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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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I. Introduction

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, flavour (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 producers in the world are China, USA, 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).

Biostimulants can enhance the growth, yield, and quality of crops significantly. Several types of plant/algal biostimulant provide added benefit to plants when applied by foliar spray or drenching. Studies in this field have been undertaken for close to 70 years but many things are still remained unknown which limit the use of biostimulants. Some plant/algal residues such as chitosan and seaweed (*Ascophyllum nodosum*) are believed to be good sources of biostimulants (Khan *et al.,* 2009).

Chitosan, a name given 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 degraded 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 the protection of 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 and yield 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 *etal*. 2009). Seaweed is now recognized as an excellent source of natural plant growth regulators. (Khan *etal*., 2009; Cardozo *etal*. 2007), which include cytokinins and gibberellins. 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 solubilization of nutrients (Freitas *et al.*, 1997) and symbiotic nitrogen leading to enhanced fruit set, quality and general crop performance. In most strawberry farmlands in the world including Kenya, synthetic fertilizers and pest management products are applied during growth and development to maximize yield. As the use of synthetic chemicals (fertilizers and pesticides) in crop production and protection increases, the threat to both environment and human health is also increasing. 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). Therefore, the present study was aimed to investigate the effects of biozyme and chitosan on growth, yield of 'Chandler' strawberry under field condition.

II. Materials And Methods

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 seas 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 1200 to 1400 mm. The soils are well-drained sandy-loam-vintric mollic Andosols (Jaetzold and Schmidt*.*, 2012).

Materials used in the study

Disease free strawberry (*Fragaria x ananassa* Duch) splits were obtained from Thika strawberry farm in Thika while biozyme was obtained from Arysta Lifescience (K) Ltd which is located in Tulip House, 2nd Floor, Mombasa Road Nairobi. Chitosan, on the other hand was sourced from Kobian Scientific Kenya Limited.

Preparation of the biozyme and chitosan concentrations for used in the study

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).

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 1 litre of distilled water before used.

Experimental design and treatments

A factorial experiment was carried out in a Randomized Complete Block Design (RCBD) with 3 replications. Nine treatments were used in the study. These were;0ppm biozyme+0ppm chitosan, 25ppm chitosan, 50ppm chitosan, 25ppm biozyme, 25ppm biozyme+25ppm chitosan, 25ppm biozyme+50ppm chitosan, 50ppm biozyme, 50ppm biozyme+25ppm chitosan, 50ppm biozyme+50ppm chitosan. Biozyme was applied at three concentrations (0ppm, 25ppmland 75ppm per liter of distilled water), chitosanwas applied at three levels (0ppm, 25ppmland 75ppm per liter of distilled water).The blocks were used to minimize the occurrence of experimental errors caused by soil fertility. The experiment covered an area of 23.4 m by 9.2 m with individual blocks measuring 23.4 m by 2 m separated by a 1m path. Individual experimental units within a block measured 2 m by 2.4 m with an inter-plot spacing of 0.6 m. The treatments were applied three weeks after planting, during flowering and one week before harvest.

Data collection and analysis

Data were collected on the number of leaves, plant height, number of flowers, number of fruits, fruit length, fruit diameter, fruit weight and yield after 28, 42,56 and 70 days of planting. The number of the leaves was determined by counting all fully grown leaves after three weeks of their establishment. Plant height was determined using a ruler from the base to the top of the plant. Fruit length and diameter were determined using a Vernier caliper (cm). The freshly harvested berries from randomly selected four plants in each treatment per replicate were collected and weighted using a digital balance (HANGPING JA 12002, Japan).

Data were subjected to Analysis of Variance (ANOVA) and significant means separated using Tukey's honestly significant difference (Tukey's HSD) test at $p \leq 0.05$. The SAS statistical package (SAS Institute, 2005) was used for data analysis.

III. Results

Number of leaves

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 1). Over the two 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 1. Treated plots produced the highest number of the 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 trial 2

Table 1: Effect of biozyme and chitosan rates on the number of the leaves of 'Chandler' Strawberry(*FragariaX 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 $p \leq 0.05$.**

Plant height

The application of biozymeand chitosan significantly ($p \leq 0.05$) influenced plant height throughout the growing period (Table 2). The highest 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 lowest plant height (14.14cm for trial1 and 14.55cm for trial 2 at 70 DAP) was recorded from the strawberry plants treated with the distilled water (control) (Table 2). 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 2).

Table 2: Effect of biozyme and chitosan rates on plant height leaves 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≤0.05.**

Number of flowers

Application of biozymeand chitosanresulted in a significant (p≤0.05) increase in the number of flowers per plant (Table 3). 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 3). However, there were no significant differences in plots treated with both 25ppm biozyme and 25ppm chitosan in both trials (Table 3). 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 3: 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	0.18b	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≤0.05.**

Number of fruits per plant

There was significant (P≤0.05) influence of biozyme and chitosan in the number of fruits per plant (Table 4). 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 trial 1 and 2 respectively. There was no significance difference in the number of the fruits per plants in the interactions 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 4: Effect of biozyme and chitosan rates on the number fruits per plantof '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 $p \leq 0.05$.**

Fruit length

The results presented in the Table (5) clearly revealed that the application of biozyme and chitosan as a foliar significantly ($p \leq 0.05$) influenced the fruit length in both trails. 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 trail 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 trail 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 trails. Furthermore, there was no significance difference in fruit length from the plots treated with 25ppm biozyme in all sampling days in trail 1. In trail 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 5: Effect of biozyme and chitosan rates on fruit lengthof 'Chandler' Strawberry (*Fragaria X Ananassa* Duch.)

Treatments	Trail	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≤0.05.**

Fruit diameter

The production of strawberry using biozymeand chitosansignificantly (P≤0.05) influenced fruit diameter in the study (Table 6). 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 6: Effect of biozyme and chitosan rates on Fruit diameterof '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≤0.05.**

Fruit weight

Fruit weight was significantly (p≤0.05) influenced by the application of biozyme and chitosan in the experiment study (Table 7). 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 7). 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 7: Effect of biozyme and chitosan rates on Fruit weight in gramsof '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 cht1		9.67ab	11abc	11bcd	10.17bc
25ppm bzy1		10.83ab	12.93abc	13.83ab	12.67ab
25ppm cht+25ppm bzy1		13.33a	16.50a	16.45a	14.83a
25ppm bzy+50ppm cht1		8.77b	8.83bc	8.83d	8.83c
50ppm bzy1		10ab	9.83bc	10.50bcd	9.50bc
50ppm bzy+25ppm cht1		7.67b	9.33bc	9.83cd	8.50c
50ppm bzy+50ppm cht1		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≤0.05.**

Fruit yield per plant

The application of biozyme and chitosan at different rates significantly (p≤0.05) influenced the yield of strawberry fruits per plant (Table 8). 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 attrial 2.

Table 8: Effect of biozyme and chitosan rates on the fruit yield 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≤0.05.**

IV. Discussion

In the present study, the foliar application of biozyme and chitosan on the strawberry plant at different ratessignificantly influenced its the growth and yield. Sharma (1990) found the 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. 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. 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.

Biozyme has shown positive influence on the growth and vigour of the plant.This might be due to higher uptake of plant nutrient as well as quicker relocation of plant metabolites in the plant canopy. Chitosan on the other hand 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 promotes 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 application of biozyme and chitosanin the present studysignificantly enhanced growth of the plant giving taller strawberry plants compared to control. These results are in agreement with Cuibu and Shiayama (2001) who reported positive effects of chitosan incorporated into the soil on early growth stages of soybean, mini-tomato, upland rice and lettuce. This improvement included plant height, leaf area, and dry weight of plants. The results obtained for vegetative growth characteristics in the present study are also in agreement with those reported by Abou El-Yazied *et al.*, (2012)andon 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.

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.

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). 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). Reduced flower drops 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 and Bhosale, 2007) and enhance resistance to water as well as nutrient stress. Biozyme also enhanced photosynthesis and mobilization of metabolites to the flowers (Bhatia and 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 *etal*. (2009) on gladioli. 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. 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 and 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 promotes 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.

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. 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 (Taiz and Zeiger, 2006; Chaudhary *et al*., 2006). Increased fruit size is in corroboration with the findings of (Singh, 2008) and (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.

Fruit weight

In the present study, the application of biozyme and chitosan significantly influenced the fruit weight of strawberry in both seasons. 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 seasons. 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 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 and thus fruit yield.

V. Conclusions and Recommendations

Based on the findings, it can be concluded that; Use of biozyme and chitosaninfluenced the growth and yield of strawberry fruits.The use of 25ppm biozyme and 25ppm chitosanincombination,therefore be recommended for use in strawberry production as an alternative to inorganic chemicals

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