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Effect of Chitosan and Seaweed Extracts on Fruiting of Flame Seedless Grapevines Grown Under Sandy Soil Condition

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Abstract:

During 2021 and 2022 seasons, Flame seedless grapevines were treated three times with chitosan at 100 to 400 ppm and / or seaweed extracts at 0.05 to 0.2% . The merit of this study was examining the effect of single and combined application of chitosan and seaweed extracts at different concentration on growth, vine nutritional status, yield as well as physical and chemical characters of Flame seedless grapes.

Spraying the vines with chitosan at 100 to 400 ppm and / or seaweed extracts at 0.05 to 0.2% three times during season was very effective in enhancing growth aspects, leaf pigments and nutrients, yield and both physical and chemical characteristics of the berries over the control treatment. The promotion was associated with increasing the concentrations. Negligible promotion on these parameters was observed among the higher two concentration. Using chitosan was greatly superior than using seaweed extract in all parameters.

Conclusively.

According to the obtained data, it is suggested to use a mixture of chitosan at 200 ppm and seaweed extracts at 0.1% three times at growth start, just after berry setting and at one month later gave the best with regard to growth, yield and berries quality of Flame seedless grapevines grown under sandy soil

Keywords: Flame seedless grapevines – chitosan seaweed extracts – yield – fruit quality.

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Introduction:

Grape (*Vitis Vinifera l*) is considered the first fruit yield in both area production all over the world. In Egypt it is the third main fruit after citrus and mango. Flame seedless grapes is very important grape cultivar grown in Egypt. It is one of the most delicious, refreshing and nourishing subtropical fruits. The berries are good source of vitamins, sugars, minerals and organic acids. The berries are consumed in fresh forms as a table fruit and in the processed form as wine, fresh juice and raisin . Flame seedless grapes is gaining more popularity both as table purpose and raisin making because of its high total soluble solids, thin skin and desired shape.

Chitosan is considered a biopolymer produced from chitin and is very safe for human being it has bioactivity and biocompatibility (**Dias *et al.*, 2013**) using it in plants resulted in improving the yield and reducing transpiration (**Dzung *et al.*, 2011 and Mondal *et al.*, 2012**).

Chitosan coating on caller fresh fruit provide modified atmosphere strong and decrease quality changes through control of the internal gas composition of the fruits . The coating offers a protective barrier against bacterial contamination and moisture transfer to extend the shelf life (**Ghasemnezhard *et al.*, 2013) (Jiang and Li, 2001**) reported that chitosan coating help to reduce transpiration and control weight loss to slow down ripening and expand shelf life by controlling respiration rate and ethylene production.(**Adwiger, 2013**) should that the agricultural and Horticultural uses for chitosan , primarly for plant defense and yield increase, are based on how this glucosamine polymer influences that biochemistry and molecular biology of that plant cell.

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Recently, some researches reported that chitosan enhanced plant development such as (**Shehata *et al.*, 2012; Shiri *et al.*, 2013 and Wafaa *et al.*, 2014**).

Seaweed extracts being organic and biodegradable in nature is considered as an important source of some nutrition for sustainable agriculture (**Cassan *et al.*, 1992**) seaweed extracts contain Various trace elements (Zn, Fe, Mn, Cu, Co. and Mo), amino acids, vitamins and plant growth hormones (IAA, Cytokinins and IBA) which cause many beneficial effects on plant growth and development.(**Metting *et al.*, 1990, Spinelli *et al.*, 2009 and Abdel. Mawgoud *et al.*, 2010**)

The extract of seaweeds has been reported to induce many positive changes in treated plants such as improved crop yield Increased postharvest, shelf life , increased seed germination and reduced incidence of Fungal and insect attack (**Metting *et al.*, 1990)**

The aim of this study was to assess the effects of different concentration of application of chitosan and seaweed extract on the growth, yield and berries quality of Flame seedless grapevines.

MATERIALS AND METHODS

This study was carried out during two consecutive seasons 2021 and 2022 on sixty uniform in vigour 11- years old Flame seedless grapevines. The selected vines are grown in a private vineyard located at west Samalout El- Minia Governorate. Where the texture of the soil is sandy (Table 1): soil analysis was done according to procedures that outlines by (**Wilde *et al.*, 1985**).

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The selected vines are planted at 2.0 x 3.0 meters apart (700 vines / fedd.). The chosen vines were short pruned (spur pruning) during the 2st week of Dec. during both seasons leaving 72 eyes on the basis of (20 fruiting spurs x three eyes plus 6 replacement spurs x two eyes) Drip Irrigation system was followed using water containing 960 ppm salinity.. The selected vines (60 vines) received the regular agricultural and horticultural practices that were already applied in the vineyard except application of chitosan and seaweed extract.

Table (1) : Analytical data of the tested soil

Constituents	value	content	value
Sand %	83.0	Total N%	0.04
Silt %	12.5	$CaCO_3$ %	8.2
Clay %	4.5	K (meq/ 100g soil)	0.65
Texture	Loamy sand	P ppm	8.5
O.M	0.55	Fe ppm	6.3
pH(1:2.5 extract)	7.91	Zn ppm	0.50
E.C.(1:2.5 mmhos/1cm)	1.7	Mn ppm	0.90

This study included the following ten treatments from chitosan and seaweed extract

1- control
2- spraying seaweed extract at 0.05%
3- spraying seaweed extract at 0.1%
4- spraying seaweed extract at 0.2%
5- spraying chitosan at 100 ppm
6- spraying chitosan at 200 ppm

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7- spraying chitosan at 400 ppm

8- spraying seaweed extract at 0.05% and chitosan at 100 ppm

9- spraying seaweed extract at 0.1% and chitosan at 200 ppm

10-spraying seaweed extract at 0.2% and chitosan at 400 ppm

Each treatment was replicated three times, two vine per replicated . The total vines selected for achieving this study was 60 vines. Triton B as a wetting agent was added at 0.05% few drops of 0.1 N NaOH was added to the known weight of chitosan to facilitate the solubility and seaweed extract and the control vines received water containing Triton B and few drops of 0.1 N NaOH spraying was done till runoff.

Randomized Completed Block Design (RCBD) was used for statistical analysis of the present study .

During both seasons, the following parameters were recorded.

1- Some vegetative growth characteristics namely the main shoot length (cm.), number of leaves / shoot and leaf area (cm^2) (**Ahmed and Morsy , 1999**) .

2- Chlorophylls A, B, total chlorophylls and total carotenoids (as mg/ g F.W.) (**Von- Wettstein, 1957).**

3- Percentages of N, P and K as well as Fe, Zn and Mn (as ppm) in the leaves (on dry weight basis) (**Summer, 1985 and Wilde *et al.*, 1985**).

4- Yield expressed in weight (kg.) and number of clusters / vine as well as weight (g.) , length and shoulder of cluster (cm.)

5- Percentage of berries colouration.

6- Physical and chemical characteristics of the berries namely weight (g) longitudinal and equatorial of berry (cm.), TSS%, total acidity % (as g tartaric acid / 100 ml juice) (**A.O.A.C., 2000**) and TSS/ acid. Statistical analysis was done using new L.S.D at 5% (**Mead *et al.*,1993**)

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RESULTS AND DISCUSSION

1-Vegetative growth characteristics:

It is clear from obtained data in Table (2) that spraying the vines three times with chitosan at 100 to 400 ppm and / or seaweed extracts at 0.05 to 0.2% significantly enhanced three growth aspects namely the main shoot length, number of leaves / shoot and leaf area relative to the control. The promotion was associated with increasing concentration of chitosan from 100 to 400 ppm and seaweed extract from 0.05 to 0.2% . Combined application of chitosan and seaweed extracts significantly increased these growth aspects than using each material alone . Using chitosan was significantly superior than using seaweed extracts in stimulating these growth traits. Increasing concentration of chitosan from 200 to 400 ppm and seaweed extracts from 0.1 to 0.2% had no significant promotion on these growth traits.

The maximum values of main shoot length (123.0, 123.5 cm) number of leaves per shoot (21.0, 22.0 leaf) and leaf area (128.5, 129.0 cm^2) were recorded on the vines that received three sprays of a mixture of chitosan at 400 ppm and seaweed extracts at 0.2 % during both seasons, respectively. The untreated vines produced the minimum values of the main shoot length (98.5, 99.0cm) number of leaves / shoot (13.5, 14.0 leaf) and leaf area (101.0, 103.0 cm^2) during both seasons, respectively . These results were true during 2021 and 2022 seasons.

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The beneficial effects of chitosan on enhancing enzymes, antioxidants, hormones the resistance to diseases and microorganisms, levels of ABA which play a key role in the regulation of water use due to the closure of stomata availability and uptake of water and essential nutrients through adjusting osmotic pressure in plant cells and in descending order water loss, transpiration the accumulation of harmful free ridicules (**Hadwiger *et al.*, 2002**) could explain the present results.

The results of chitosan are in harmony with those found by (**Hadwiger, 2013; Ali *et al.*, 2017; El- Kenawy, 2017; Hussein- Esraa, 2017; Khalil *et al.*, 2020 and Refaai and Silem, 2021)**.

The promoting effect of seaweed extract on growth characters might be attributed to its positive role in supplying vines with their requirements from all mineral nutrients and organic at balanced rate.

The higher own content of seaweed extract from natural antioxidants and hormones which encourage cell division could give another explanation (**Subba,Rao,1984**) These results are in agreement with those obtained by (**El.Saman,2010,Abdel-Hameed et al.2010,Gad El-kareem and Abd El-Rahman , 2013, khalaf ,2017 and Amin –Sarah 2020**).

2-leaf chemical composition:

Tables (3, 4) show the effect of single and combined applications of chitosan and seaweed extracts on the leaf chemical composition namely chlorophylls a, b, total chlorophylls , total carotenoids, N, P and K (as 1%) and Fe , Zn and Mn (as ppm) in the leaves of Flame seedless grapevines during 2021 and 2022 seasons .

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One cane state from the obtained data that subjecting Flame seedless grapevines three times with chitosan and / or seaweed extracts was significantly followed by stimulating chlorophylls a, b, total chlorophylls, total carotenoids, N, P, K, Fe, Zn and Mn relative to the control treatment.

The stimulation of these leaf chemical composition was in proportional to the increase in concentrations of each material. Employing chitosan at 100 to 400 ppm significantly was accompanied with enhancing these leaf chemical composition than using seaweed extracts at 0.05 to 0.2% combined applications were significantly superior than using each material. Negligible promotion on these leaf pigments and nutrients were observed among the higher two concentrations of each material. Using the higher concentrations of chitosan namely 400 ppm and seaweed extracts namely 0.2% gave the highest values of chlorophyll a (2.81, 2.82 mg/ g. F.W) chlorophyll b (1.29, 1.25 mg/g F.W.), Total chlorophylls (4.05, 4.07 mg/g F.W) , total carotenoids (1.35, 1.36 mg/g F.W.); N (1.89, 1.91%) , P(0.43 , 0.44%)K (1.36, 1.37%); Zn (58.3, 58.9 ppm) Fe (57.2 , 58.0 ppm), Mn (56.1, 56.3 ppm) during both seasons respectively. The untreated vines produces the lowest values. The results were true during 2021 and 2022 seasons.

The positive action of chitosan and seaweed extract on enhancing root development and up take of leaf pigments and nutrients in the leaves could explain the present results (**El-Saman, 2010; Abd El-Hameeed *et al.*, 2010; Gad El-Kareem and Abd El-Rahman , 2013; Ali *et al.*, 2017; Husein- Esraa, 2017; Khalaf, 2017; El-Kenawy, 2017; Amin-Sarah, 2020, Khalil *et al.*, 2020 and Refaai and Silem, 2021).**

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3- Yield as well as cluster weight and dimensions:

It is evident from the data in Table (5) that supplying the vines with chitosan at 100 to 400 ppm and / or seaweed extracts at 0.05 to 0.2% significantly was followed by improving yield expressed in weight (kg.) and number of clusters per vine and weight, length and shoulder of cluster relative to the control treatment. There was a progressive promotion on these parameters with increasing concentrations of each material.

Significant differences on these parameters were observed between all concentrations and materials except among the higher two concentrations of each material. Therefore from economical point of view it is necessary to use the material. Combined were favorable than using each material alone in this respect using chitosan significantly preferable than using seaweed extracts in improving yield and cluster characteristics.

From economical point of view, using chitosan at 200 ppm plus seaweed extracts at 0.1% resulted in the highest yield, under such promised treatment, yield per vine reached 10.88 and 13.20 kg during both seasons, respectively. The untreated vine gave yield reached 9.12 and 9.53 kg during both seasons, respectively.

The percentage of increment on the yield due to application of the previous treatment over the control treatment reached 19.3 and 38.5 % during 2021 and 2022 seasons, respectively. These results were nearly same during both seasons.

The beneficial effect of chitosan on berry setting might be attributed to their positive action on growth, vine nutritional status and pigments. The promotion on the yield was attributed to their positive action non berry setting and cluster weight and dimensions.

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The promoting effect of chitosan on yield and cluster weight was emphasized by (**Ali *et al.*, 2017; El- Kenawy, 2017; Amin – Sara, 2020 and Khalil *et al.*, 2020)**. The increase in bunches weight may be due to increased synthesis of photosynthates in the treated grapevines. The obtained results are in line with (**Norrie and Keathley, 2006**) who reported that application of seaweed extract to Thompson seedless grapevines increased the number of primary cluster/ vine. The increase of cluster weight and yield may be related to the availability of and Mg in seaweed extracts. Both nutritional are known to enhance chlorophylls content and photosynthesis rate (**Khan *et al.*, 2012**).

4- Some physical and chemical characteristics of the berries:

One can state from the data in Table (6) that treating the vines with chitosan at 100 to 400 ppm and / or seaweed extracts at 0.05 to 0.2% was significantly very effective in enhancing quality of the berries in terms of increasing berry colouration %, berry weight and dimensions (longitudinal and equatorial) TSS % and TSS / acid ratio and decreasing total acidity % relative to the control treatment. The promotion was depended on increasing concentrations of each material. Application of chitosan surpassed the application of seaweed extracts in this connection. Combined application were significantly preferable than using each alone in enhancing berries quality. These results were true in during 2021 and 2022 seasons.

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These results regarding the effect of chitosan on promoting berries quality might be ascribed to their positive action on enhancing leaf pigments and total anthocyanins in the berries . These results regarding the promoting effect of chitosan on berries quality are in harmony with those obtained by (**Hadwiger *et al* ., 2002, Ali *et al.*, 2017, Hussein- Esraa, 2017, El-Kenawy 2017, Khalil *et al.*, 2020 and Refaai and Silem 2021**)

The beneficial effect of seaweed extracts on enhancing cell division and the biosynthesis of TSS % and total sugars % may help in advancing maturity stages (**Adem, 1999**)

Application of seaweed extract significantly improved TSS % (**Khan *et al.*, 2012)**

The results are conformity with those obtained by (**El- Saman, 2010, Gad El –Kareem and Abd El-Rahman, 2013, Khalaf, 2017 and Amin –Sarah, 2020**).

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Table (2): Effect of single and combined applications of seaweed extract and chitosan on some vegetative growth characteristics of Flame seedless grapevines during 2021 and 2022 seasons.

Characters / Treatments	Main shoot length (cm)		No. of leaves / shoot (leaf)		Leaf area $(cm)^2$	
	2021	2022	2021	2022	2021	2022
T_1-Control	98.5	99.0	13.0	14.0	101.0	103.0
T_2- Spraying seaweed extract at 0.05 %	103.0	105.0	14.5	16.0	105.5	106.0
T_3- Spraying seaweed extract at 0.1 %	109.0	110.0	16.5	17.0	112.0	113.2
T_4- Spraying seaweed extract at 0.2 %	111.0	112.5	17.0	18.0	116.0	118.0
T_5- Spraying chitosan at 100 ppm	105.0	106.0	15.0	16.0	109.0	110.0
T_6- Spraying chitosan at 200 ppm	110.5	112.0	16.5	18.0	113.5	115.0
T_7- Spraying chitosan at 400 ppm	113.0	113.5	18.5	19.0	117.5	118.0
T_8- Spraying seaweed extract and chitosan at low conc.	108.0	110.0	17.0	18.0	113.0	114.0

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T_9- Spraying seaweed extract and chitosan at mid. conc.	121.0	122.0	20.0	20.5	126.0	127.0
T_{10}- Spraying seaweed extract and chitosan at high conc.	123.0	123.5	21.0	22.0	128.0	129.0
New L.S.D. at 5%	1.2	1.3	1.1	1.1	1.3	1.4

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Table (3): Effect of single and combined applications of seaweed extract and chitosan on some leaf pigments in the leaves of Flame seedless grapevines during 2021 and 2022 seasons.

Characters / Treatments	Chlorophyll a (mg/ g. F.W.)		Chlorophyll b (mg/ g. F.W.)		Total chlorophylls (mg/ g. F.W.)		Total carotenoids (mg/ g. F.W.)	
	2021	2022	2021	2022	2021	2022	2021	2022
T_1-Control	2.50	2.48	1.03	1.05	3.53	3.53	1.12	1.14
T_2- Spraying seaweed extract at 0.05 %	2.58	2.60	1.11	1.12	3.69	3.72	1.20	1.21
T_3- Spraying seaweed extract at 0.1 %	2.66	2.68	1.16	1.17	3.82	3.85	1.25	1.26
T_4- Spraying seaweed extract at 0.2 %	2.71	2.72	1.18	1.19	3.88	3.91	1.27	1.28
T_5- Spraying chitosan at 100 ppm	2.60	2.62	1.13	1.14	3.73	3.76	1.22	1.23
T_6- Spraying chitosan at 200 ppm	2.69	2.71	1.18	1.19	3.87	3.90	1.27	1.28
T_7- Spraying chitosan at 400 ppm	2.72	2.73	1.21	1.22	3.93	3.95	1.30	1.31
T_8- Spraying seaweed extract and chitosan at low conc.	2.65	2.66	1.17	1.19	3.82	3.85	1.26	1.27

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T_9- Spraying seaweed extract and chitosan at mid. conc.	2.73	2.74	1.21	1.23	3.94	3.97	1.31	1.33
T_{10}- Spraying seaweed extract and chitosan at high conc.	2.81	2.82	1.24	1.25	4.05	4.07	1.35	1.36
New L.S.D. at 5%	0.06	0.07	0.03	0.04	0.08	0.09	0.04	0.05

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Table (4): Effect of single and combined applications of seaweed extract and chitosan on the leaf content of N, P and K (as %) and Fe, Mn and Zn (as ppm) of Flame seedless grapevines during 2021 and 2022 seasons.

Characters / Treatments	Leaf N %		Leaf P %		Leaf K %		Leaf Fe (ppm		Leaf Mn (ppm)		Leaf Zn (ppm)	
	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
T_1-Control	1.58	1.60	0.19	0.21	1.08	1.10	50.0	50.2	49.0	49.2	51.0	51.3
T_2- Spraying seaweed extract at 0.05 %	1.66	1.68	0.28	0.29	1.16	1.18	51.2	51.8	50.3	50.5	52.4	52.9
T_3- Spraying seaweed extract at 0.1 %	1.73	1.75	0.33	0.34	1.22	1.24	53.5	53.8	52.4	52.6	54.6	54.9
T_4- Spraying seaweed extract at 0.2 %	1.75	1.76	0.35	0.36	1.24	1.25	54.8	55.0	53.7	53.8	55.8	56.1
T_5- Spraying chitosan at 100 ppm	1.68	1.70	0.31	0.32	1.18	1.20	52.0	52.5	51.0	51.2	53.1	53.6
T_6- Spraying chitosan at 200 ppm	1.76	1.77	0.35	0.36	1.25	1.26	54.0	55.0	53.0	53.2	55.0	56.0
T_7- Spraying chitosan at 400 ppm	1.78	1.79	0.38	0.39	1.28	1.29	54.5	55.5	53.4	53.6	55.5	56.6
T_8- Spraying seaweed extract and chitosan at low conc.	1.77	1.78	0.37	0.38	1.27	1.28	54.2	55.0	53.1	53.3	55.3	56.1
T_9- Spraying seaweed extract and chitosan at mid. conc.	1.85	1.86	0.41	0.42	1.33	1.34	56.5	57.0	55.4	55.6	57.6	58.1

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T_{10}- Spraying seaweed extract and chitosan at high conc.	1.89	1.91	0.43	0.44	1.36	1.37	57.2	58.0	56.1	56.3	58.3	58.9
New L.S.D. at 5%	0.07	0.08	0.03	0.04	0.05	0.06	0.9	1.1	0.8	0.9	1.4	1.3

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Table (5): Effect of single and combined applications of seaweed extract and chitosan on yield as well as number clusters / vine cluster weight and dimensions of Flame seedless grapevines during 2021 and 2022 seasons.

Characters / Treatments	No. of cluster / vine		Yield/ vine (kg.)		Cluster weight (g.)		Cluster length (cm.)		Cluster shoulder (cm.)	
	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
T_1-Control	24.0	25.0	9.12	9.53	380.0	381.0	16.5	16.8	11.5	12.0
T_2- Spraying seaweed extract at 0.05 %	25.0	26.0	9.88	10.40	395.0	400.0	17.2	17.5	12.3	12.5
T_3- Spraying seaweed extract at 0.1 %	25.0	27.0	10.13	11.07	405.0	410.0	17.5	17.8	12.5	12.9
T_4- Spraying seaweed extract at 0.2 %	25.0	28.0	10.20	11.54	408.0	412.0	17.7	17.9	12.8	13.0
T_5- Spraying chitosan at 100 ppm	25.0	27.0	10.18	11.07	407.0	410.0	17.6	17.8	12.7	12.8
T_6- Spraying chitosan at 200 ppm	25.0	29.0	10.38	12.18	415.0	420.0	18.4	18.6	13.5	13.6
T_7- Spraying chitosan at 400 ppm	25.0	30.0	10.50	12.90	420.0	430.0	18.6	18.8	13.7	13.9
T_8- Spraying seaweed extract and chitosan at low conc.	25.0	29.0	10.45	12.24	418.0	422.0	18.5	18.7	13.6	13.8

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T_9- Spraying seaweed extract and chitosan at mid. conc.	25.0	30.0	10.88	13.20	435.0	440.0	19.6	19.8	14.5	14.8
T_{10}- Spraying seaweed extract and chitosan at high conc.	25.0	31.0	11.13	13.95	445.0	450.0	20.0	21.0	14.9	15.2
New L.S.D. at 5%	NS	1.0	0.65	0.78	9.5	10.2	0.6	0.7	0.4	10.5

Table (6): Effect of single and combined applications of seaweed extract and chitosan on some physical and chemical characteristics of Flame seedless grapevines during 2021 and 2022 seasons.

Characters / Treatments	Berries colouration %		Av. Berry weight (g.)		Av. Berry longitudinal (cm.)		Av. Berry equatorial (cm.)		TSS%		Total acidity		TSS/ acid	
	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
T_1-Control	77.5	78.0	3.00	3.05	2.05	2.10	1.80	1.82	17.8	17.8	0.695	0.695	25.6	25.6
T_2- Spraying seaweed extract at 0.05 %	83.5	84.0	3.22	3.25	2.10	2.15	1.90	1.90	18.4	18.5	0.650	0.645	28.3	28.7
T_3- Spraying seaweed extract at 0.1 %	86.0	86.5	3.29	3.31	2.16	2.18	1.95	1.96	18.9	19.2	0.630	0.625	30.0	30.7
T_4- Spraying seaweed extract at 0.2 %	87.5	88.0	3.32	3.33	2.18	2.19	1.98	1.99	19.2	19.4	0.610	0.600	31.5	32.3
T_5- Spraying chitosan at 100 ppm	86.5	87.0	3.28	3.30	2.17	2.19	1.96	1.98	19.0	19.0	0.625	0.615	30.4	30.9

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T_6- Spraying chitosan at 200 ppm	91.0	91.5	3.35	3.36	2.26	2.28	2.02	2.04	19.6	19.7	0.605	0.595	32.4	33.1
T_7- Spraying chitosan at 400 ppm	92.5	93.0	3.37	3.38	2.28	2.29	2.05	2.07	19.8	19.9	0.590	0.580	33.6	33.7
T_8- Spraying seaweed extract and chitosan at low conc.	91.5	92.0	3.35	3.37	2.26	2.29	2.03	2.05	19.7	19.8	0.595	0.590	33.1	33.5
T_9- Spraying seaweed extract and chitosan at mid. conc.	95.0	95.0	3.46	3.48	2.33	2.35	2.10	2.11	20.5	20.6	0.570	0.560	35.9	36.8
T_{10}- Spraying seaweed extract and chitosan at high conc.	96.5	97.0	3.50	3.55	2.36	2.38	2.12	2.14	21.0	21.2	0.565	0.560	37.2	37.9
New L.S.D. at 5%	0.8	0.9	0.04	0.05	0.03	0.04	0.02	0.03	0.4	0.5	0.016	0.014	1.3	1.4