

ORIGINAL ARTICLES

Response of Strawberry Plants to Foliar Spraying of Chitosan

El-Miniawy, S.M., M.E. Ragab, S.M. Youssef and A.A. Metwally

Horticulture Dept., Faculty of Agriculture, University of Ain Shams, Egypt.

ABSTRACT

The study was carried out to investigate the effect of foliar sprayings of chitosan extract (2.5 or 5.0 ml/l) with different number of applications (once, twice or three times) on growth, chlorophyll, mineral content of leaves, some fruit-quality parameters and yield of strawberry during the two successive seasons of 2009/2010 and 2010/2011. Results revealed that all tested foliar applications of chitosan increased all vegetative growth characteristics (plant length, number of leaves/plant, leaf area, root and vegetative growth fresh and dry weights), and yield attributes (fruit weight, early and total yields/plant). However, there was no significant effect for the tested treatments on leaf chlorophyll content and most of fruit quality characters. The most effective treatment in enhancing growth, fruit quality and yield of cold stored strawberry cv. Sweet Charlie was found to be 5.0 ml/l chitosan spraying three times.

Key words: Strawberry, Chitosan, Growth, Yield, Quality.

Introduction

Strawberry (*Fragaria x ananassa* Duch.) is a small fruit crop of great nutritional and medicinal values (Maas *et al.*, 1991) and is one of the most popular fruits worldwide. In the last two decades, strawberry has become one of the very important horticultural vegetable crops for local fresh consumption, food processing and export, in Egypt. Total annual production amounted to 96,640 tons in 2010/2011 season from cold stored transplants (Central Administration of Horticulture, Ministry of Agriculture and Land Reclamation, Egypt). Strawberries are unique with highly desirable taste, flavor, and excellent dietary sources of ascorbic acid, potassium, fiber and simple sugar sources of energy (Perez *et al.*, 1997). Crop yield and early harvests are of primary importance to the growers, while fruit quality is the most important to the consumers.

Chitosan is a linear polysaccharide composed of randomly distributed β -(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). It is a polysaccharide called 2-Amino-2-deoxybeta-D-glucosamine (Peniston and Johnson, 1980). Chitosan can be extracted from the marine crustacean like shrimps, cramp, and pinfish or from the exoskeletons of most insects under the name of chitin which can be transformed into chitosan by extracting the acetyl group and turn it into amino (Sugiyama *et al.*, 2001).

Chitosan has been widely used in agricultural applications mainly for stimulation of plant defense (Yu and Meuhlbaauer, 2001; Hadwiger *et al.*, 2002; Bautista-Baños *et al.*, 2003; Naeem *et al.*, 2010). Also, many efforts were done to study the effect of chitosan on plant growth, development and productivity. In this respect, chitosan promoted growth of roots, shoots and leaves and improved yield components of various vegetable crops including soybean sprouts (Lee *et al.*, 2005), cucumber (Farouk *et al.*, 2008; Shehata *et al.*, 2012), tomato (El-Tantawy, 2009), sweet pepper (Ghoname *et al.*, 2010), radish (Farouk *et al.*, 2011), beans (Sheikha and Al-Malki, 2011), chili (Chookhongkha *et al.*, 2012), cowpea (El-Tanahy *et al.*, 2012; Farouk and Ramadan, 2012), chinese garlic (Fawzy *et al.*, 2012), okra (Mondal *et al.*, 2012) and mungbean (Mondal *et al.*, 2013). However, the influence of the foliar applications of chitosan on growth and yield of strawberry has not been well studied yet. Therefore, this experiment was conducted to investigate the effect of foliar sprayings of chitosan (2.5 and 5.0 ml/l) with different number of applications (once, twice or three times) on growth, chlorophyll and mineral content of leaves, some fruit-quality parameters and yield of strawberry.

Materials and Methods

Experimental site, cultivar and cultivation:

The study was conducted in a Private Farm in Mit Kenana village, Sheebin El-Qanater Center, Qalubia Governorate, Egypt, during the two successive seasons of 2009/2010 and 2010/2011. Cold-stored bare rooted strawberry transplants (*Fragaria x ananassa* Duch. cv. Sweet Charlie) with one well-developed crown of

diameter 8-10 mm were planted. Sweet Charlie is an important strawberry cultivar which planted widely in Egypt. The transplants were obtained from the Strawberry and Non-Traditional Crop Improvement Center of the Faculty of Agriculture, Ain Shams University.

Strawberry transplants were planted on October 10th and 4th in the first and second growing seasons, respectively. The frigo transplants were cultivated in raised beds of 15-20 cm height and 120 cm wide. The transplants were planted 30 cm apart in a four-row system under drip irrigation system. The soil type was loam with pH of 7.44 and EC of 0.41 mmhos/cm.

In both seasons, all cultural practices (irrigation, fertilization, weeding, and pest control) were performed according to the recommendations of the Egyptian Ministry of Agriculture.

Experimental design:

The experiment was conducted to investigate the effect of the foliar applications of the commercial chitosan extract (Chito-care) (2.5 and 5.0 ml/l) with different number of applications (once, twice or three times) on growth, chlorophyll and mineral content of leaves, some fruit-quality parameters and yield. The chemical composition of the commercial chitosan (Chito-care) used is presented in Table 1. Spraying of each concentration was done: once at 30 days, twice at 30 and 60 days, and thrice at 30, 60 and 90 days after transplanting. In order to avoid interferences with different moisture levels, the same amount of distilled water was sprayed to the control plants at a given time. The lower leaf surface was sprayed until wetted as well as upper surface since it was reported that absorption by the lower leaf surface was rapid and effective (Hull *et al.*, 1975).

Table 1: The chemical composition of commercial chitosan extract (Chito-care) used.

Component	Concentration	Component	Concentration
N	1000 ppm	Zn	100 ppm
P ₂ O ₅	500 ppm	Cu	50 ppm
K ₂ O	500 ppm	Mn	50 ppm
Fe	100 ppm	B	50 ppm

The experimental design was randomized complete block with 7 treatments [(2 concentrations x 3 number of applications) + control] with 3 replicates and the plot area was 3 m² included 40 plants.

Data recorded:

Vegetative growth:

A random sample of ten plants from the two inner rows of each experimental plot was taken at 120 days after planting for vegetative growth data. Plant length and number of leaves/plant were recorded. Leaf area was estimated using the disk method according to Moursi *et al.* (1968). The plants were removed with a shovel, to prevent damage to the root system. The excess soil attached to the roots was carefully removed. In the laboratory, the plants were washed, and root and vegetative growth fresh weights were recorded. They were dried in an oven at 70°C until constant weight to record the root and vegetative growth dry weights.

Chlorophyll:

A portable chlorophyll meter (SPAD-502, Konica Minolta Sensing, Inc., Japan) was used to measure leaf greenness of the plants. At 120 days after planting, measurements were taken at four locations on each leaf; two on each side of the midrib on the youngest fully expanded leaves of randomly selected five plants per plot and then averaged (Khan *et al.*, 2003).

Crown carbohydrate:

Total carbohydrate of crowns was determined at 120 days after planting using phenol sulphuric acid method (Dubois *et al.*, 1956).

Mineral analysis of leaves:

Leaf samples were taken at 120 days from planting and oven-dried at 70°C until constant weight and ground to pass a 1 mm sieve then 0.1 g of the dry samples was taken and digested using a mixture of sulphuric acid and hydrogen peroxide as described by Thomas *et al.* (1967). All the studied elements were assayed in the digest of the concerned plant samples. Total nitrogen was determined using Kjeldahl method as described by

Piper (1950). Phosphorus content was measured spectrophotometrically using the ascorbic acid method (AOAC, 2005). Potassium was measured by flame photometer as described by Page *et al.* (1982).

Yield components:

Marketable fruits were harvested at 2–3 day intervals during the growing season, counted, and weighed to record average fruit weight. The early yield/plant was determined as weights of all harvested fruit during the first four harvests. Total yield/plant was calculated.

Fruit quality:

Thirty full mature fruits were collected randomly from each treatment in the middle of the growing season (April in both seasons) as subsamples for fruit quality. Fruit firmness was measured using Shatillon penetrometer. Soluble solid content (SSC) was determined using a hand refractometer. Titratable acidity and ascorbic acid content were determined according to A.O.A.C. (2005). The SSC/titratable acidity ratio was calculated.

Statistical analysis:

The statistical analysis was conducted using the COSTAT package program. Data were subjected to analysis of variance (ANOVA). The differences among means of data were compared by Duncan's Multiple Range Test (Waller and Duncan, 1969). All statistical determinations were made at $P = 0.05$.

Results and Discussion

Vegetative growth:

Data in Table 2 clearly show that all tested treatments of chitosan significantly increased plant length, number of leaves/plant and leaf area compared with the control treatment in both seasons. However, these increments were not significant at 2.5 ml/l chitosan for leaf area in the first season. Foliar spraying of chitosan at 5.0 ml/l three times gave significantly the highest plant length, number of leaves/plant and leaf area compared with control in both seasons.

Table 2: Effect of foliar application of chitosan-extract concentrations with different number of sprays on some vegetative growth characters of strawberry plants in 2009/2010 and 2010/2011 seasons.

Treatments	Plant length (cm)		Number of leaves/plant		Leaf area (cm ²)	
	1 st Season	2 nd Season	1 st Season	2 nd Season	1 st Season	2 nd Season
0.0 ml/l (Control)	19.13 d	17.66 e	13.00 c	15.60 c	56.47 b	50.83 c
2.5 ml/l (once)	21.26 c	20.33 cd	15.86 b	16.60 b	59.93 ab	58.91 b
2.5 ml/l (twice)	22.06 bc	20.16 d	16.00 b	17.26 b	60.32 ab	61.24 ab
2.5 ml/l (thrice)	22.40 ab	21.26 bc	17.40 a	17.00 b	60.57 ab	60.31 b
5.0 ml/l (once)	22.53 ab	21.46 b	16.93 a	16.93 b	63.83 a	61.70 ab
5.0 ml/l (twice)	22.86 ab	21.96 b	17.20 a	17.13 b	63.08 a	60.71 ab
5.0 ml/l (thrice)	23.13 a	23.83 a	17.66 a	18.20 a	62.77 a	63.52 a

Means within a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Data in Table 3 show that foliar spraying of chitosan increased fresh and dry weights of roots and vegetative growth of strawberry plants as compared to the control. Chitosan spraying at 5.0 ml/l two or three times gave the highest significant values of fresh and dry weights of roots and vegetative growth in both seasons.

Table 3: Effect of foliar application of chitosan-extract concentrations with different number of sprays on root and vegetative growth weights of strawberry plants in 2009/2010 and 2010/2011 seasons.

Treatments	Root fresh weight (g)		Root dry weight (g)		Vegetative growth fresh weight (g)		Vegetative growth dry weight (g)	
	1 st Season	2 nd Season	1 st Season	2 nd Season	1 st Season	2 nd Season	1 st Season	2 nd Season
0.0 ml/l (Control)	8.30 e	8.16 d	1.28 e	2.36 d	37.10 c	47.82 b	9.52 c	10.69 b
2.5 ml/l (once)	9.90 d	11.03 bc	2.27 d	2.77 bc	44.49 c	50.84 ab	12.32 ab	12.85 a
2.5 ml/l (twice)	10.10 d	11.73 ab	2.31 cd	2.99 ab	44.92 bc	54.08 a	11.58 b	13.44 a
2.5 ml/l (thrice)	10.40 cd	11.30 bc	2.41 cd	2.86 bc	50.18 a	51.93 a	13.10 a	13.38 a
5.0 ml/l (once)	10.70 bc	10.40 c	2.47 bc	2.65 c	46.26 bc	53.01 a	12.48 ab	13.12 a
5.0 ml/l (twice)	11.10 ab	11.06 bc	2.59 b	2.79 bc	48.60 ab	51.84 a	12.79 a	12.85 a
5.0 ml/l (thrice)	11.50 a	12.46 a	2.76 a	3.14 a	51.88 a	54.86 a	13.32 a	13.51 a

Means within a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Chitosan has been reported as a high potential bio-molecule that increases plant growth and development (Khan *et al.*, 2002; Chibu and Shibayama, 2003; Gornik *et al.*, 2008). Hadwiger *et al.* (2002) reported that chitosan had molecular signals that served as plant-growth promoters. The obtained results of vegetative growth characteristics are in agreement with those reported by Lee *et al.* (2005) on soybean sprouts, Farouk *et al.* (2008) and Shehata *et al.* (2012) on cucumber, El-Tantawy (2009) on tomato, Abdel-Mawgoud *et al.* (2010) on strawberry, Ghoname *et al.* (2010) on sweet pepper, Farouk *et al.* (2011) on radish, Sheikha and Al-Malki (2011) on beans, Choohongkha *et al.* (2012) on chili, El-Tanahy *et al.* (2012) and Farouk and Ramadan (2012) on cowpea, Fawzy *et al.* (2012) on chinese garlic, Mondal *et al.* (2012) on okra and Mondal *et al.* (2013) on mungbean. The stimulating effect of chitosan on plant growth may be attributed to an increase in the availability and uptake of water and essential nutrients through adjusting cell osmotic pressure, and reducing the accumulation of harmful free radicals by increasing antioxidants and enzyme activities (Guan *et al.*, 2009) or may be attributed to an increase in the key enzyme activities of nitrogen metabolism (nitrate reductase, glutamine synthetase and protease) and improved the transportation of nitrogen (N) in the functional leaves as well as increased photosynthesis which enhanced plant growth and development (Mondal *et al.*, 2012).

Chlorophyll content:

Data in Table 4 show that there was no influence on SPAD readings as a result of treatments with chitosan on strawberry compared with control in both seasons.

Table 4: Effect of foliar application of chitosan-extract concentrations with different number of sprays on leaf chlorophyll readings and crown carbohydrates of strawberry plants in 2009/2010 and 2010/2011 seasons.

Treatments	Chlorophyll content (SPAD Reading)		Crown carbohydrates (mg/g dry weight)	
	1 st Season	2 nd Season	1 st Season	2 nd Season
0.0 ml/l (Control)	50.47 a	48.85 a	137.66 d	145.66 d
2.5 ml/l (once)	48.99 a	47.85 a	205.33 c	214.66 c
2.5 ml/l (twice)	48.19 a	48.87 a	206.33 c	213.00 c
2.5 ml/l (thrice)	47.96 a	47.29 a	217.33 bc	222.33 c
5.0 ml/l (once)	46.85 a	44.64 a	235.66 ab	237.66 b
5.0 ml/l (twice)	49.53 a	48.89 a	239.66 a	247.33 ab
5.0 ml/l (thrice)	47.40 a	50.17 a	245.33 a	256.00 a

Means within a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Crown carbohydrate content:

Total crown carbohydrates were increased as a result of chitosan spraying in all treatments compared with control in both seasons (Table 4). The best treatment which gave the highest significant values was 5.0 ml/l chitosan for three times in both seasons. This increment in total carbohydrates in crowns may be considered as a result to the increment of vegetative growth characteristics, i.e. plant length, number of leaves/plant, leaf area and fresh and dry weights of roots and vegetative growth as found in Tables 2 and 3, or may be resulted of the increase in phosphorous content of leaves (Table 5) which is an essential nutrient playing an important role in the biosynthesis and translocation of carbohydrates (Nijjar, 1985).

Mineral analysis of leaves:

As for the nitrogen content of leaves, there was a significant increase for the tested treatments of chitosan compared with the control plants in the first season while chitosan spraying did not affect it in the second season (Table 5). On the other hand, all tested chitosan sprayings not only increased phosphorus content but also potassium content of leaf tissues compared with the control treatment. Chitosan spraying at 5.0 ml/l (one, two or three times) gave the highest significant values of phosphorus and potassium percent of leaf tissues in both seasons. These results are in agreement with those reported by Abdel-Mawgoud *et al.* (2010) on potassium content in strawberry leaves, Farouk and Ramadan (2012) on phosphorus content in cowpea leaves.

Table 5: Effect of foliar application of chitosan-extract concentrations with different number of sprays on mineral analysis of strawberry leaves in 2009/2010 and 2010/2011 seasons.

Treatments	Nitrogen (%)		Phosphorus (%)		Potassium (%)	
	1 st Season	2 nd Season	1 st Season	2 nd Season	1 st Season	2 nd Season
0.0 ml/l (Control)	3.12 b	3.64 a	0.10 b	0.13 b	1.06 c	1.00 b
2.5 ml/l (once)	3.36 a	3.78 a	0.15 ab	0.15 ab	1.47 a	1.13 ab
2.5 ml/l (twice)	3.53 a	3.54 a	0.13 ab	0.15 ab	1.41 ab	1.32 a
2.5 ml/l (thrice)	3.55 a	3.59 a	0.11 b	0.15 ab	1.13 bc	1.17 ab
5.0 ml/l (once)	3.48 a	3.59 a	0.14 ab	0.15 ab	1.44 a	1.28 a
5.0 ml/l (twice)	3.51 a	3.40 a	0.14 ab	0.16 ab	1.47 a	1.36 a
5.0 ml/l (thrice)	3.53 a	3.36 a	0.18 a	0.19 a	1.46 a	1.33 a

Means within a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Yield components:

Average fruit weight response to the tested chitosan sprayings had an oscillating trend. Spraying of chitosan at 2.5 ml/l once and 5.0 ml/l once or twice gave the highest significant average fruit weight in the first season, while 2.5ml/l once or thrice in the second season exhibited the highest significant ones (Table 6).

In general, data in Table 6 show that except for 2.5 ml/l once and twice and 5 ml/l twice in the first season, and 2.5 ml/l once in the second season, all tested treatments of chitosan significantly increased early yield/plant compared with the control treatment. Except for 2.5 ml/l once and twice in the first season, and 5.0 ml/l once in the second season, all tested treatments of chitosan significantly increased total yield/plant compared with control treatment.

These results agree with those reported by El-Tantawy (2009) on tomato, Abdel-Mawgoud *et al.* (2010) on strawberry, Ghoname *et al.* (2010) on sweet pepper, Chookhongkha *et al.* (2012) on chili, El-Tanahy *et al.* (2012) and Farouk and Ramadan (2012) on cowpea, Fawzy *et al.* (2012) on chinese garlic, Shehata *et al.* (2012) on cucumber, Mondal *et al.* (2012) on okra and Mondal *et al.* (2013) on mungbean. These influences in yields may be due to the increase in vegetative growth characteristics, i.e. plant length, number of leaves/plant, leaf area, and fresh and dry weights of roots and vegetative growth (Tables 2 and 3), and may be also attributed to the increase in the crown carbohydrate content (Table 4).

Table 6: Effect of foliar application of chitosan-extract concentrations with different number of sprays on average fruit weight and fruit yield of strawberry plants in 2009/2010 and 2010/2011 seasons.

Treatments	Average fruit weight (g)		Early yield/plant (g)		Total yield/plant (g)	
	1 st Season	2 nd Season	1 st Season	2 nd Season	1 st Season	2 nd Season
0.0 ml/l (Control)	10.37 c	9.19 b	174.72 b	140.58 b	349.38 d	375.93 c
2.5 ml/l (once)	12.79 ab	11.62 a	180.18 b	174.43 ab	368.58 cd	464.25 ab
2.5 ml/l (twice)	11.38 bc	10.63 ab	176.54 b	210.16 a	360.77 cd	475.77 a
2.5 ml/l (thrice)	11.07 c	11.42 a	208.66 a	191.20 a	419.22 ab	451.12 ab
5.0 ml/l (once)	12.75 ab	9.77 ab	212.54 a	206.60 a	449.50 a	421.43 bc
5.0 ml/l (twice)	13.06 a	10.29 ab	184.18 b	204.18 a	395.99 bc	458.75 ab
5.0 ml/l (thrice)	11.84 abc	10.07 ab	208.16 a	199.50 a	431.49 ab	453.04 ab

Means within a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Fruit quality:

Data in Table (7) reveal that chitosan spraying did not affect fruit firmness, titratable acidity and ascorbic acid content in both seasons. Also, data show that there was no significant difference in fruit soluble solids content between chitosan sprayings and the control treatment except for 5.0 ml/l three times gave lower values than control fruits in the first season and 2.5 ml/l one or two times in the second season which exhibited higher significant values than control. As for the SSC/titratable acidity ratio, there was no significant difference between chitosan sprayings and the control treatment except for 2.5 ml/l chitosan twice in both seasons.

These results are in agreement with those reported by Abdel-Mawgoud *et al.* (2010) who found that total soluble solids (TSS) showed tendency to increase in response to chitosan application however, these results were not significantly different among each other. Also, total acidity and total sugars in fruits significantly increased in response to chitosan application compared to the control treatment. The two parameters increased as the chitosan applied concentration increased until 2 cm³/l then the response started to decline.

Table 7: Effect of foliar application of chitosan-extract concentrations with different number of sprays on fruit quality of strawberry in 2009/2010 and 2010/2011 seasons.

Treatments	Fruit firmness (g/cm ²)		SSC (Brix)		Titratable acidity (%)		SSC/Titratable acidity ratio		Ascorbic acid content (mg /100 g fw)	
	1 st Season	2 nd Season	1 st Season	2 nd Season	1 st Season	2 nd Season	1 st Season	2 nd Season	1 st Season	2 nd Season
0.0 ml/l (Control)	220.00 a	229.25 a	8.80 a	8.34 c	0.27 a	0.28 a	32.21 ab	29.82 b	62.91 a	69.63 a
2.5 ml/l (once)	246.33 a	239.33 a	8.93 ab	9.83 ab	0.28 a	0.27 a	31.17 ab	35.52 ab	86.37 a	59.01 a
2.5 ml/l (twice)	253.66 a	243.66 a	9.23 a	10.58 a	0.27 a	0.27 a	34.12 a	38.27 a	65.81 a	54.79 a
2.5 ml/l (thrice)	226.33 a	253.33 a	9.33 a	9.64 abc	0.28 a	0.28 a	33.25 ab	34.51 ab	76.28 a	60.03 a
5.0 ml/l (once)	247.33 a	238.66 a	9.22 a	9.01 bc	0.28 a	0.27 a	32.98 ab	32.93 ab	58.32 a	67.27 a
5.0 ml/l (twice)	237.66 a	245.33 a	9.08 a	8.90 bc	0.27 a	0.28 a	33.29 ab	31.48 b	86.72 a	76.54 a
5.0 ml/l (thrice)	235.33 a	245.66 a	8.38 b	8.99 bc	0.29 a	0.29 a	28.95 b	31.40 b	80.71 a	74.92 a

Means within a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Conclusion:

In conclusion, this study demonstrated that foliar spraying of chitosan induced positive effects on the plant growth and fruit yield of plants produced from cold-stored strawberry transplants cv. Sweet Charlie. The most effective treatment was found to be chitosan spraying at 5.0 ml/l three times. Further studies are required in order to determine the effect of chitosan on the net photosynthetic rate, water relations, antioxidant compounds, enzyme activity and endogenous phytohormones.

References

- Abdel-Mawgoud, A.M.R., A.S. Tantawy, M.A. El-Nemr, Y.N. Sassine, 2010. Growth and yield responses of strawberry plants to chitosan application. *European Journal of Scientific Research*, 39(1): 161-168.
- AOAC (Association of Official Analytical Chemists-International), 2005. *Official Methods of Analysis*. 18th edn., eds.: W. Hortwitz, G. W. Latimer, AOAC-Int. Suite 500, 481 North Frederick Avenue, Gaithersburg, Maryland, USA.
- Bautista-Baños, S., M. Hernández-López, E. Bosquez-Molina and C.L. Wilson, 2003. Effects of chitosan and plant extracts on growth of *Colletotrichum gloeosporioides*, anthracnose levels and quality of papaya fruit. *Crop Protect.*, 22: 1087-1092.
- Chibu, H. and H. Shibayama, 2003. Effects of chitosan application on the growth of several crops, In: T. Urugami, K Kurita, and T. Fukamizo (eds.), *Chitin and chitosan in life science.* Yamaguchi, Japan, pp: 235-239.
- Chookhongkha, N., S. Miyagawa, Y. Jirakiattikul and S. Photchanachai, 2012. Chili growth and seed productivity as affected by chitosan. *International Conference on Agriculture Technology and Food Sciences (ICATFS'2012)* Nov. 17-18, 2012 Manila, Philippines.
- Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, 26: 350-356.
- El-Tanahy, A.M.M., A.R. Mahmoud, M.M. Abde-Mouty and A.H. Ali, 2012. Effect of chitosan doses and nitrogen sources on the growth, yield and seed quality of cowpea. *Aust. J. Basic & Appl.Sci.*, 6(4): 115-121.
- El-Tantawy, E.M., 2009. Behavior of tomato plants as affected by spraying with chitosan and aminofort as natural stimulator substances under application of soil organic amendments. *Pak. J. Biol. Sci.*, 12: 1164-1173.
- Farouk, S. and A.A. Ramadan, 2012. Improving growth and yield of cowpea by foliar application of chitosan under water stress. *Egyptian Journal of Biology*, 14: 14-26.
- Farouk, S., A.A. Mosa, A.A. Taha, H.M. Ibrahim and A.M. El-Gahmery, 2011. Protective effect of humic acid and chitosan on radish (*Raphanus sativus* L. var. *sativus*) plants subjected to cadmium stress. *Journal of Stress Physiology and Biochemistry*, 7(2): 99-116.
- Farouk, S., K.M. Ghoneem and A.A. Ali, 2008. Induction and expression of systematic resistance to downy mildew disease in cucumber plant by elicitors. *Egypt. J. Phytopathol.*, 1-2: 95-111.
- Fawzy, Z.F., Z.S. El-Shal, L. Yunsheng, O. Zhu and O.M. Sawan, 2012. Response of garlic (*Allium Sativum* L.) plants to foliar spraying of some bio-stimulants under sandy soil condition. *J. Appl. Sci. Res.*, 8(2): 770-776.
- Ghoname, A.A., M.A. El-Nemr, A.M.R. Abdel-Mawgoud and W.A. El-Tohamy, 2010. Enhancement of sweet pepper crop growth and production by application of biological, organic and nutritional solutions. *Res. J. Agric. & Biol. Sci.*, 6(3): 349-355.
- Gornik, K., M. Grzesik and B.R. Duda, 2008. The effect of chitosan on rooting of grapevine cuttings and on subsequent plant growth under drought and temperature stress. *J. Fruit Ornament. Plant Res.*, 16: 333-343.
- Guan, Y.J., J. Hu, X.J. Wang and C.X. Shao, 2009. Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress. *Journal of Zhejiang University Science B.*, 10(6): 427-433.
- Hadwiger, L.A., S.J. Klosterman and J.J. Choi, 2002. The mode of action of chitosan and its oligomers in inducing plant promoters and developing disease resistance in plants, In: K. Suchiva, S. Chandkrachang, P. Methacanon and M.G. Peter (eds.), *Advances in chitin science*, vol. 5, Bangkok, pp: 452-457.
- Hull, H.M., H.L. Morton and J.R. Wharrie, 1975. Environmental influence on cuticle development and resultant foliar penetration. *Bot. Rev.*, 41: 421-451.
- Khan, M.H., K.L.B. Singha and S.K. Panda, 2002. Changes in antioxidant levels in *Oryza sativa* L. roots subjected to NaCl salinity stress. *Acta Physiologia Plantarum*, 24: 145-148.
- Khan, W., B. Prithviraj and D.L. Smith, 2003. Photosynthetic responses of corn and soybean to foliar application of salicylates. *J. Plant Physiol.*, 160: 485-492.

- Lee, Y.S., Y.H. Kim and S.B. Kim, 2005. Changes in the respiration, growth, and vitamin C content of soybean sprouts in response to chitosan of different molecular weights. *HortScience*, 40: 1333-1335.
- Maas, J.L., S.Y. Wang and G.J. Galletta, 1991. Evaluation of strawberry cultivars for allelic acid content. *HortScience*, 26: 66-68.
- Mondal, M.M.A., M.A. Malek, A.B. Puteh, M.R. Ismail, M. Ashrafuzzaman and L. Naher, 2012. Effect of foliar application of chitosan on growth and yield in okra. *A.J.C.S.*, 6: 918-921.
- Mondal, M.M.A., M.A. Malek, A.B. Puteh and M.R. Ismail, 2013. Foliar application of chitosan on growth and yield attributes of mungbean (*Vigna radiata* (L.) Wilczek). *Bangladesh J. Bot.*, 42(1): 179-183.
- Moursi, M.A., H.A. Tawfik and A. Abdel-Gawad, 1968. Principles of agricultural researches (in Arabic). Dar El-Hanna printing House, Cairo, pp: 418.
- Naeem, M., A. Hassan, M. Ahmed and A. El-Sayed, 2010. Radiation-induced degradation of chitosan for possible use as a growth promoter in agricultural purposes. *Carbohydrates Polymers*, 79: 555-562.
- Nijjar, G.S., 1985. Nutrition of fruit trees. Usha Raji Kumar, Kalyani, New Delhi, India.
- Page, A.L., R.H. Miller and D.R. Keeney, 1982. Methods of soil analysis-chemical and microbiology properties, SSSA Inc., Mad., WI., USA.
- Peniston, Q.P. and E. Johnson, 1980. Process for the manufacture of chitosan. US Patent No. 4, 195, 175, 5pp.
- Perez, A.G., R. Olias, J. Espeda, J.M. Olias and C. Sanz, 1997. Rapid determination of sugars, nonvolatile acids, and ascorbic acid in strawberry and fruits. *J. Agr. Food Chem.*, 45: 3545-3549.
- Piper, C.S., 1950. Soil and plant analysis. 1st Ed. Interscience Publishers Inc., New York, USA, pp: 30-59.
- Shehata, S.A., Z.F. Fawzy and H.R. El-Ramady, 2012. Response of cucumber plants to foliar application of chitosan and yeast under greenhouse conditions. *Aust. J. Basic and Appl. Sci.*, 6(4): 63-71.
- Sheikha, S.A. and F.M. Al-Malki, 2011. Growth and chlorophyll responses of bean plants to the chitosan applications. *European Journal of Scientific Research*, 50(1): 124-134.
- Sugiyama, H., K. Hisamichi, K. Sakai, T. Usui, J.I. Ishiyama, H. Kudo, H. Ito and Y. Senda, 2001. The conformational study of chitin and chitosan oligomers in solution. *Bioorganic and Medicinal Chemistry*, 9: 211-216.
- Thomas, R.L., R.W. Sheard and J.R. Moyer, 1967. Comparison of conventional and automated procedures for nitrogen, phosphorus and potassium analysis of plant materials using a single digestion. *Agron. J.*, 59: 240-243.
- Waller, R.A. and D.B. Duncan, 1969. A Bayes rule for the symmetric multiple comparison problem. *J. Amer. Statist. Assoc.*, 64: 1485-1503.
- Yu, G. and G. Meuhlbauer, 2001. Benzothiadiazole-induced gene expression in wheat spikes does not provide resistance to *Fusarium* head blight. *Physiological and Molecular Plant Pathology*, 59: 129-139.