	2.00ab
50ppm bzy+50ppm cht	2	2.00cd	2.50bc	2.08b	1.58b

*****Means within a column followed by the same letter are not significantly different according to Tukey's Honestly Significant Difference test at $p \leq 0.05$.

Key: ppm-parts per million, cht-chitosan, bzy- biozyme.

4.1.6 Number of the flowers per plant

Application of biozyme and chitosan resulted in a significant ($p \leq 0.05$) increase in the number of flowers per plant (Table 8). The highest number of flowers (0.92 and 1.17) were recorded at the plot treated with both 25ppm biozyme and 25ppm chitosan in trial 1 and 2 respectively (Table 8). However, there were no significant differences in plots treated with both 25ppm biozyme and 25ppm chitosan in both trials (Table 8). The lowest flower number (0.12 and 1.17) was recorded in the plots treated with the highest rates of both biozyme and chitosan (50ppm) in both trials.

Table 8: Effect of biozyme and chitosan rates on the number of flowers of 'Chandler' Strawberry (*Fragaria X Ananassa* Duch.)

Treatments	Trial	Days After Planting			
		28	42	56	70
Control (distilled water)	1	0.03b	0.07b	0.15b	0.22cd
25ppm cht	1	0.25b	0.33b	0.42ab	0.68ab
50ppm cht	1	0.18b	0.28b	0.32b	0.53bc
25ppm bzy	1	0.25b	0.33b	0.43ab	0.45bcd
25ppm cht+25ppm bzy	1	0.67a	1a	1.08a	0.92a
25ppm bzy+50ppm cht	1	0.07b	0.17b	0.25b	0.33bcd
50ppm bzy	1	0.15b	0.37b	0.42ab	0.45bcd
50ppm bzy+25ppm cht	1	0.11b	0.18b	0.18cd	

50ppm bzy+50ppm cht	1	0.03b	0.13b	0.17b	0.12d
Control (distilled water)	2	0.58d	0.67b	0.38b	0.25b
25ppm cht	2	2.17ab	0.92b	1.17ab	0.75ab
50ppm cht	2	1.42abcd	0.83b	0.45b	0.62ab
25ppm bzy	2	1.75abc	0.92b	0.92ab	0.63ab
25ppm cht+25ppm bzy	2	2.42a	2.12a	1.78a	1.17a
25ppm bzy+50ppm cht	2	1cd	0.67b	0.67ab	0.58ab
50ppm bzy	2	1.08cd	1.08ab	0.67ab	0.73ab
50ppm bzy+25ppm cht	2	1.33bcd	0.83b	0.3b	0.38b
50ppm bzy+50ppm cht	2	0.75cd	0.33b	0.25b	0.23b

*****Means within a column followed by the same letter are not significantly different according to Tukey's Honestly Significant Difference test at $p \leq 0.05$.

Key: ppm-parts per million, cht-chitosan, bzy- biozyme.

4.1.7 Number of fruits per plant

There was significant ($P \leq 0.05$) influence of biozyme and chitosan in the number of fruits per plant (Table 9). The highest and the lowest number of fruits per plant were recorded in the treated and the untreated plots respectively. The treatment with both 25ppm biozyme and 25ppm chitosan recorded the highest number of fruits per plant (3.50 and 2.58) in both trials respectively. However, the untreated plots (control) recorded the lowest number of fruits per plant (0.90 and 1.08) in trials 1 and 2 respectively. There was no significance difference in the number of the fruits per plants in the interaction of 25ppm biozyme and 25ppm chitosan at all sampling days in both trials while in the treatment with 25ppm biozyme, there was no significance difference in the number of the fruits per plants as at 42 DAP, 56 DAP and 70 DAP in trial 2.

Table 9: Effect of biozyme and chitosan rates on the number fruits per plant of 'Chandler' Strawberry (*Fragaria X Ananassa* Duch.)

Treatments	Trial	Days After Planting			
		28	42	56	70
Control (distilled water)	1	0.07b	0.25c	0.5b	0.90b
25ppm cht	1	0.42ab	1.58ab	1.52b	1.92b
50ppm cht	1	0.42ab	1.25abc	1.83ab	2.08ab
25ppm bzy	1	0.33ab	1.25abc	1.67b	1.98ab
25ppm cht+25ppm bzy	1	1a	2.33a	3.08a	3.50a
25ppm bzy+50ppm cht	1	0.22b	0.75bc	0.92b	1.67b
50ppm bzy	1	0.22b	1bc	1.30b	1.33b
50ppm bzy+25ppm cht	1	0.15b	0.75bc	1.05b	1.17b
50ppm bzy+50ppm cht	1	0.12b	0.67bc	1.13b	0.92b
Control (distilled water)	2	0.33c	1.23c	2.07b	1.08c
25ppm cht	2	1.58ab	2.13abc	2.67ab	2.25ab
50ppm cht	2	1.08bc	2.12abc	2.73ab	1.75abc
25ppm bzy	2	1.08bc	2.32ab	2.80ab	2.23ab
25ppm cht+25ppm bzy	2	2.42a	2.73a	3.50a	2.58a
25ppm bzy+50ppm cht	2	1.33abc	1.67bc	2.02b	1.58abc
50ppm bzy	2	0.92bc	1.45bc	2.58ab	1.92abc
50ppm bzy+25ppm cht	2	1bc	2abc	2.28b	1.50abc
50ppm bzy+50ppm cht	2	1bc	1.5bc	2.02b	1.27bc

*Means within a column followed by the same letter are not significantly different according to Tukey's Honestly Significant Difference test at $p \leq 0.05$.

Key: ppm-parts per million, cht-chitosan, bzy- biozyme.

4.1.8 Fruit length

The results presented in the Table (10) clearly revealed that the application of biozyme and chitosan as a foliar significantly ($p \leq 0.05$) influenced the fruit length in both trials. The interaction of 25ppm biozyme and 25ppm chitosan recorded the highest fruit length (3.62cm and 3.47cm) on 70 DAP compared with the rest of the treatments in trial 1 and 2 respectively. However, the strawberry plants treated with both 50ppm biozyme and 50ppm chitosan recorded the lowest fruit length (2.22cm and 2.13cm) in trial 1 and 2 respectively. There was no

significance difference in fruit length from the plots treated with both 25ppm biozyme and 25ppm chitosan at all sampling days in both trials. Furthermore, there was no significance difference in fruit length from the plots treated with 25ppm biozyme in all sampling days in trial 1. In trial 2, there was no significance difference in fruit length from the plots with the interaction of 50ppm biozyme and 25ppm chitosan and also 50ppm biozyme and 50ppm chitosan in all sampling days.

Table 10: Effect of biozyme and chitosan rates on fruit length of 'Chandler' Strawberry (*Fragaria X Ananassa* Duch.)

Treatments	Trial	Days After Planting			
		28	42	56	70
Control (distilled water)	1	2.70b	2.45bc	2.18d	2.28b
25ppm cht	1	3.08ab	2.88ab	2.87bc	2.78ab
50ppm cht	1	2.83ab	2.85ab	2.88bc	2.97ab
25ppm bzy	1	2.95ab	2.83ab	3.33ab	3.00ab
25ppm cht+25ppm bzy	1	3.38a	3.23a	3.57a	3.62a
25ppm bzy+50ppm cht	1	2.67b	2.83ab	2.85bc	2.70b
50ppm bzy	1	2.92ab	2.85ab	2.65cd	2.53b
50ppm bzy+25ppm cht	1	2.48b	2.60bc	2.62cd	2.52b
50ppm bzy+50ppm cht	1	2.63b	2.22c	2.37cd	2.22b
Control (distilled water)	2	2.50c	2.50bc	2.58c	2.15c
25ppm cht	2	3.37ab	2.90abc	3.05abc	2.75abc
50ppm cht	2	3.27abc	2.97ab	3.05abc	2.73bc
25ppm bzy	2	2.93abc	2.73bc	3.22ab	3.00ab
25ppm cht+25ppm bzy	2	3.68a	3.37a	3.38a	3.47a
25ppm bzy+50ppm cht	2	2.93abc	2.57bc	2.87abc	2.70bc
50ppm bzy	2	3.17abc	2.62bc	2.87abc	2.38bc
50ppm bzy+25ppm cht	2	2.68bc	2.48bc	2.73bc	2.38bc
50ppm bzy+50ppm cht	2	2.49c	2.40c	2.60c	2.13c

*Means within a column followed by the same letter are not significantly different according to Tukey's Honestly Significant Difference test at $p \leq 0.05$.

Key: ppm-parts per million, cht-chitosan, bzy- biozyme.

4.1.9 Fruit diameter

The production of strawberry using biozyme and chitosan significantly ($P \leq 0.05$) influenced fruit diameter in the study (Table 11). The highest fruit diameter (2.68cm and 2.80 at 70 DAP) was recorded in plots treated with the interaction of 25ppm biozyme and 25ppm chitosan compared with the other treatments in both trials. However, the lowest fruit diameter (1.70cm and 1.80cm) was recorded from the plots treated with the interaction of 50ppm biozyme and 50ppm chitosan. In trial 1, there was no significance difference in the fruit

diameter in the plots treated with the combination of 25ppm biozyme and 25ppm chitosan at all sampling days while in trial 2, there was significance difference in the fruit diameter in the plots treated with 25ppm chitosan and the combination of both 25ppm biozyme and 25ppm chitosan at all sampling days.

Table 11: Effect of biozyme and chitosan rates on Fruit diameter of 'Chandler' Strawberry (*Fragaria X Ananassa* Duch.)

Treatments	Trial	Days After Planting			
		28	42	56	70
Control (distilled water)	1	2.13c	2.12bc	1.97cde	1.98bc
25ppm cht	1	2.25bc	2.48ab	2.45ab	2.25abc
50ppm cht	1	2.28abc	2.58a	2.43abc	2.43ab
25ppm bzy	1	2.40abc	2.33ab	2.37abcd	2.30ab
25ppm cht+25ppm bzy	1	2.58a	2.70a	2.68a	2.68a
25ppm bzy+50ppm cht	1	2.37abc	2.13bc	1.95de	1.97bc
50ppm bzy	1	2.53ab	2.30ab	2.23abcde	2.03bc
50ppm bzy+25ppm cht	1	2.12c	2.27ab	2.07bcde	2.15abc
50ppm bzy+50ppm cht	1	2.12c	1.82c	1.87e	1.70c
Control (distilled water)	2	2.02c	2.02c	2.15b	2.12bc
25ppm cht	2	2.65ab	2.40ab	2.40ab	2.35ab
50ppm cht	2	2.33bc	2.23abc	2.37ab	2.45ab
25ppm bzy	2	2.37bc	2.23abc	2.45ab	2.35ab
25ppm cht+25ppm bzy	2	2.77a	2.53a	2.72a	2.80a
25ppm bzy+50ppm cht	2	2.20c	2.08bc	2.17b	1.97bc
50ppm bzy	2	2.28bc	2.40ab	2.13b	2.12bc
50ppm bzy+25ppm cht	2	2.25c	2.06c	2.20b	2.17bc
50ppm bzy+50ppm cht	2	2.10c	1.92c	2.07b	1.80c

*Means within a column followed by the same letter are not significantly different according to Tukey's Honestly Significant Difference test at $p \leq 0.05$.

Key: ppm-parts per million, cht-chitosan, bzy- biozyme.

4.1.9 Fruit weight

Fruit weight was significantly ($p \leq 0.05$) influenced by the application of biozyme and chitosan in the experiment study (Table 12). The highest fruit weight (14.83grams and 14.67grams) was recorded in plots treated with the combination of both 25ppm biozyme and 25ppm chitosan at 70 DAP in both trials respectively. The lowest fruit weight (8.78grams and 7.00grams) was recorded from the plots treated with the combination of 50ppm biozyme and

50ppm chitosan at 70 DAP in both trials respectively. There was no significance difference in the fruit weight from the plots treated with both 25ppm biozyme and 25ppm chitosan in all sampling days (Table 12). There was no significance difference in fruit weight from the plots treated with both 25ppm biozyme and 25ppm chitosan at all sampling days in both trials.

Table 12: Effect of biozyme and chitosan rates on Fruit weight in grams of 'Chandler' Strawberry (*Fragaria X Ananassa* Duch.)

Treatments	Trial	Days After Planting			
		28	42	56	70
Control (distilled water)	1	7.08b	9.33bc	8d	8.83c
25ppm cht	1	10.33ab	13.60ab	12.83abc	12.67ab
50ppm cht	1	9.67ab	11abc	11bcd	10.17bc
25ppm bzy	1	10.83ab	12.93abc	13.83ab	12.67ab
25ppm cht+25ppm bzy	1	13.33a	16.50a	16.45a	14.83a
25ppm bzy+50ppm cht	1	8.77b	8.83bc	8.83d	8.83c
50ppm bzy	1	10ab	9.83bc	10.50bcd	9.50bc
50ppm bzy+25ppm cht	1	7.67b	9.33bc	9.83cd	8.50c
50ppm bzy+50ppm cht	1	7.50b	7.50c	7.77d	8.78c
Control (distilled water)	2	7.17b	6.17a	7.33b	7.33d
25ppm cht	2	8.83ab	8.50a	9.67ab	12.00abc
50ppm cht	2	8.50ab	9.17a	9.17ab	10.50abcd
25ppm bzy	2	8.50ab	8.00a	10.17ab	12.83ab
25ppm cht+25ppm bzy	2	10.33a	12.33a	11.83a	14.67a
25ppm bzy+50ppm cht	2	7.17b	8.17a	8.33ab	9.00bcd
50ppm bzy	2	8.00ab	8.25a	8.67ab	9.00bcd
50ppm bzy+25ppm cht	2	7.67b	9.44a	8.00b	8.33cd
50ppm bzy+50ppm cht	2	7.83b	7.75a	8.50ab	7.00d

*Means within a column followed by the same letter are not significantly different according to Tukey's Honestly Significant Difference test at $p \leq 0.05$.

Key: ppm-parts per million, cht-chitosan, bzy- biozyme.

4.1.10 Fruit yield per plant

The application of biozyme and chitosan at different rates significantly ($p \leq 0.05$) influenced the yield of strawberry fruits per plant (Table 13). The treated plots produced the highest yield of fruits per plant compared to the control. The highest fruit yield per plant (61.117 grams and 49.167 grams) was recorded in plots treated with the combination of both 25ppm biozyme and 25ppm chitosan in both trials respectively. The lowest fruit yield (33.250 grams and 28.000 grams) was recorded from the control. There was significance difference in fruit yield from all the treated plots except plots treated with 25ppm bzy+50ppm cht, 50ppm bzy, 50ppm bzy+25ppm cht and 50ppm bzy+50ppm cht at trial 2.

Table 13: Effect of biozyme and chitosan rates on the fruit yield (production) per plant of 'Chandler' Strawberry (*Fragaria X Ananassa* Duch.)

Treatments	Trial 1	Trial 2
Control (distilled water)	33.250de	28.000c
25ppm cht	48.933bc	39.000ab
50ppm cht	41.833bcd	37.333bc
25ppm bzy	50.267b	39.500ab
25ppm cht+25ppm bzy	61.117a	49.167a
25ppm bzy+50ppm cht	35.267de	32.667bc
50ppm bzy	39.833cde	33.917bc
50ppm bzy+25ppm cht	35.333de	33.444bc
50ppm bzy+50ppm cht	31.544e	31.083bc

*Means within a column followed by the same letter are not significantly different according to Tukey's Honestly Significant Difference test at $p \leq 0.05$.

Key: ppm-parts per million, cht-chitosan, bzy- biozyme.

4.2 Response of biozyme and chitosan on fruit quality of 'Chandler' Strawberry (*Fragaria X Ananassa* Duch.)

The results on the response of biozyme and chitosan on total soluble solids (TSS) and titratable acidity (TA)

4.2.1 Total soluble solids (TSS)

The results obtained showed that the fruit total soluble solids content was significantly different ($p \leq 0.05$) by the application of biozyme and chitosan in the experiment (Figure 3). The

highest percentage of the total soluble solids (11.7% brix and 13.5% brix) was recorded in the plot treated with the combination of 25ppm biozyme and 25ppm chitosan in both trials. However, the lowest percentage of the total soluble solids (8.4% brix and 7.9% brix) was recorded in the plot treated with the control (distilled water). There were no significance differences of total soluble solids in the untreated plot, plot treated with 25ppm chitosan, 25ppm biozyme, 25ppm biozyme and 25ppm chitosan and plot treated the combination of 50ppm biozyme and 50ppm chitosan in the experiment.

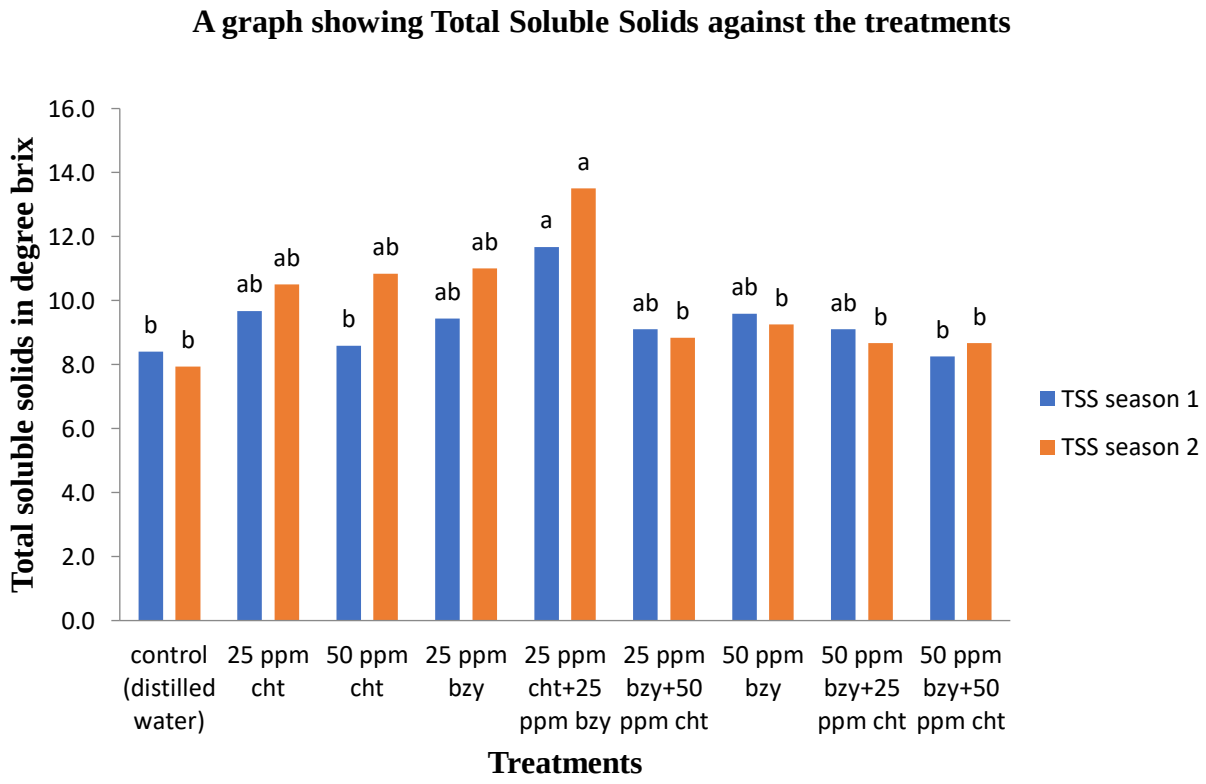

Figure 6. Biozyme and chitosan effects on Total soluble solids in brix %. Means followed by the same letter are not significantly different according to Tukey's Honestly Significant Difference test at $p \leq 0.05$.

Key: ppm-parts per million, cht-chitosan, bzy- biozyme.

4.2.2 Titratable acidity (TA)

The titratable acidity in strawberry fruit was significantly influenced by the application of biozyme and chitosan rates (Figure 4). From the results, the highest percentage of the titratable acidity (0.29% and 0.28%) was recorded in the plot treated with the

combination of 50ppm biozyme and 50ppm chitosan in trial 1 and 2 respectively. However, the lowest percentage of titratable acidity (0.20% and 0.14%) was recorded in the plot treated with the combination of 25ppm biozyme and 25ppm chitosan trial 1 and 2 respectively. (Figure 4). There were no significance differences of titratable acidity in the untreated plot and plot treated the combination of 50ppm biozyme and 50ppm chitosan in the experiment.

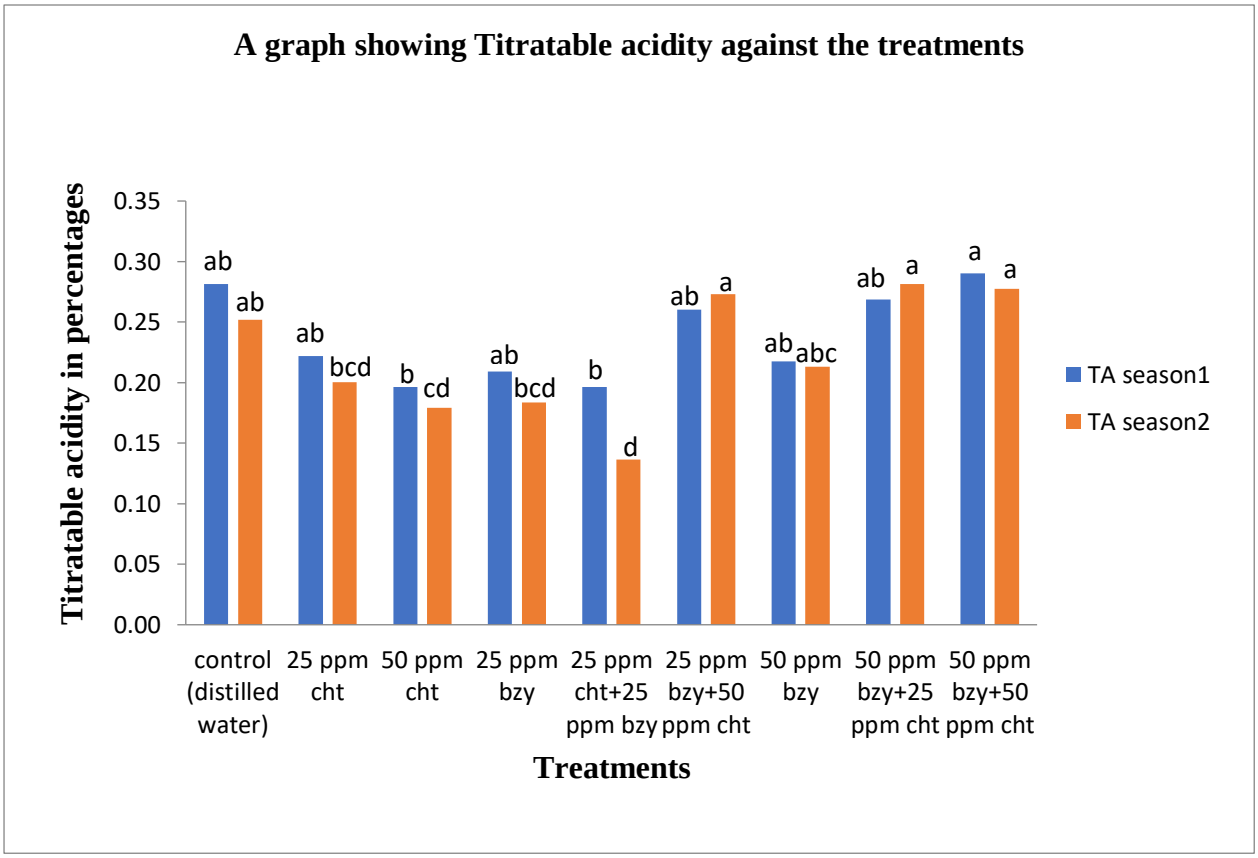

Figure 7. Biozyme and chitosan effects on titratable acidity in percentages. Means followed by the same letter are not significantly different according to Tukey's Honestly Significant Difference test at $p \leq 0.05$.

Key: ppm-parts per million, cht-chitosan, bzy- biozyme.

4.2.3 Fruit shelf-life

Strawberry shelf-life was significantly ($p \leq 0.05$) influenced by the application of biozyme and chitosan in the experiment study (Figure 5). The plot that was treated with the combination of 25ppm and 25ppm chitosan recorded the highest number of the days (13.3 days and 12.3 days) in trial 1 and 2 respectively when their fruits were stored in a regulated refrigerator under 2 ± 1^{o}C and 90% humidity. However, the fruits from the untreated plots

recorded the lowest number of days (5.5 days and 5.7 days) in trial 1 and 2 respectively when they were stored under the same conditions. There was no significance difference in the number of the days from the plot treated with the combination of 25ppm and 25ppm chitosan.

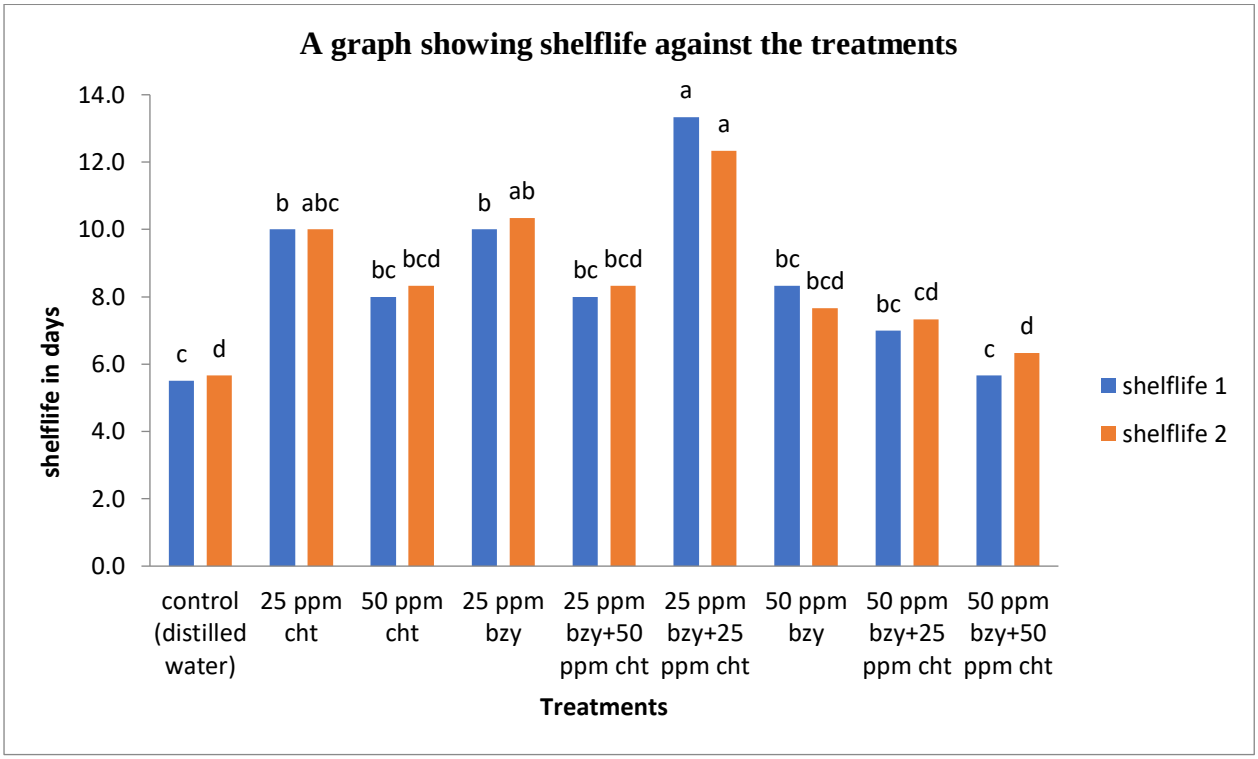

Figure 8. Biozyme and chitosan effects on strawberry shelf-life. Means followed by the same letter are not significantly different according to Tukey's Honestly Significant Difference test at $p \leq 0.05$.

Key: ppm-parts per million, cht-chitosan, bzy- biozyme.

CHAPTER FIVE
DISCUSSION

This chapter presents a discussion of the results. The layout of this chapter follows sequentially the order in which the results were presented in chapter four of this document.

5.1 Response of biozyme and chitosan on growth and yield of strawberry

5.1.1 Leaves number

The number of the leaves was significantly influenced by the application of biozyme and chitosan in both trials. The combination of both 25ppm biozyme and 25ppm chitosan in trial 1 and 2 produced the highest number of the leaves from all the treated plots with the lowest number of the leaves obtained from the untreated plots. Mandal and Kumar (1989) and Sharma (1990) found same results in guava and apple respectively with the application of Biozyme crop plus and Protozyme in different concentrations. Thus, it is apparent that Biozyme, which consist of precursor of auxins, enzyme, protein and micronutrients positively improve vegetative growth and in turn yield of crop.

Biozyme has shown positive influence on the growth and vigour of the plant and this might be due to higher uptake of plant nutrient as well as quicker relocation of plant metabolites in the plant canopy. The biostimulant, chitosan is known to promote plant growth and development, and provide enhanced disease suppression capability to plants through multiple mechanisms including induced systemic resistance (Malerba *et al*., 2018). Foliar application of varying doses of chitosan on strawberry canopy in this study stimulated all aspects of vegetative growth (leaf number, leaf length and plant height) with concurrent improvement of fruit yield and fruit quality compared with untreated plants.

Many investigators have reported that chitosan controls numerous pre- and post-harvest diseases, and increase yield of various ornamental as well as horticultural commodities in different parts of the world (Bautista-Baños *et al*., 2006). Results from this study indicate that the rate of chitosan at which it promote growth and yield of field grown strawberries is milligram per liter concentration, which is in full agreement with previous findings (Mukta *et al*., 2017). A similar positive influence of chitosan on plant vegetative features was observed in multiple genera from family *Orchidaceae*, such as *Cymbidium* (Nahar *et al*., 2012) or *Dendrobium* (Tantasawat *et al.*, 2010). The research by Lee *et al*. (2005) showed a positive chitosan influence on soy seedling growth and the stimulating action of chitosan was directly proportional to the molecular weight of the compound used in the experiment.

The obtained results of vegetative growth characteristics in the current study are in agreement with those reported by Spinelli *et al.* (2010) and Abdel-Mawgoud *et al.* (2010) on watermelon, Shehata *et al.* (2011) on celeriac, Abou El-Yazied *et al.* (2012) on snap bean and Fawzy *et al.* (2012) on garlic. All the earlier researchers found that seaweed extract when applied in foliar form increases plant height, number of leaves per plant, leaf area and fresh and dry weight of biomass of these crops. Also, these results are in accordance with the earlier studies which indicated that seaweed extract applications promote root growth and development in plants (Thompson *et al*., 2004). An improved root system could be influenced by endogenous auxins as well as other compounds in the extracts (Crouch *et al*., 1992). The improved vegetative growth traits could be due to endogenous growth substances as well as other compounds in the extracts (Durand *et al*., 2003) which affect cellular metabolism in treated plants leading to enhanced growth and crop yield. Moreover, seaweed extracts improve nutrient uptake by roots (Crouch, 1990) resulting in root systems with improved water and nutrient efficiency, thereby causing enhanced general plant growth and vigour.

5.1.2 Leaf length and width

The results obtained in this study on the length and width of the leaves of strawberry revealed that they were significantly influenced with the application of biozyme and chitosan. Foliar application of varying doses of chitosan on strawberry canopy in this study stimulated all aspects of vegetative growth (leaf length, leaf number, shoot and root dry weights) with concurrent improvement of fruit yield and fruit quality compared with untreated control (Malerba *et al.*, 2018). One of the interesting findings of this study is that foliar application of chitosan at different doses improved growth of strawberry plants to some extent compared to untreated control. The biostimulants, biozyme and chitosan are known to promote plant growth and development, and provide enhanced disease suppression capability to plants through multiple mechanisms including induced systemic resistance (Malerba *et al.*, 2018).

Foliar application of varying doses of chitosan on strawberry canopy in this study stimulated all aspects of vegetative growth (leaf length, leaf number, shoot and root dry weights) with concurrent improvement of fruit yield and fruit quality compared with untreated control. Many investigators have reported that chitosan controls numerous pre- and post-harvest diseases and increase yield of various ornamental as well as horticultural commodities in different parts of the world (Pichyangkura *et al*., 2015). Results from this study further indicated that the rate of chitosan to promote growth and yield of field grown strawberries is milligram per liter concentration, which is in full agreement with previous findings (Mukta *et*

al., 2017). Chibu and Shibayama (1999) studied also chitosan application on early growth of four crops: soybean, lettuce, tomato and rice. The results showed that chitosan at 0.1 or 0.5% increased leaf area, leaf dry weight and leaf length of soybean, lettuce and rice whereas chitosan at 0.1% showed positive effects on leaf area, leaf length and dry weight of tomato.

5.1.3 Plant height

In the present study, the application of both biozyme and chitosan significantly enhanced plant growth giving taller strawberry plants compared to control. These results are in agreement with Cuibu and Shibayama (1999) who reported positive effects of chitosan incorporated into the soil on early growth stages of soybean, mini-tomato, upland rice and lettuce. These improvement included plant height, leaf area, and dry weight of plants. Also Harada *et al*. (1995) reported that application of biozyme and chitosan in the field increased plant height, branch length, node number per plant and seed yield of soybean and total root length per plant increased by biozyme and chitosan application in the pot experiment.

The results obtained for vegetative growth characteristics in the present study are in agreement with those reported by Abou El-Yazied *et al.* (2012) on snap bean, Fawzy *et al.* (2012) on garlic who found that seaweed extract foliar spray also increased plant height, number of leaves per plant, leaf area and fresh and dry weight of biomass of these crops. Also, these results are in accordance with the earlier studies which indicated that seaweed extract applications promote growth and development in plants (Metting *et al.*, 1990).

Mohamed *et al*. (2018), revealed that chitosan may additionally provide a few amino compounds required for plant growth that led to increase total N content increasing in leaves or higher capacity of plant absorption of N from soil as chitosan would possibly increase key enzymes of nitrogen metabolism and promote transportation of N within the functional leaves. Also, chitosan may increase the availability, uptake and transport of essential nutrients via adjusting cell osmotic pressure and thereby progresses plant growth and development e.g. plant shoots, number of leaves, leaf area and total leaf area per plant thus reversing in increasing its fresh and dry weight.

5.1.4 Number of flowers and fruits

The number of flowers and fruit per plant were significantly affected by the application of biozyme and chitosan rates. More flowers and fruits were observed in treated plants compared to control. In this concern, Ohta *et al*. (1999) found that flower number of *Eustoma grandiflorum* was greatest in plants grown in chitosan treated. A stimulating effect of chitosan on the number of flowers was observed in plants such as gerbera (Wanichpongpan *et al*., 2001)

and gladioli (Ramos-Garcia *et al*., 2009). Salachna and Zawadzińska (2014) working on 'Gompey' freesia, reported that the chitosan-treated plants (0.5%) had more leaves and flowered earlier as well as had higher relative chlorophyll content. Reeta *et al.* (2010) observed increased number of flowers and fruits in tomato with application of seaweed liquid fertilizers. Similarly, increased number of fruits per plant and per cent fruit set in tomato with the application of biozyme was also observed by Ofosu-anim *et al.* (2007).

Similar results was found by Sharma (1990) who recorded increased fruit yield with Biozyme crop plus and Protozyme application in Apple cv. Red Delicious. Reduced flower drop and increase in fruit set might be due to delay in abscission (the effect of cytokinins and auxins) through preservation of loss of pectin material in middle lamella (Kachave & Bhosale ,2007) and enhance resistance to water as well as nutrient stress (Fujioka, 1997), enhanced photosynthesis and mobilization of metabolites to the flowers (Bhatia & Kaur, 1997). The results are in agreement with the findings of Gore *et al.* (2007) and Kumar *et al.* (2000) who reported that application of biozyme significantly increased the yield of chilli and bell pepper.

Improvement in yield with different treatments over control was due to the direct or indirect effects of growth and yield attributes including; number of branches per plant, number of flowers per plans, fruit length, fruit setting percentage and number of fruits per plant and also fruit weight. Thus, it can be inferred that the biozyme which consist of precursors of auxin, enzyme, protein and micronutrients may have some beneficial role in improving growth and productivity of chilli.

These results are in line with Ahmed *et al*. (2016) who studied pre-harvest foliar application of Washington navel orange tree by chitosan. They declared that there was a significant increase in total number of flowers /tree and fruit set percentage over controls especially at low concentration. The positive effect of chitosan in stimulating flowering and increasing its number was reported by Wanichpongpan *et al*. (2001) on gerbera and Ramos-Garcia *et al*. (2009) on gladioli, beside Salachna and Zawadzińska (2014) who reported that "Gompey" freesia corms treated with 0.5% chitosan solution had more leaves, flowered earlier and formed more flowers. According to what was previously mentioned, chitosan may provide a few amino compounds that led to increasing total N content in leaves or higher capacity of plant absorption of nitrogen from soil. Iqbal *et al.* (2004) stated that the rate of leafy inflorescence formation and its ovaries growth was determined by various nitrogenous compounds since these show a higher polyamine content. Abdel-Aziz and El-Azazy (2016), indicated that (NH_3) ammonia and (NH^{4+}) ammonium ion accumulated during stress-as winter

low temperature (that induced flowering in citrus) resulted in stimulation of new arginine biosynthesis and the accumulation of putrescine at an early stage of floral organogenesis, followed by rapid metabolism of these compounds during flower development.

Moreover, many investigators observed that exogenous application of chitosan had a promotive effect on increasing fruit set of citrus trees; (El-Sese, 2005) on Balady mandarin, (Abd El-moneim *et al*., (2007); Abd El-Rahman *et al.* (2012) on washington navel orange and (Baghdady *et al*. (2014) on Valencia orange. Plant biostimulants, particularly chitosan and biozyme had an important role on flowering and fruit set of different crops, since it promote fruit set and reduce fruit drop in many citrus species and varieties. In this concern, Ghoname *et al*. (2010) observed that foliar application of chitosan on sweet pepper significantly increased the number of fruits per plant and the mean weight of fruit, as well as fruit quality characteristics.

5.1.5 Fruit length and diameter

Fruit length and diameter of the strawberry fruits were significantly influenced by application of biozyme and chitosan. Treated plots with the lowest rates of biozyme and chitosan produced the longest fruit length and diameter over the control. The present results are in conformity with the findings of Eris *et al.* (1995) who observed that fruit length and fruit diameter significantly increased with the increased application of seaweed extract in pepper. This increase in fruit size with the application of biozyme and chitosan could be due to nature of auxins (NAA) to stimulate cell division and cell enlargement of the fruits (Chaudhary *et al*., 2006; Taiz and Zeiger, 2006). Increased fruit size is in corroboration with the findings of (Singh, 2008); (Hoang, 2003) who reported that application of NAA increased fruit size of pomegranate. Biozyme not only provided required nutrients for cell activation but also stimulate cellular differentiation, ensuring the number and strength of floral buds that contribute to a higher number of fruits. The application of biozyme during fruit growth results in the formation of more epidermis cells, allowing the fruits to increase their size and commercial grade consistency.

5.1.6 Fruit weight and yield

In the present study, the application of biozyme and chitosan significantly influenced the fruit weight of strawberry in both trials. The strawberry fruit weight was higher from the plots treated with the combination of 25ppm biozyme and 25ppm chitosan over the other treated plots. However, plots treated with the highest combination rates of biozyme and chitosan (50ppm) recorded the lowest fruit weight in both trials. These results are in agreement

with those reported by Agnieszka *et al.* (2004) and Roussos *et al.* (2009) on soybean, Abdel-Mawgoud *et al.* (2010) on watermelon. These increases in fruit weight may be closely linked to the increase in vegetative growth characteristics (Table 3 and 4). In this concern, Ghoname *et al*. (2010) observed that foliar application of chitosan on sweet pepper significantly increased the number of fruits per plant and the mean weight of fruit, as well as yield and fruit quality characteristics.

Foliar application of varying doses of chitosan on strawberry canopy in this study stimulated all aspects of vegetative growth (leaf length, leaf number and height) with concurrent improvement of fruit yield and fruit quality compared with untreated control. Many investigators reported chitosan to control numerous pre- and post-harvest diseases, and increase yield of various ornamental as well as horticultural commodities in different parts of the world (Pichyangkura *et al*., 2015). Kossak and Dyki (2008) showed more numerous and larger xylem cells and phloem vascular bundles in the stems of tomato plants treated with biostimulants compared to the control plants. This phenomenon can contribute to a more effective transport of mineral elements, water, and assimilates, and, consequently, can increase fruit weight.

5.2 Response of biozyme and chitosan on the quality of strawberry fruit

5.2.1 Total Soluble Solids and Titratable Acidity

The amount of total soluble solids and titratable acidity of the strawberry fruit were significantly influenced by the application of biozyme and chitosan in both trials. This result was consistent with Ahmed *et al*. (2016) who stated that TSS % of navel orange fruits were affected significantly by pre-harvest chitosan spray at two different locations under study in comparison with control. The same observation was observed by Saif Eldeen *et al.* (2014) on artichoke. This result are in agreement with the findings of Reeta *et al*. (2010) who observed significant increase in total soluble sugars with the application of seaweed liquid fertilizer. These results are in harmony with (Youssef *et al*., 2015; EL-Eleryan, 2015; Ahmed *et al*., 2016) whose found that Navel orange fruits treated with chitosan showed the significant increase in total soluble solids percentage over the storage periods.

Titratable acidity of the strawberry fruit was significantly influenced by the application of the biozyme and chitosan throughout the growing period in both trials. These results are in line with those obtained by (Scalon *et al*., 2012; Plácido *et al*., 2016) they found that fruits treated with chitosan had low titratable acidity. This might be due to chitosan formation a semipermeable layer regulate gas exchanges on fruit surface and inhibiting respiration (Shiri

et al., 2013), thus reduce production of compounds responsible for acidity (organic acids) in fruits and release hydrogen ions, contributing to decrease fruit titratable acidity and delay the senescence stage progress. Moreover, the representing results of chitosan were in line with Zagzog *et al*. (2017), who showed that acidity % statistically reduced by chitosan foliar application in comparison with control.

5.2.2 Shelf-life

Shelf-life of strawberry fruit was significantly influenced by the application of biozyme and chitosan throughout the growing periods in both trials. Previous similar studies on Chitosan revealed its benefits from reducing decay in peach, litchi and sweet cherry (Li and Yu, 2000; Hernandez-Munoz *et al.,* 2006; Hernandez-Munoz *et al.,* 2008; Sun *et al.,* 2010; Chailoo and Asghari, 2011). El-Ghaouth *et al.,* (1991) suggested that Chitosan induces chitinase, a defense enzyme, which catalyzes the hydrolysis of Chitin, a common component of fungal cell walls (Hou *et al.,* 1998), thus preventing the growth of fungi on the fruit. The results suggest that Chitosan coating is an effective way of preserving fruits and slowing down the oxidation process. It has also been found that strawberry fruits treated with biozyme performed better in storage (Norrie and Keathley, 2006). Numerous studies have revealed a wide range of beneficial effects of seaweed extract applications on plants, such as enhanced postharvest shelf-life of perishable products (Norrie *et al*., 2006).

CHAPTER SIX

CONCLUSSIONS AND RECOMMENDATIONS

6.1 Conclusions

Based on the findings, it can be concluded that;

i. Application of 25ppm biozyme and 25 ppm chitosan on the growth of field grown strawberry plants significantly improved growth and yield of strawberry fruit when compared to untreated control.
ii. Use of 25ppm of biozyme and 25ppm chitosan on strawberry plant produced fruits with the highest quality of strawberry fruits when compared with the other treatments.
iii. The application of 25ppm biozyme and 25ppm chitosan in combination enhanced the growth, yield and quality of strawberry plant.

6.2 Recommendations

Based on the results of this study, the following recommendations can be made;

i. The growth and yield of strawberry plants can be enhanced by the application of biozyme and chitosan at their lowest rates of 25ppm of each biozyme and chitosan.
ii. The quality of the strawberry fruits can be increased by the foliar spray of 25ppm biozyme and 25 ppm chitosan.
iii. The growth, yield and quality of strawberry plants can be improved by the combination foliar spray of 25ppm biozyme and 25ppm chitosan. Since biozyme and chitosan improved growth, yield and quality of strawberry, further research should be done on the response of these biostimulants on other soft fruits which are consumed when fresh.

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APPENDICES

Appendix A: Research permit

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Appendix B: Published paper

IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS) e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 15, Issue 10 Ser. I (October 2022), PP 39-49 www.iosrjournals.org DOI: 10.9790/2380-1510013949 www.iosrjournals.org 39 | Page

Biozyme and Chitosan effect on Growth and Yield of 'Chandler' Strawberry (*Fragaria X Ananassa* Duch.)

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Abstract

Biozyme and chitosan are emerging category of biostimulants that target to increase the production of strawberry and are used as alternative to agrochemicals due to the growing interest of consumers on healthy and safe products. However, due to exclusion of agrochemicals, strawberry production has declined by between 5-34% due to limited nutrition. The purpose of the study was to investigate the response of biozyme and chitosan on the growth, yield and quality of strawberry fruit. The experimental design was randomized complete block design with nine treatments replicated three times. The results showed that the use of 25ppm biozyme and 25ppm chitosan positively influenced the growth and yield of strawberry plant. Maximum number of leaves, plant height, flower number, fruits number, fruit weight, fruit length and diameter were achieved with the use of 25ppm biozyme and 25ppm of chitosan in combination. Thus, findings from the current study however, indicate that biozyme, a seaweed extract and chitosan biostimulant obtained from naturally abundant chitin of crustaceans and fungal cell walls at their lowest rates could be used as environment-friendly agent for sustainable production of high quality strawberry with no use of synthetic inputs.

Key words*: biostimulants, biozyme, chitosan, foliar spray, plant-growth enhancer, strawberry,* ----------

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Date of Submission: 14-10-2022 Date of Acceptance: 29-10-2022 ----------

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Appendix C: Response of biozyme and chitosan on the number of leaves of strawberry plant at 14 DAP

Sources	DF	Type III SS	MSE	F Ratio	P>F
Total	26	83.792			
Block	2	2.675	1.337	2.33	0.1297

Biozyme	2	29.859	14.929	25.97	<0.0001
Chitosan	2	19.914	9.957	17.32	<0.0001
Biozyme *chitosan.	4	22.145	5.536	9.63	0.0004
Error	16	9.199	0.5749		

Appendix D: Response of biozyme and chitosan on leaf length of strawberry plant at 28 DAP

Sources	DF	Type III SS	MSE	F Ratio	P>F
Total	26	149.793			
Block	2	39.324	19.662	15.54	0.0002
Biozyme	2	14.950	7.475	5.91	0.0120
Chitosan	2	37.214	18.6072	14.71	0.0002
Biozyme *chitosan.	4	38.062	9.515	7.52	0.0013
Error	16	20.243	1.265		

Appendix E: Response of biozyme and chitosan on leaf width of strawberry plant at 14 DAP

Sources	DF	Type III SS	MSE	F Ratio	P>F
Total	26	22.939			
Block	2	7.900	3.950	14.60	0.0002
Biozyme	2	3.060	1.530	5.65	0.0139
Chitosan	2	2.138	1.069	3.95	0.0403
Biozyme *chitosan.	4	5.511	1.377	5.09	0.0077
Error	16	4.330	0.2706		

Appendix F: Response of biozyme and chitosan on plant height of strawberry plant at 14 DAP

Sources	DF	Type III SS	MSE	F Ratio	P>F

Total	26	78.161			
Block	2	24.701	12.350	41.77	<0.0001
Biozyme	2	4.777	2.388	8.08	0.0038
Chitosan	2	20.108	10.054	34.00	<0.0001
Biozyme *chitosan.	4	23.841	5.960	20.16	<0.0001
Error	16	4.730	0.2956		

Appendix G: Response of biozyme and chitosan on number of flowers of strawberry plant at 14 DAP

Sources	DF	Type III SS	MSE	F Ratio	P>F
Total	26	11.416			
Block	2	0.125	0.062	0.50	0.6157
Biozyme	2	2.000	1.000	8.00	0.0039
Chitosan	2	4.625	2.312	18.50	<0.0001
Biozyme *chitosan.	4	2.666	0.666	5.38	0.0063
Error	16	2.000	0.125		

Appendix H: Response of biozyme and chitosan on number of fruits of strawberry plant at 28 DAP

Sources	DF	Type III SS	MSE	F Ratio	P>F
Total	26	7.846			
Block	2	0.375	0.187	1.54	0.2413
Biozyme	2	1.642	0.821	6.74	0.0075
Chitosan	2	2.023	1.011	8.31	0.0034
Biozyme *chitosan.	4	1.855	0.463	3.81	0.0233
Error	16	1.952	0.122		

Appendix I: Response of biozyme and chitosan on fruit weight on strawberry plant at 56 DAP

Sources	DF	Type III SS	MSE	F Ratio	P>F
Total	26	262.911			
Block	2	35.290	17.645	10.43	0.0013
Biozyme	2	62.783	31.391	18.56	<0.0001
Chitosan	2	67.017	33.508	19.81	<0.0001
Biozyme *chitosan.	4	70.752	17.688	10.46	0.0002
Error	16	27.056	1.691		

Appendix J: Response of biozyme and chitosan on fruit length on strawberry plant at 56 DAP

Sources	DF	Type III SS	MSE	F Ratio	P>F
Total	26	5.347			
Block	2	0.106	0.053	1.17	0.3365
Biozyme	2	2.614	1.307	28.66	<0.0001
Chitosan	2	0.558	0.279	6.12	0.0106
Biozyme *chitosan.	4	1.338	0.334	7.33	0.0015
Error	16	0.720	0.045		

Appendix K: Response of biozyme and chitosan on fruit diameter on strawberry plant at 56 DAP

Sources	DF	Type III SS	MSE	F Ratio	P>F
Total	26	2.641			
Block	2	0.351	0.175	6.55	0.0084
Biozyme	2	0.394	0.197	7.35	0.0055
Chitosan	2	0.467	0.233	8.71	0.0028
Biozyme *chitosan.	4	0.997	0.249	9.28	0.0004
Error	16	0.432	0.027		

Appendix L: Response of biozyme and chitosan on TSS of strawberry fruit at 28 DAP

Sources	DF	Type III SS	MSE	F Ratio	P>F
Total	26	95.930			
Block	2	5.700	2.850	2.45	0.1180
Biozyme	2	23.100	11.550	9.93	0.0016
Chitosan	2	12.966	6.483	5.57	0.0146
Biozyme *chitosan.	4	35.544	8.886	7.64	0.0012
Error	16	18.624	1.164		

Appendix M: Response of biozyme and chitosan on TA on strawberry fruit at 14 DAP

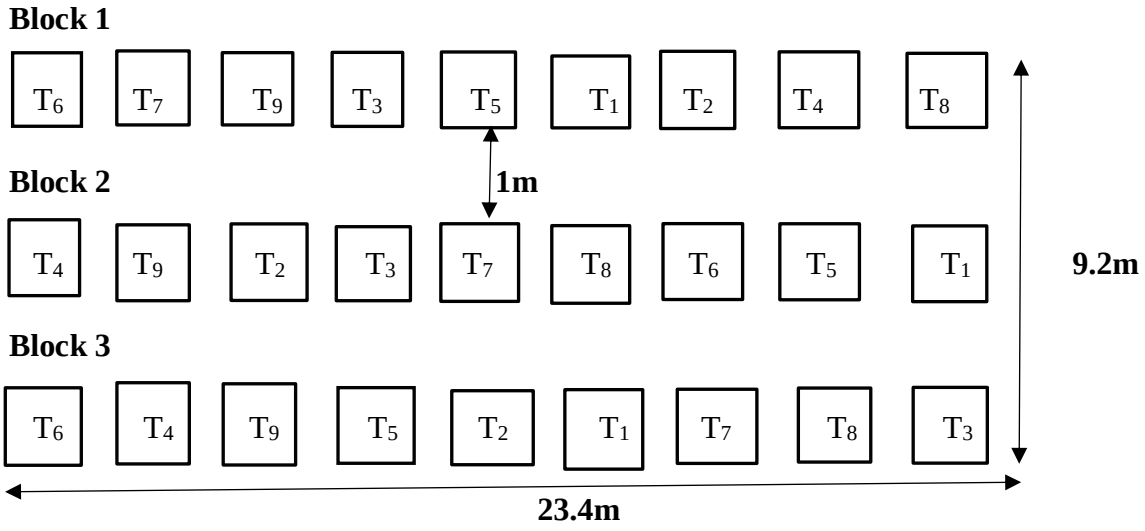
Sources	DF	Type III SS	MSE	F Ratio	P>F
Total	26	0.080			
Block	2	0.003	0.001	2.52	0.1118
Biozyme	2	0.017	0.008	12.40	0.0006
Chitosan	2	0.012	0.006	9.13	0.0023
Biozyme *chitosan.	4	0.034	0.008	12.31	<0.0001

Error	16	0.010	0.0006

Appendix N: Response of biozyme and chitosan on shelf-life of strawberry fruit at 14 DAP

Sources	DF	Type III SS	MSE	F Ratio	P>F
Total	26	154.740			
Block	2	2.740	1.370	1.27	0.3076
Biozyme	2	63.629	31.814	29.49	<0.0001
Chitosan	2	37.851	18.925	17.55	<0.0001
Biozyme *chitosan.	4	33.259	8.314	7.71	0.0002
Error	16	17.264	1.079		

Appendix O: Field layout



Experimental layout and randomization of the treatments

KEY: T_1-0ppm bzy+0ppm cht, **T_2**-0ppm bzy+25ppm cht, **T_3**-0ppm bzy+50ppm cht, **T_4**-25ppm bzy+0ppm cht, **T_5**-25ppm bzy+25ppm cht, **T_6**-25ppm bzy+50ppm cht, **T_7**-50ppm bzy+0ppm cht, **T_8**-50ppm bzy+25ppm cht, **T_9**-50ppm bzy+50ppm cht. **Bzy**-biozyme, **cht**-chitosan