



## SOAKING AND FOLIAR APPLICATION WITH CHITOSAN AND NANO CHITOSAN TO ENHANCING GROWTH, PRODUCTIVITY AND QUALITY OF ONION CROP

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### Abstract

This study was conducted at the Experimental Farm of Sakha Agricultural Research Station during the two successive winter seasons of 2018/2019 and 2019/2020, to assess the potential effect of soaking and foliar spraying with chitosan and Nano chitosan, on the growth, yield quality of onion bulbs. Results showed that the maximum values of growth characteristics (plant height, No. of leaves/plant, dry weight of plant, specific leaf area, leaf area index and crop growth rate), average bulb weight, marketable and total yields were obtained by soaking seedling with Nano chitosan at the rate of 75 ppm, while the minimum values were recorded under soaking with water (control), in both seasons. Spraying with Nano chitosan at the rate of 50 ppm resulted the highest values of all growth characters, average bulb weight, marketable and total yields and bulb quality parameters (bulb diameter, TSS% and DM %) as compared with the other spraying treatments or the control. From the results of this study, based on the onion productivity in addition to the economic analysis of the results, it is clear the promising role of Nano chitosan as an aid to increase the efficiency of traditional fertilizers and increase the net return per fed.

**Keywords :** Chitosan, onion, Nano chitosan, foliar application, onion bulb yield.

### Introduction

Onion is the most used flavorings vegetable in the world. In Egypt, onion is considered one of the most important vegetable crops, for local markets and export as fresh or dried. The productivity of onion is influenced by several factors, such as fertilization, as onion plants is a highly nutrient responsive. Conventional fertilizations have undoubtedly helped in improving both bulb yield and quality of onion, but lately, it was arise many novel trends in fertilizations. Using of chitosan as a carrier for slow fertilizer release is one of these trends, as it can improve the efficiency of the fertilization process (Lei *et al.*, 2011). Chitosan is a member of the polysaccharides which is considered a useful natural polymer and is produced by alkaline N-deacetylation of chitin. Chitosan is the second most abundant natural polymer on earth and is a component of the cell walls of many fungi and insects as well as some algae. Chitosan was first categorized as an elicitor in plants activating genes that underlie the biosynthetic pathways of secondary metabolites. Chitosan can be used both in vivo and in vitro and can be sprayed on plant aerial organs to induce the accumulation of bioactive secondary metabolites (Yin *et al.*, 2011).

Chitosan, poly [ $\beta$ -(1-4)-linked-2-amino-2-deoxy-D-glucose], is the N-deacetylated product of chitin, which is currently obtained from the outer shell of crustaceans such as crabs and shrimps and it has been used in agriculture as plant growth promoter (Sandford, 1989 and Katiyaret *al.*, 2014). Chitin and chitosan are polysaccharides, chemically similar to cellulose differing only by the presence or absence of nitrogen. The agricultural and horticultural uses for chitosan, primarily for plant defense and yield increase, are based on how this glucosamine polymer influences the biochemistry and molecular biology of the plant cell. Chitosan is an anti-transpirant compound that has proved to be effective in many crops (khan *et al.*, 2002 and Karimi *et al.*, 2012). It was used to protect plants against oxidative stress (Guan *et al.*, 2009) and to stimulate plant growth (Farouk *et al.*, 2011). Chitosan is a natural low toxic and inexpensive compound that is biodegradable and environmentally friendly with various applications in agriculture. Chitin and chitosan have been improved soil fertility, and enhanced the mineral nutrient uptake of plant (Dzung, 2007). Increased the content of chlorophylls, photosynthesis and chloroplast enlargement, escalating nitrogen fixing nodes of species of leguminous plants (Dzung and Thang, 2004). Reduced the effects of abiotic stress on plants (like drought stress), by increase the key enzymes related to the closure of the plants stomata resulting in reduction of water loss (Song *et al.*, 2006). The beneficial effect of chitosan is generally depending on its concentration, application

methods, environmental conditions, and growth status. There are many investigations about the influences of chitosan application on different plants. As, chitosan foliar application affect the growth and yield of basil (*Ocimum ciliatum*) (Pirbalouti *et al.* 2017). Bittelli *et al.* (2001) reported that foliar application of chitosan reduced water use of pepper plants by 26–43% while maintaining biomass production and yield. They suggested that chitosan might be an effective anti transpirate to conserve water use in agriculture. In addition, Sheikha and Al-Malki (2011) indicated that chitosan enhanced bean shoot and root length, fresh and dry weights of shoot, root, and leaf area as well as the level of chlorophylls. Results of previous investigations (Farouk *et al.*, 2008 and Ghoname *et al.*, 2010) indicated that foliar application of chitosan resulted in higher vegetative growth and improvement in fruit quality of pepper, radish, and cucumber. Nano-chitosan is a natural material with excellent physicochemical properties. It is environmentally friendly and bioactive. Nano-chitosan has been prepared by several approaches, including physical cross linking by ionic gelation between chitosan and specific negatively charged macromolecules (Calvo *et al.*, 1997). Moreover, chitosan and chitosan nanoparticle films and coatings can be used as a vehicle for incorporating natural or chemical antimicrobial agents, antioxidants, enzymes, or functional substances such as plant extracts, probiotics, minerals, or vitamins (Ojagh *et al.*, 2010). Nevertheless, data on the utilization of chitosan for onion are meager in Egypt. In this way, this study was carried to consider the impact of soaking and foliar spraying of chitosan and Nano chitosan on onion cultivar of Giza red, uniquely the adjustment in yield and quality properties.

### Material and Method

#### Experimental treatments:

A field experiments was conducted at the Experimental Farm of Sakha Agricultural Research Station in Kafr El-Sheikh Governorate at North Nile Middle Delta Region, during the two successive growing seasons of 2018/2019 and 2019/2020 to study the response of onion plant to soaking and foliar spraying with chitosan and Nano chitosan. Soil samples were collected at depths (0-20, 20-40 and 40-60) before experiments. Salinity was determined in the saturated soil poste extract according to Page (1982). Soil bulk density and total porosity as described by Campbell (1994). In filtration rate was determined using double cylinder infiltrometers as described by Garcia (1978). Organic matter content was determined according to Walkaly and Black method as described by Hesse (1971). To study the soil texture, the soil texture, the particle size distribution was determined according to the international method (Klute, 1986). Chemical analysis in both seasons are shown in Table 1. Phosphorus and potassium fertilizer

were applied during soil preparation as recommended and nitrogen fertilizer was applied at rate of 120 kg/fed. as ammonium nitrate (33.5%) was side dressed at two equal doses, at 30 and 60 days from transplanting. Onion seed were sown within the period of 10th and

17th of October in 2018/2019 and 2019/2020 seasons, respectively. Onion seedlings cv. Giza red were transplanted on December 10th and 19th for 1st and 2nd seasons, respectively.

**Table 1 :** The initial of some soil properties for the experimental field loads

Soil depth (cm)	Particle size distribution			Texture class	OM (%)	Bulk density (g/cm <sup>3</sup> )	Total porosity (E%)	Basic I R (cm/hr)
	Sant (%)	Silt (%)	Clay (%)					
0 -20	15.96	28.21	55.83	Clayey	1.65	1.19	55.09	0.75
20 - 40	17.69	29.13	53.18	Clayey	1.51	1.29	51.32	
40 - 60	16.93	31.48	51.59	Clayey	1.27	1.34	49.43	
Mean	16.87	29.60	53.53	Clayey	1.48	1.27	51.95	
Soil depth (cm)	Ec (dsm <sup>-1</sup> )	SAR	Field capacity (%)	Wilting Point	Available water (%)	Available nutrients (ppm)		
						N	P	K
0 - 20	2.37	7.59	43.65	22.87	20.78	33.17	8.21	383
20 - 40	3.22	8.85	40.18	20.96	20.38	40.81	9.55	410
40 - 60	3.87	9.70	38.67	19.75	18.92	36.45	8.15	329
Mean	3.15	8.71	40.83	21.19	20.03	36.81	8.64	374

Before transplanting on permanent soil, onion seedlings were soaked on chitosan or Nano chitosan solution according to the treatment dose. During the two experimental seasons, spraying with chitosan or Nano chitosan was conducted at three times; at 50, 65 and 80 days from transplanting. The experiment treatments were arranged at split-plot design with four replicates. The main plots were randomly assigned with the five soaking application treatments, whereas foliar application treatments were randomly distributed in sub plots. The experimental plot area was 10.5 m<sup>2</sup> (included 5 ridges, 60 cm width and 3.5 meter long). All the cultural operations like nursery raising, main field preparation, transplanting, fertilization, irrigation; weeding, plant protection etc. were carried out as recommended. This investigation includes the following treatments:

#### Main plots: Soaking seedlings with chitosan form:

S<sub>0</sub>. Control (soaking with water)., S<sub>1</sub>.Soaking with 100 ppm of chitosan (ch 100)., S<sub>2</sub>.Soaking with 150 ppm of chitosan (ch 150).

S<sub>3</sub>.Soaking with 50 ppm of Nano chitosan (Nano 50)., S<sub>4</sub>. Soaking with 75 ppm of Nano chitosan (Nano 75).

#### Sub plots: Foliar spraying with chitosan form:

F<sub>0</sub>. Control (spraying with water)., F<sub>1</sub>.Foliar spraying with chitosan at 100 ppm (ch 100)., F<sub>2</sub>.Foliar spraying with chitosan at 150 ppm (ch 150)., F<sub>3</sub>.Foliar spraying with Nano chitosan at 50 ppm (Nano 50)., F<sub>4</sub>.Foliar spraying with Nano chitosan at 75 ppm (Nano 75).

#### Preparation of Nano chitosan particles:

Chitosan, poly [ $\beta$ -(1-4)-linked-2-amino-2-deoxy-D-glucose]. Nano chitosan solution was obtained from Nanotech Company (Gate3, Dreamland, 6th October, Cairo- Egypt). The chitosan nanoparticles can be prepared according to (Zhen *et al.*, 2007).

#### Crop data collection:

##### A. Growth parameters:

A representative sample of five plants was randomly taken from the 2nd row of each plot at 110 DAT (days after transplanting) to estimating the following data:

**A.1. Plant height (cm):** It was measured in cm from the base of swelling sheath to the tip of longest tubular blades.

**A.2. Number of leaves/plant:** It is expressed as the average of number of leaves that appeared on each individual plant.

**A. 3. Plant dry weight:** The fresh matter of 5 plants was taken and oven-dried at 70°C till a constant weight to obtain the dry weight of plant, according to the methods described in A.O.A.C. (1975).

**A.4. Specific leaf area per plant cm<sup>2</sup>/g:** It was calculated according to the following formula:

$$SLA = \text{Leaf area} / \text{leaf dry weight}$$

**A.5. Leaf area index per plant** was determined according to the following formula:

$$LAI = \text{Leaf area per plant (cm}^2\text{)} / \text{Land area per plant (cm}^2\text{)}$$

**A.6.Crop growth rate (CGR, g/week)** using the formula of:

$$CGR = (W_2 - W_1) / (T_2 - T_1)$$

Where:

W<sub>2</sub>-W<sub>1</sub> is the difference in dry matter accumulation of whole plants between two samples, T<sub>2</sub>-T<sub>1</sub> is the number of weeks between two successive sample) 90-110 DAT).The parameters of specific leaf area, leave area index and crop growth rate were determined according to Hunt (1990).

#### A. Photosynthetic pigments assay:

Chlorophyll was extracted in 85% acetone from fresh leaf sample according to the method of Metzner *et al.* (1965). The concentration of the different pigment fractions (chlorophyll a, chlorophyll b and carotenoids) was determined in mg/g fresh weight.

#### B. Yield components:

At harvest time, bulbs from each experimental plot were collected and cured for 15 days, and then dried leaves were removed. After that, average bulb weight (g), marketable yield (t/fed.), culls yield (t/fed.), and total yield (t/fed.) were estimated.

#### D. Onion quality:

Samples of five bulbs from each plot were randomly selected to determine bulb diameter (cm), total soluble solids percentage (TSS %), dry matter percentage (DM %) and protein content. TSS% was determined immediately after harvest by a hand refractometer in the same representative sample of the 5 bulbs according to A.O.A.C. (1975). The protein content of bulbs was estimated quantitatively in the borate buffer extract using the method described by Bradford (1976). The protein content was calculated as ( $\mu\text{g/g}$  dry weight).

#### E- Economic feasibility study:

The economic feasibility of treatments was calculated as follows: **1.** Total costs of onion production (L.E./fed.): as affected by different treatments., **2.** Total income (L.E./fed.) = Selling rate (L.E./ ton)  $\times$  Yield (ton/fed.), **3.** Net farm return (L.E./fed.) = Total

income - Total costs. And 4. Benefit/Cost ratio (B/C) = Total income/ Total cost. Prevailing market prices were used for different outputs and inputs. One ton of marketable onion =2000 L.E. and one ton of culls onion =800 L.E. as an average of the two seasons. Economical evaluation was conducted using the formulas described by CIMMYT, 1988.

#### Statistical analysis:

All data collected were subjected to statistical analysis as described by Snedecor and Cochran (1980) at 5% of significance level and the means were compared using LSD test to check difference. All statistical analyses were performed with a software package Costat® Statistical Software, ver. 6.311 (CoStat Software, 2005); a product of, Cohort Software, Monterey, California.

**Table 2:** Plant height (cm), number of leaves/plant and plant dry weight (g) as influenced by soaking and foliar spraying with chitosan and Nano chitosan, and their interaction at 110 DAT during 2018/2019 and 2019/2020 seasons.

Treatment	2018/2019			2019/2020			
	Plant height (cm)	No. of leaves/plant	Plant dry weight(g)	Plant height (cm)	No. of leaves/plant	Plant dry weight(g)	
<b>A. Soaking of seedlings with chitosan (ppm)</b>							
Control	70.16	7.99	25.34	68.82	8.75	18.81	
Ch 100	76.14	8.34	27.07	73.74	9.33	20.33	
Ch 150	77.85	8.68	28.44	76.22	10.01	21.19	
Nano 50	80.12	8.93	30.21	80.70	11.11	21.96	
Nano 75	83.33	9.60	31.57	86.76	12.02	23.19	
LSD <sub>(0.05)</sub>	<b>1.04</b>	<b>0.21</b>	<b>0.69</b>	<b>0.99</b>	<b>0.86</b>	<b>0.74</b>	
<b>B. Foliar spraying of seedlings with chitosan (ppm)</b>							
Control	73.62	7.72	25.71	72.91	9.33	19.10	
Ch 100	76.69	8.46	27.39	75.84	9.96	20.44	
Ch 150	78.97	9.02	29.84	79.28	10.79	21.71	
Nano 50	81.27	9.52	30.86	80.83	11.12	23.32	
Nano 75	77.06	8.82	28.84	77.38	10.03	20.91	
LSD <sub>(0.05)</sub>	<b>0.89</b>	<b>0.16</b>	<b>0.43</b>	<b>0.68</b>	<b>0.63</b>	<b>0.60</b>	
<b>Interaction (A x B):</b>							
Control	Control	67.44	7.16	22.82	64.46	7.47	15.48
	Ch 100	69.08	7.67	24.84	67.12	8.80	18.97
	Ch 150	71.29	8.38	26.91	70.55	9.10	19.79
	Nano 50	72.88	8.57	27.12	72.15	9.53	20.46
	Nano 75	70.14	8.17	25.04	69.81	8.88	19.36
Ch 100	Control	70.99	7.33	24.06	69.21	8.26	18.11
	Ch 100	75.87	7.96	26.24	72.82	9.41	19.60
	Ch 150	78.03	8.67	28.83	75.45	9.80	21.55
	Nano 50	79.53	9.23	28.90	76.68	9.75	21.90
	Nano 75	76.28	8.50	27.34	74.54	9.41	20.48
Ch 150	Control	74.29	7.58	25.12	71.76	9.83	19.55
	Ch 100	78.94	8.56	27.39	75.28	9.75	20.22
	Ch 150	79.51	9.00	29.49	77.78	10.33	21.67
	Nano 50	80.45	9.50	31.30	79.44	10.83	23.32
	Nano 75	76.06	8.75	28.90	76.82	9.33	21.21
Nano 50	Control	75.69	7.90	27.98	76.47	10.17	20.91
	Ch 100	79.24	8.66	28.00	79.64	10.5	21.54
	Ch 150	81.73	9.16	31.62	82.71	11.75	21.97
	Nano 50	83.15	10.00	32.38	83.98	12.00	24.03
	Nano 75	80.81	8.92	31.07	80.71	11.16	21.35
Nano 75	Control	79.66	8.61	28.59	82.62	10.91	21.47
	Ch 100	80.33	9.45	30.48	84.37	11.33	21.85
	Ch 150	84.33	9.86	32.38	89.91	13.00	23.58
	Nano 50	90.33	10.28	34.59	91.89	13.50	26.88
	Nano 75	82.00	9.78	31.83	85.01	11.33	22.17
LSD <sub>(0.05)</sub>	<b>1.98</b>	<b>N.S</b>	<b>0.97</b>	<b>1.52</b>	<b>N.S</b>	<b>1.33</b>	

LSD<sub>(0.05)</sub>: Least significant difference and N.S indicate not significant at P: 0.05 probability.

Foliar spraying of onion plants with 75 ppm of Nano chitosan was significantly increased plant height, number of leaves/plant and plant dry weight, in both seasons. In the same trend, results revealed that foliar spraying with 50 ppm of Nano chitosan gave the highest significant increment in these parameters as compared to other treatments. Kalteh et al. (2014) and Siddiqui et al. (2014) reported that soil addition of chitosan increased plant height, canopy diameter, and leaf area of *Capsicum annum* L. Data presented in Tables (2) show that the interaction effect between different soaking and foliar application doses with chitosan was significant on plant height and plant dry weight in both seasons. While it did not reach the level of significance on

## Results and Discussion

### A. Growth parameters:

Data in table (2) reveal that plant height, number of leaves/plant, plant dry weight were significantly affected by soaking with chitosan, in both seasons. The highest values of these traits were observed by soaking onion seedlings with Nano chitosan at rate of 75 ppm, while the lowest values were observed under control treatment (soaking with water). These results were true in both seasons. The increase in onion growth parameters by soaking with chitosan was probably due to that chitosan plays an important role in promoting and improving plant vegetative growth.

number of leaves/plant in both seasons. Generally, it could be stated that soaking of onion seedlings with 75 ppm Nano chitosan, plus foliar spraying plants with 50 ppm Nano chitosan gave the highest values of plant height, number of leaves/plant and plant dry weight as compared to other combinations. While, soaking seedlings and spraying with water (control) gave the lowest values. This may be due to the absorption of Nano chitosan by soaking plus foliar spraying which further utilized for various physiological processes to influence favorably the growth parameters under study. In addition, the values obtained with soaking and spraying with chitosan were less than Nano chitosan treatments. These results were true in the two seasons. For

growth analysis, data in Table (3) indicate that specific leaf area (SLA), leaf area index (LAI) and crop growth rate (CGR), were significantly affected by soaking treatments in both seasons, except for LAI in the second season. Soaking of seedlings with 75 ppm Nano chitosan recorded the maximum values of growth analysis parameters, followed by soaking with 50 ppm Nano chitosan; whereas soaking with water (control) recorded the minimum values. The obtained results showed significant effect on SLA, LAI and CGR as response to foliar spraying, in both seasons, except for LAI in the second season (Table, 3). The highest values of these traits were obtained by foliar spraying with 50 ppm Nano chitosan. While, the lowest values were obtained under

control treatment, in both seasons. The interaction between the two factors had significant effect on SLA in both seasons; and on LAI and CGR in the first season. Soaking with 75 ppm Nano chitosan plus foliar spraying with 50 ppm Nano chitosan appeared the highest values of SLA, LAI and CGR, while the control treatments of the two factors appeared the lowest values.

**B. Pigments component:**

Chlorophyll a+b and carotenoids contents as affected by studied treatments are shown in Table, 4. Soaking with 75ppm Nano chitosan had the highest values of these traits,

**Table 3:** Specific leaf area (SLA), leaf area index (LAI) and crop growth rate (CGR), as influenced by soaking and foliar spraying with chitosan and Nano chitosan, and their interaction at 110 DAT during 2018/2019 and 2019/2020 seasons.

Treatment		2018/2019			2019/2020		
		SLA (Cm <sup>2</sup> /g)	LAI	CGR (g/week) (90-110DAT)	SLA (Cm <sup>2</sup> /g)	LAI	CGR (g/week) (90-110DAT)
<b>A. Soaking of seedlings with chitosan (ppm)</b>							
Control		145.67	0.807	12.24	142.85	0.816	16.62
Ch 100		170.74	0.929	13.01	160.64	0.869	17.76
Ch 150		178.14	1.134	13.53	175.14	0.898	18.51
Nano 50		189.63	1.239	14.27	193.26	1.059	19.47
Nano 75		207.91	1.589	14.99	212.79	1.153	20.31
LSD (0.05)		<b>7.04</b>	<b>0.095</b>	<b>0.47</b>	<b>2.45</b>	<b>N.S</b>	<b>0.67</b>
<b>B. Foliar spraying of seedlings with chitosan (ppm)</b>							
Control		142.58	0.857	12.59	135.10	0.759	16.25
Ch 100		162.21	1.007	12.98	162.40	0.842	17.38
Ch 150		189.66	1.299	13.94	194.48	1.026	19.33
Nano 50		222.45	1.421	15.04	208.21	1.193	20.82
Nano 75		175.18	1.113	13.48	184.50	0.975	18.88
LSD (0.05)		<b>6.66</b>	<b>0.079</b>	<b>0.43</b>	<b>2.90</b>	<b>0.199</b>	<b>0.85</b>
<b>Interaction (A x B):</b>							
Control	Control	114.83	0.438	11.19	104.21	0.625	14.72
	Ch 100	123.47	0.766	11.95	116.65	0.696	15.94
	Ch 150	163.34	0.954	12.90	156.96	0.925	17.45
	Nano 50	181.37	1.003	12.95	178.67	0.962	17.90
	Nano 75	145.32	0.874	12.18	157.76	0.873	17.05
Ch 100	Control	130.01	0.668	11.75	110.83	0.638	16.01
	Ch 100	157.47	0.807	12.38	136.50	0.723	16.34
	Ch 150	190.95	1.044	13.56	189.62	1.027	18.20
	Nano 50	206.99	1.225	13.87	191.25	1.095	20.98
	Nano 75	168.28	0.901	13.48	175.01	0.861	17.28
Ch 150	Control	135.47	0.949	12.29	124.07	0.719	15.01
	Ch 100	167.48	1.024	12.78	175.33	0.822	17.23
	Ch 150	194.76	1.229	13.81	184.87	0.785	19.57
	Nano 50	215.35	1.302	15.15	214.01	1.215	21.31
	Nano 75	177.63	1.162	13.65	177.41	0.946	19.42
Nano 50	Control	156.27	1.074	13.79	154.29	0.861	17.41
	Ch 100	174.45	1.115	13.84	180.10	0.876	17.68
	Ch 150	195.46	1.325	14.16	212.11	1.162	20.49
	Nano 50	237.78	1.517	15.64	222.92	1.320	21.83
	Nano 75	184.16	1.167	13.91	196.88	1.073	19.91
Nano 75	Control	176.28	1.157	13.93	182.09	0.951	18.09
	Ch 100	188.19	1.327	13.97	203.42	1.088	19.67
	Ch 150	203.76	1.943	15.26	228.81	1.232	20.92
	Nano 50	270.77	2.057	17.58	234.20	1.369	22.08
	Nano 75	200.54	1.461	14.18	215.43	1.122	20.77
LSD (0.05)		<b>14.89</b>	<b>0.177</b>	<b>0.96</b>	<b>6.50</b>	<b>N.S</b>	<b>N.S</b>

LSD (0.05): Least significant difference and N.S indicate not significant at P: 0.05 probability.

followed by soaking with 50 ppm Nano chitosan. While, soaking with water (control) appeared the lowest values. These results were true in both seasons. These results reflect the role of chitosan compounds on increasing the photosynthetic pigments through their effects on physiological function on the plants. There were significant increase in Chlorophyll a+b and carotenoids contents when spraying plants with chitosan compounds. Foliar spraying with 50 ppm Nano chitosan gave the highest values these traits followed by foliar spraying with 150 ppm chitosan. While, the control treatment gave the lowest value. These results were true in the two seasons. Chlorophyll and carotenoids contents were resulted as a direct effect of chitosan and Nano chitosan on plant growth viability. The increase in photosynthetic pigments by applying of chitosan forms may be due to that Nano chitosan enhancing

endogenous levels of cytokinins, which stimulated chlorophyll synthesis and growth or to the greater availability of amino compounds released from chitosan (Chibu and Shibayama, 2001). Farouk and Aman (2012) reported that foliar application of chitosan, especially at 250 mg.l-1, significantly increased these parameters compared to the untreated plants under stress. It has been reported that nanoparticle treatment could induce higher chlorophyll contents in Asparagus and Sorghum (Namasivayam and Chitrakala 2011). Purvis (1980) reported that higher ethylene causes an increase in activity of chlorophyllase enzyme and destruction of internal chloroplast membranes. The implied inhibition of ethylene action by Nano chitosan is responsible for higher chlorophyll contents in the treated treatments.

**Table 4:** Photosynthetic pigments of leaves (mg/g fresh weight), as influenced by soaking and foliar spraying with chitosan and Nano chitosan, and their interaction at 110 DAT during 2018/2019 and 2019/2020 seasons.

Treatment		2018/2019		2019/2020	
		Chlorophyll a + b	Carotenoids	Chlorophyll a + b	Carotenoids
(mg/g fresh weight)					
<b>A. Soaking of seedlings with chitosan (ppm)</b>					
Control		1.239	0.141	1.629	0.349
Ch 100		1.318	0.221	1.699	0.362
Ch 150		1.542	0.310	1.778	0.385
Nano 50		1.908	0.404	1.950	0.394
Nano 75		2.809	0.515	2.115	0.565
LSD (0.05)		<b>0.176</b>	<b>0.114</b>	<b>0.068</b>	<b>0.046</b>
<b>B. Foliar spraying of seedlings with chitosan (ppm)</b>					
Control		1.006	0.238	1.610	0.355
Ch 100		1.255	0.259	1.731	0.366
Ch 150		2.152	0.369	1.937	0.410
Nano 50		2.705	0.428	2.079	0.556
Nano 75		1.698	0.259	1.814	0.368
LSD (0.05)		<b>0.169</b>	<b>0.077</b>	<b>0.035</b>	<b>0.031</b>
<b>Interaction (A x B):</b>					
Control	Control	0.484	0.119	1.413	0.327
	Ch 100	0.807	0.133	1.477	0.331
	Ch 150	1.474	0.145	1.749	0.367
	Nano 50	2.353	0.167	1.899	0.375
	Nano 75	1.080	0.139	1.610	0.347
Ch 100	Control	0.564	0.165	1.462	0.331
	Ch 100	0.927	0.182	1.584	0.350
	Ch 150	1.790	0.267	1.824	0.387
	Nano 50	2.023	0.302	1.941	0.400
	Nano 75	1.286	0.185	1.686	0.344
Ch 150	Control	0.773	0.232	1.532	0.359
	Ch 100	1.139	0.225	1.726	0.374
	Ch 150	1.956	0.325	1.898	0.387
	Nano 50	2.414	0.463	1.972	0.443
	Nano 75	1.425	0.304	1.759	0.363
Nano 50	Control	1.287	0.255	1.764	0.365
	Ch 100	1.559	0.306	1.865	0.379
	Ch 150	2.107	0.530	2.052	0.410
	Nano 50	2.846	0.556	2.134	0.438
	Nano 75	1.742	0.375	1.934	0.379
Nano 75	Control	1.925	0.416	1.879	0.393
	Ch 100	1.843	0.452	2.001	0.396
	Ch 150	3.431	0.576	2.164	0.501
	Nano 50	3.890	0.652	2.452	1.125
	Nano 75	2.955	0.480	2.082	0.407
LSD (0.05)		<b>0.289</b>	<b>N.S</b>	<b>0.079</b>	<b>0.069</b>

LSD (0.05): Least significant difference and N.S indicate not significant at P: 0.05 probability.

The interaction between the two factors had significant effect on Chlorophyll a+b content in the two seasons and carotenoids content in the second season. The greatest values of chlorophyll a+b and carotenoids contents were obtained by soaking with 75 ppm Nano chitosan and foliar spraying with 50 ppm Nano chitosan, followed by spraying with 150 ppm chitosan. While, the smallest values were obtained under control treatments of soaking and foliar spraying.

#### C. Yield components:

Results in Table (5) revealed that soaking with chitosan significantly increased average bulb weights, marketable yield/fed. and total yield /fed. in the two seasons as compared to control treatment. Soaking with 75 ppm Nano chitosan attained the highest values. The obtained results showed a significant effect on onion yield and yield component as response to foliar spraying. The highest values of average bulb weight, marketable yield/fed and total yield/fed, were obtained by foliar spraying with 50 ppm Nano chitosan during both seasons. Culls yield/fed take an opposite direction in responding to chitosan foliar spraying. The lowest values of culls yield/fed. were obtained under foliar spraying with 50 and 75 ppm Nano chitosan, in the first and second seasons, respectively. The different combinations between soaking and foliar spraying with chitosan appeared a significant effect on onion yield in both seasons, except for average bulb weight and culls

yield in the first season (Table, 5). Soaking with 75 ppm Nano chitosan and spraying with 50 ppm Nano chitosan gave the highest values of average bulb weight, marketable yield/fed. and total yield /fed. While, soaking and spraying with water (control treatments) gave the lowest values. For culls yield/fed., the lowest values were obtained under soaking with 50 ppm Nano chitosan and spraying with 50 ppm Nano chitosan, in the second seasons. The highest values of culls yield/fed. were obtained under control treatments of the two factors.

#### D. Onion quality:

The results in Table (6) demonstrate that bulb diameter, total soluble solids percentage (TSS %), dry matter percentage (DM %) and protein content were significantly affected by soaking with chitosan in the two seasons. Soaking with 75 ppm Nano chitosan treatment attained the highest values of all the above onion quality parameters. While, soaking with water attained the lowest values. From the obtained results, it can be noticed that Nano chitosan compounds surpassed chitosan compound in respect to their effects on all onion quality parameters, in both seasons. The superiority of average bulb weight, marketable yield/fed. and total yield/fed. under soaking or foliar spraying with chitosan reflect the role of chitosan in improving onion growth characters. This role of chitosan might be due to an increase in stomata conductance and net photosynthetic CO<sub>2</sub>-fixation activity (Khan et al., 2002).

**Table 5:** Average bulb weight (g), marketable yield/fed., culls yield/fed. and total yield/fed., as influenced by soaking and foliar spraying with chitosan and Nano chitosan and their interaction during 2018/2019 and 2019/2020 seasons.

Treatment	2018/2019				2019/2020				
	Average bulb weight (g)	Market. yield (t/fed)	Culls yield (t/fed.)	Total yield (t/fed.)	Average bulb weight (g)	Market. yield (t/fed)	Culls yield (t/fed.)	Total yield (t/fed.)	
<b>A. Soaking of seedlings with chitosan (ppm)</b>									
Control	76.61	12.09	2.30	14.39	82.67	11.12	2.04	13.16	
Ch 100	83.79	13.10	1.86	14.95	84.26	12.78	1.85	14.62	
Ch 150	86.32	14.37	1.73	16.10	94.15	13.84	1.83	15.66	
Nano 50	93.19	16.11	1.66	17.77	106.81	14.67	1.73	16.41	
Nano 75	109.26	19.21	1.46	20.66	116.11	16.51	1.66	18.17	
LSD (0.05)	<b>4.08</b>	<b>0.33</b>	<b>0.21</b>	<b>0.24</b>	<b>2.48</b>	<b>0.25</b>	<b>N.S</b>	<b>0.21</b>	
<b>B. Foliar spraying of seedlings with chitosan (ppm)</b>									
Control	79.26	13.62	2.19	15.82	77.49	12.52	2.13	14.65	
Ch 100	85.51	14.63	1.87	16.49	92.27	13.31	1.90	15.21	
Ch 150	94.59	15.56	1.65	17.21	103.57	14.32	1.61	15.93	
Nano 50	101.21	16.18	1.51	17.69	113.84	15.00	1.64	16.64	
Nano 75	88.60	14.89	1.78	16.67	96.85	13.77	1.82	15.59	
LSD (0.05)	<b>4.04</b>	<b>0.34</b>	<b>0.22</b>	<b>0.30</b>	<b>3.28</b>	<b>0.28</b>	<b>0.14</b>	<b>0.25</b>	
<b>Interaction (A x B):</b>									
Control	Control	65.45	10.00	3.09	13.09	58.12	9.57	2.68	12.26
	Ch 100	73.64	11.88	2.26	14.14	79.54	10.64	2.24	12.87
	Ch 150	81.11	12.85	2.09	14.9j	86.15	11.95	1.49	13.45
	Nano 50	85.15	13.30	1.98	15.28	99.59	12.04	1.78	13.82
	Nano 75	77.69	12.42	2.08	14.50	89.96	11.39	1.99	13.38
Ch 100	Control	73.87	11.7	2.13	13.92	63.41	11.78	2.17	13.95
	Ch 100	79.48	12.78	1.98	14.77	81.38	12.37	1.94	14.31
	Ch 150	86.76	13.12	1.67	14.7j	90.55	13.57	1.58	15.15
	Nano 50	94.75	14.63	1.67	16.29	101.08	13.59	1.62	15.21
	Nano 75	84.10	13.17	1.86	15.03	84.85	12.58	1.91	14.49
Ch 150	Control	75.31	13.15	2.03	15.18	79.28	12.41	2.07	14.48
	Ch 100	82.54	14.13	1.83	15.96	88.87	13.30	1.87	15.18
	Ch 150	91.64	15.17	1.53	16.71	98.17	14.55	1.71	16.26
	Nano 50	96.25	15.25	1.48	16.73	110.53	15.33	1.61	16.94
	Nano 75	85.86	14.15	1.77	15.92	94.12	13.57	1.88	15.45
Nano 50	Control	82.01	15.35	2.03	17.38	91.76	13.47	1.91	15.38
	Ch 100	87.70	15.45	1.76	17.22	99.74	14.25	1.84	16.09
	Ch 150	96.63	16.95	1.47	18.42	117.38	15.08	1.67	16.75
	Nano 50	110.09	17.37	1.32	18.69	122.48	16.18	1.40	17.59
	Nano 75	89.51	15.44	1.71	17.16	102.64	14.37	1.83	16.21
Nano 75	Control	99.67	17.80	1.70	19.50	94.91	15.34	1.84	17.18
	Ch 100	104.21	18.88	1.52	20.40	111.78	15.98	1.61	17.59
	Ch 150	116.81	19.71	1.46	21.17	125.62	16.47	1.57	18.04
	Nano 50	119.80	20.38	1.11	21.49	135.56	17.84	1.77	19.61
	Nano 75	105.82	19.27	1.50	20.77	112.68	16.92	1.50	18.42
LSD (0.05)	<b>N.S</b>	<b>0.78</b>	<b>N.S</b>	<b>0.68</b>	<b>7.32</b>	<b>0.61</b>	<b>0.30</b>	<b>N.S</b>	

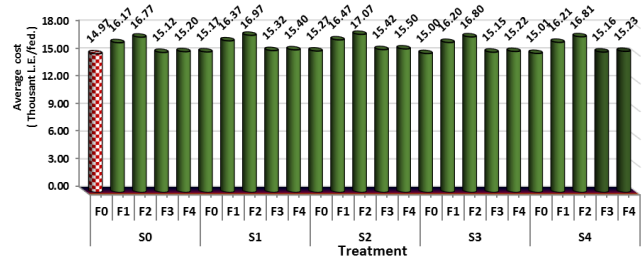
LSD (0.05): Least significant difference and N.S indicate not significant at P: 0.05 probability.

This compound is able to increase leaf resistance to water vapor loss, thus improving plant water use and increasing biomass or yield (Tambussi et al., 2007). Some Researchers stated that chitosan NPs at 10, 25 or 100 ppm increased spike length, plant height, grain yield, and harvest index of wheat compared to the control (Abdel-Aziz et al., 2016). Ohta et al., 2004 found that the highest concentration is necessary when the chitosan is amended in the soil. It was 1.0% (w/w) chitosan in the soil mixture that could stimulate seedling growth. There were significant increase in bulb diameter, TSS%, DM% and protein content in all different concentrations of chitosan when compared with control treatment. Foliar spray with 75 ppm Nano chitosan surpassed all other foliar treatments, followed by foliar spray with 50 ppm Nano chitosan treatment. Increasing of onion bulbs quality parameters under soaking or foliar spraying with chitosan compounds may be due to the N content of chitosan that plays important role in the synthesis of protein. Similar results were obtained by Xianling et al. (2002) who observed that mulberry grains were coated with chitosan solution increased the respiration rate of germination seeds, chlorophyll, protein content and peroxidase in seedlings. Lizarraga-Paulin et al. (2013) stated that chitosan sprinkling increased protein content in maize varieties. The interaction between treatments indicated that spraying with 50 ppm Nano chitosan plus soaking with 75 ppm Nano chitosan significantly increased TSS% and DM% in both seasons; and bulb diameter and protein content in the first seasons. Soaking with 75 ppm Nano chitosan and spraying with 50 ppm Nano chitosan significantly gave the highest and the same value of protein content, in the second season.

**E- Economic feasibility study:**

The results of the partial budget analysis (Figs. 1, 2, 3 and 4) show that the total cost, which calculated as fixed cost (rental cost land preparation, seeding and planting, irrigation, fertilizers, weeding, harvesting, transportation and other expenses) total income and cost/benefit ratio were

estimated. The main findings of this study show that soaking of Nano chitosan at the rate of 75 ppm (S4), with foliar spraying with 50 ppm (F3) resulted in the highest values of gross and net return per fed and benefit-cost ratio with the lowest cost of cultivation. S4 x F3 possessed the maximum gross and net return, as well as B: C ratio. This could be attributed to their role in increasing the marketable onion bulb yield. Increases in gross return, net return and B:C ratio amounted to 79.94, 250.36 and 78.08 % respectively, due to soaking of Nano chitosan at the rate of 75 ppm with foliar spraying with 50 ppm than control. Therefore gross return and net return were highest in Nano chitosan whether spraying or soaking with highest benefit cost ratio (2.60). The results agreed with the results obtained by Geries et al.(2016) who indicated that spraying onion plants with water treatment (control) gave the lowest values of gross return, net return and benefit: cost ratio (12470, 4290 L.E/ fed. and 1.52, respectively).

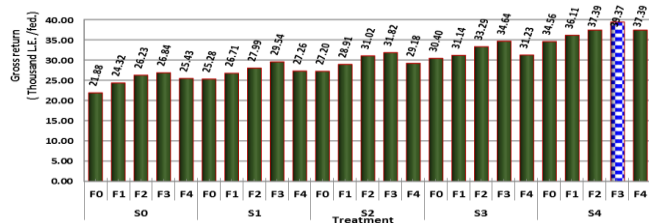


**Figure 1:** Cost cultivation (Thousand L.E./fed.) of onion yield as influenced by soaking and foliar with chitosan and Nano chitosan as overall mean values through the two growing seasons.

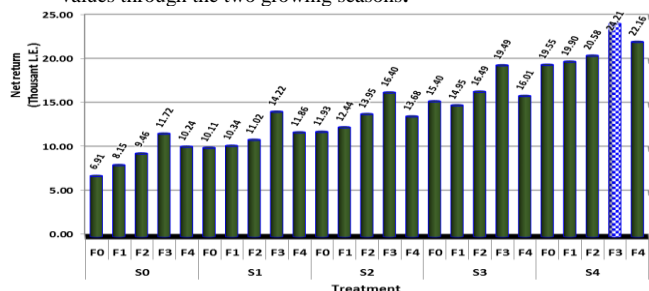
**Table 6:** Bulb diameter, total soluble solids percentage (TSS %), dry matter percentage (DM %) and protein content as influenced by soaking and foliar spraying with chitosan and Nano chitosan and their interaction during 2018/2019 and 2019/2020 seasons.

Treatment	2018/2019				2019/2020				
	Bulb diameter (cm)	Total soluble solids (%)	Dry matter (%)	Protein (µg/g.d.wt)	Bulb diameter (cm)	Total soluble solids (%)	Dry matter (%)	Protein (µg/g.d.wt)	
<b>A. Soaking of seedlings with chitosan (ppm)</b>									
Control	5.70	12.91	14.91	0.718	5.69	10.87	14.93	0.713	
Ch 100	6.58	13.73	15.29	0.779	6.57	12.19	15.29	0.775	
Ch 150	7.54	14.77	16.03	0.787	7.38	13.24	15.88	0.783	
Nano 50	7.69	15.06	16.61	0.794	7.73	13.91	16.29	0.794	
Nano 75	8.59	15.74	16.79	0.804	8.61	15.12	16.51	0.825	
LSD (0.05)	0.16	0.40	0.26	0.006	0.34	0.12	0.33	0.005	
<b>B. Foliar spraying of seedlings with chitosan (ppm)</b>									
Control	6.55	12.82	15.08	0.727	6.33	12.17	14.95	0.739	
Ch 100	6.93	13.53	15.43	0.762	6.89	12.72	15.58	0.751	
Ch 150	7.55	15.28	16.41	0.764	7.55	13.41	16.12	0.798	
Nano 50	7.86	16.26	16.80	0.806	8.02	14.06	16.34	0.811	
Nano 75	7.23	14.30	15.90	0.794	7.19	12.97	15.92	0.789	
LSD (0.05)	0.13	0.20	0.22	0.007	0.28	0.17	0.20	0.007	
<b>Interaction (A x B):</b>									
Soaking	Foliar spraying								
Control	Control	4.98	11.29	14.01	0.548	4.72	10.16	13.62	0.626
	Ch 100	5.23	12.26	14.27	0.666	5.33	10.54	14.54	0.659
	Ch 150	5.90	13.37	15.47	0.792	6.00	11.11	15.55	0.762
	Nano 50	6.50	14.65	15.82	0.788	6.50	11.48	15.83	0.796
	Nano 75	5.88	12.96	14.97	0.794	5.88	11.04	15.13	0.720
Ch 100	Control	5.68	12.15	14.00	0.751	5.51	11.41	14.14	0.742
	Ch 100	6.41	12.69	14.42	0.763	6.28	11.94	15.31	0.748
	Ch 150	7.12	14.88	16.35	0.797	7.12	12.75	15.67	0.789
	Nano 50	7.17	15.76	16.38	0.796	7.63	12.88	15.91	0.804
	Nano 75	6.53	13.19	15.31	0.789	6.33	12.02	15.41	0.791
Ch 150	Control	7.07	13.31	14.95	0.756	6.40	12.01	14.97	0.761
	Ch 100	7.39	13.49	15.60	0.792	7.26	12.77	15.64	0.767
	Ch 150	7.64	15.74	16.30	0.788	7.64	13.86	16.28	0.790
	Nano 50	8.05	16.63	17.19	0.803	8.05	14.54	16.36	0.805
	Nano 75	7.57	14.69	16.10	0.797	7.57	13.01	16.18	0.790
Nano 50	Control	7.08	13.38	15.98	0.791	7.08	12.97	15.97	0.784
	Ch 100	7.33	14.29	16.46	0.792	7.33	13.55	16.09	0.785
	Ch 150	8.08	15.86	16.93	0.792	8.08	14.18	16.40	0.795
	Nano 50	8.30	16.82	17.24	0.801	8.50	15.08	16.70	0.812
	Nano 75	7.67	14.97d	16.46	0.791	7.67	13.77	16.33	0.793
Nano 75	Control	7.93	13.98	16.47	0.786	7.93	14.31	16.06	0.782
	Ch 100	8.27	14.92	16.41	0.793	8.27	14.79	16.33	0.794
	Ch 150	8.98	16.54	17.03	0.801	8.92	15.17	16.68	0.854
	Nano 50	9.26	17.43	17.36	0.842	9.42	16.30	16.92	0.839
	Nano 75	8.52	15.69	16.66	0.799	8.52	15.02	16.56	0.854
LSD (0.05)	0.29	0.46	0.49	0.017	N.S	0.39	0.44	0.016	

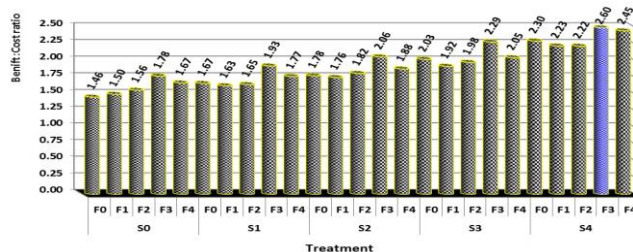
LSD (0.05): Least significant difference and N.S indicate not significant at P: 0.05 probability.



**Figure 2:** Gross return (Thousand L.E./ fed.) of onion yield as influenced by soaking and foliar with chitosan and Nano chitosan as overall mean values through the two growing seasons.



**Figure 3:** Net returns (Thousand L.E./ fed.) of onion yield as influenced by soaking and foliar with chitosan and Nano chitosan as overall mean values through the two growing seasons.



**Figure 4:** Benefit: cost ratio of onion yield as influenced by soaking and foliar with chitosan and Nano chitosan as overall mean values through the two growing seasons.

**Conclusion**

The obtained results indicated that soaking seedling or foliar application of plants with chitosan compounds had an important role in promoting and improving plant vegetative growth, and this led to increases in yield and yield components of onion. The examination inferred that using Nano chitosan to deliver highest yield in good quality with great the economic benefit was gotten from seedling soaking at 75 ppm and foliar spraying at 50 ppm.

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