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Improving Growth and Cluster Quality of Crimson Seedless Grapevines Through the Application of Effective Micro-Organisms (EM) and Seaweed Extract

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Abstract: This study was conducted for two successive seasons (2019 & 2020) in a private vineyard located at Menoufiya governorate; to evaluate the effect the application of effective micro-organisms (EM) and seaweed extract on the growth and cluster quality of Crimson Seedless grapevines. The vines were six-year-old, grown in a sandy loam soil, irrigated by the drip irrigation system, spaced at 2 X 3 meters apart, cane-pruned and trellised by Spanish Parron system. Nine treatments were carried out as follows; EM at $10cm^3$/vine, EM at $20cm^3$/vine, seaweed extract at $10cm^3$/vine, seaweed extract at $20cm^3$/vine, EM at $10cm^3$/vine + seaweed extract at $10cm^3$/vine, EM at $10cm^3$/vine + seaweed extract at $20cm^3$/vine, EM at $20cm^3$/vine + seaweed extract at $10cm^3$/vine, EM at $20cm^3$/vine + seaweed extract at $20cm^3$/vine and control (untreated vines). All treatments were soil applied at three times: at 50% bud burst stage, at fruit set stage and two weeks after fruit set. The results showed that the application of EM + seaweed extract at the high dose ($20cm^3$/vine) is the most effective among all treatments in obtaining the best vegetative growth traits *i.e.* number of leaves/shoot, leaf area, weight of prunings and leaf content of total chlorophyll as well as increasing yield and improving the physical characterizes of clusters and the physical and chemical properties of berries in both seasons.

Key words: Effective micro-organisms (EM) · Seaweed extract · Grape · Cluster quality · Vegetative growth

INTRODUCTION

Bio-stimulants are essential for the sustainable growth of horticultural crops as they reduce the need for manufactured fertilizers, increase soil fertility, and enhance crop yield through their biological activity in the rhizosphere [1].

Effective microorganisms are a mixture of useful microorganisms, mainly able to photosynthesis and lactic acid-producing bacteria, yeast, as well as and actinomycetes breaking down fungi that can be added to soil through infection to enhance the microbial diversity of the soil. This may increase soil texture and fertility, which in consequently improves the growth, yield and fruit quality of crops [2].

Seaweed extract is now applied as bio-fertilizers, it is environmentally safe and benign and has positive effects on crops, and sprayed on many different types of crops **[3].** It contains a greater amount of all minerals, amino acids, vitamins, enzymes, antioxidants, and natural hormones. As a result, plants develop faster, which results in longer shoots and roots as well as better water and nutrient intake and enhance the quality, productivity, and development of numerous fruit crops [4, 5].

One of the most promising new varieties cultivated in Egypt is Crimson Seedless, late ripening with colored berries. Some problems are related with the production of Crimson Seedless grapevines such as achieving the desired level of poor red color [6].

The aim of this investigation was to enhance growth and cluster quality of Crimson Seedless grapevines through the application of effective micro-organisms (EM) and seaweed extract.

MATERIALS AND METHODS

This study was conducted for two successive seasons (2019 & 2020) in a private vineyard located at Menoufiya governorate; to evaluate the effect the application of effective micro-organisms (EM) and seaweed extract on the growth and cluster quality of Crimson Seedless grapevines. The vines were six-year-old, grown in a sandy loam (Table 1) soil, irrigated by the

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Table 1: Physical and chemical analysis of the vineyard soil

Characters	Values
Sand (%)	71.7
Silt (%)	2.4
Clay (%)	25.9
Texture	Sandy loam
Organic carbon (%)	0.34
PH (1:2.5)	7.93
EC (Mmhos/cm)	1.41
Water holding capacity (%)	23.4
Ca Co_3 (%)	13.7
N (%)	0.97
P (%)	0.13
K (%)	0.59

drip irrigation system, spaced at 2 X 3 meters apart, cane-pruned and trellised by Spanish Parron system. The vines were pruned during the second week of January with bud load of (84 buds/vine). Eighty one uniform vines were chosen on the basis their growth depending on weight of prunings as indirect estimates for vine vigor. Each three vines acted as a replicate and each three replicates were treated by one of the following treatments.

Effective micro-organisms (EM) were constituted of mixed culture of beneficial micro organisms primarily photosynthetic and lactic acid bacteria, yeast and Streptomycetes (Table 2).

Seaweed extract was used as trade mark (Gifert) which concentrate a modified ascophllum nodosum marine plant extraction composed of alginate, protein, fats, carbohydrates, marine salts and trace elements (Table 3).

Nine treatments were carried out as follows:

T1: EM at 10cm^3/vine
T2: EM at 20cm^3/vine
T3: Seaweed extract at 10cm^3/vine
T4: Seaweed extract at 20cm^3/vine
T5: EM at 10cm^3/vine + seaweed extract at 10cm^3/vine
T6: EM at 10cm^3/vine + seaweed extract at 20cm^3/vine
T7: EM at 20cm^3/vine + seaweed extract at 10cm^3/vine
T8: EM at 20cm^3/vine + seaweed extract at 20cm^3/vine.
T9: (untreated vines)

All treatments were soil applied at three times: at 50% bud burst stage, at fruit set stage and two weeks after fruit set.

The Following Parameters Were Adopted to the Conducted Treatments:

Vegetative Growth Characteristics: At growth cessation, the following morphological and chemical determinations were evaluated on 8 shoots / the considered vine:

1 - Average number of leaves/shoot.
2 - Average leaf area (cm^2) of the apical 6th and 7th leaves using a CI-203- Laser Area-meter made by CID, Inc., Vancouver, USA.
3 - Weight of prunings (Kg/vine) was determined during winter pruning.
4 - Leaf total chlorophyll content (mg/g F.W.) was determined according to Mackinny [7].

Yield and Physical Characteristics of Clusters: Representative random samples of 12 clusters/vine were harvested at maturity when TSS reached about 16-17% according to Tourky *et al*. [8]. The following characteristics were determined:

Yield/vine (kg) was determined as number of clusters/vine X average cluster weight (g). Also, average cluster weight (g), cluster length and cluster width (cm) were determined.

Physical Properties of Berries: Average berry weight (g), average berry size (cm^3) and average berry dimensions (length and diameter) (cm) were determined.

Chemical Properties of Berries: Total soluble solids in berry juice (T.S.S.) (%) by hand refractometer and total titratable acidity as tartaric acid (%) **[9]**. Hence TSS /acid ratio and total anthocyanin of the berry skin (mg/100g fresh weight) according to Yildiz and Dikmen [10] were determined.

Experimental Design and Statistical Analysis: The complete randomized block design was adopted for the experiment. The statistical analysis of the present data

Table 2: Components of effective micro-organisms (EM)

Total bacterial	Lactic acid bacteria	Yeasts	Streptomycetes
2.5 - 9.4 X 10^4 cfu/ml	6.3 - 9.7 X 10^6 cfu/ml	10^5 - 10^6 cfu/ml	8.4 X 10^3 cfu/ml

Table 3: Components of seaweed extract

Organic matter (%)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	Zn (ppm)	Fe (ppm)	Mn (ppm)
57	1.27	0.03	12	0.06	0.05	0.2	61	135	7

was carried out according to Snedecor and Cochran [11]. Averages were compared using the new L.S.D. values at 5% level [12].

RESULTS AND DISCUSSION

Vegetative Growth Characteristics: As shown in Table 4, it is obvious that vegetative growth parameters (number of leaves per shoot, leaf area, weight of prunings and leaf total chlorophyll content) positively responded to both EM and seaweed extract application at the two doses (10 or 20cm^3/vine) either in solely or in combined form as compared to control in both seasons. The highest dose (20cm^3/vine) of EM and seaweed extract was significantly higher than the low dose (10cm^3/vine). Foliar spraying of EM and seaweed extract at the high dose (20cm^3/vine) resulted in significantly the highest values of these estimates, while the least ones were occurred by control in both seasons.

The positive influence of EM on vegetative growth might be due to their significant effect on the synthesis of compounds that control plant growth or improve the availability and uptake of nutrients from the soil, including Mg, k, N, and Fe, that contribute to the synthesis of chlorophyll and promote vegetative growth [13].

The stimulatory impact of seaweed extract on vegetative growth due to their higher content of vitamins B, GA_3, amino acids, cytokinins and minerals, which closely involved in synthesis of carbohydrates, proteins and chlorophylls [14 , 15].

The obtained results are in harmony with Sabry *et al.* [16] on Red Globe grape, Abd El-Aal *et al.* [17] on Superior grape, El-Mogy [18] on Crimson Seedless grape and Ahmed [19] on Ruby Seedless grape; they reported that vegetative growth traits were enhanced by EM application. Regarding to seaweed extract, Baneen and Al-Abbasi [20] on citrus, Alebidi *et al.* [5] on date palm and Belal *et al.* [21] on Early Sweet grapes, they reported that seaweed extract improved vegetative growth parameters.

Yield and Physical Characteristics of Clusters: The data provided in Table 5 reported that both EM and seaweed extract application at the two doses (10 or 20cm^3/vine) either in solely or in combined form markedly affected on the yield and physical characteristics of cluster, which include cluster weight, length and width in comparison to control in both seasons. Foliar spraying of EM and seaweed extract at the high dose (20cm^3/vine) was significantly superior to the low dose (10cm^3/vine). Highest significant yield and physical characteristics of

Table 4: Effect of EM + seaweed extract on vegetative growth characteristics of Crimson Seedless grapevines during 2019 and 2020 seasons.

	Number of leaves/ shoot		Leaf area (cm^2)		Weight of prunings (kg/vine)		Total chlorophyll (mg/g F.W)	
Treatments	2019	2020	2019	2020	2019	2020	2019	2020
EM at 10cm/vine (EM1)	24.1	25.5	163.3	167.9	2.54	2.62	17.1	18.7
EM at 20cm/vine (EM2)	24.8	26.7	171.7	173.6	2.71	2.73	17.6	19.1
Seaweed extract at 10cm/vine (S1)	22.8	24.7	162.9	166.7	2.49	2.58	16.6	17.9
Seaweed extract at 20cm/vine (S2)	24.4	26.2	168.2	171.1	2.67	2.64	17.6	19.0
EM1 + S1	25.5	27.1	175.4	179.5	2.75	2.77	18.3	19.6
EM1 + S2	25.8	27.5	178.5	180.3	2.84	2.91	18.6	19.9
EM2 + S1	26.6	28.7	179.8	181.7	2.89	2.97	18.9	20.1
EM2 + S2	27.4	29.2	181.9	183.1	3.04	3.09	19.1	20.4
Control	21.8	23.5	160.3	165.2	2.17	2.29	14.9	17.0
New LSD at 0.05	0.7	0.4	1.9	1.3	0.14	0.11	0.21	0.18

Table 5: Effect of EM + seaweed extract on yield and physical characteristics of clusters of Crimson Seedless grapevines during 2019 and 2020 seasons.

	Yield/vine (kg)		Cluster weight (g)		Cluster length (cm)		Cluster width (cm)	
Treatments	2019	2020	2019	2020	2019	2020	2019	2020
EM at 10cm/vine (EM1)	13.35	13.77	447.8	463.7	20.57	21.52	15.92	16.15
EM at 20cm/vine (EM2)	13.73	13.97	453.1	468.9	20.75	21.70	16.13	16.34
Seaweed extract at 10cm/vine (S1)	13.11	13.61	444.7	459.9	20.52	21.45	15.85	16.08
Seaweed extract at 20cm/vine (S2)	13.56	13.85	449.4	466.1	20.68	21.57	16.02	16.27
EM1 + S1	13.96	14.17	457.8	473.7	20.84	21.76	16.27	16.43
EM1 + S2	14.13	14.31	462.3	478.6	20.93	21.89	16.40	16.54
EM2 + S1	14.28	14.58	469.7	486.1	20.99	21.96	16.49	16.67
EM2 + S2	14.41	14.69	477.1	492.9	21.06	22.01	16.62	16.75
Control	12.79	13.35	439.9	454.3	20.42	21.33	15.74	15.95
New LSD at 0.05	0.12	0.09	7.1	6.7	0.05	0.03	0.11	0.07

clusters was obtained by foliar spraying of EM and seaweed extract, whereas the least ones were attributed to the control in both seasons.

More than 80 different types of beneficial microorganisms are primarily found in effective microorganisms (EM) bio-stimulants, which are microorganisms that can break down organic matter, which is reflected in the increase in the yield [22].

The application of seaweed extract enhances water and nutrient intake, resulting in greater and thereby activating carbohydrates metabolism, which is shown in the improved production and cluster physical characteristics [23 , 4].

The obtained results are in harmony with Sabry *et al.* [16] on Red Globe grape, Abd El-Aal *et al.* [17] on Superior grape, El-Mogy [18] on Crimson Seedless grape and Ahmed [19] on Ruby Seedless grape; they reported that yield and its attributes were enhanced by EM application. Regarding to seaweed extract, Baneen and Al-Abbasi [20] on citrus, Alebidi *et al.* [5] on date palm and Belal *et al.* [21] on Early Sweet grapes, they reported that seaweed extract enhanced yield and physical characteristics of clusters.

Physical Properties of Berries: As shown in Table 6, it is obvious that all physical properties of berries (berry weight, size, length and diameter) positively responded to both EM and seaweed extract application at the two doses (10 or 20cm^3/vine) either in solely or in combined form as compared to control in both seasons. The highest dose (20cm^3/vine) of EM and seaweed extract was significantly higher than the low dose (10cm^3/vine). Foliar spraying of EM and seaweed extract at the high dose (20cm^3/vine) induced significantly the highest values of these determinations, while the least ones were occurred by control in both seasons.

The positive effect of EM on physical characteristics of berries might be due to their significant influence on the synthesis of compounds that control plant growth or improve the availability and uptake of nutrients from the soil, including Mg, k, N, and Fe, that contribute to the synthesis of chlorophyll which it reflect on improving berry physical properties [13].

The promoting effect of seaweed extract on physical characteristics of berries may be due to promote nutrient uptake, photosynthetic activation, and cell expansion by seaweed extract, which is considered as a source of cytokinin which in turn enhancing the physical properties of berries [24].

The obtained results are in harmony with Sabry *et al.* [16] on Red Globe grape, Abd El-Aal *et al.* [17] on Superior grape, El-Mogy [18] on Crimson Seedless grape and Ahmed [19] on Ruby Seedless grape; they reported that physical characteristics of berries were enhanced by EM application. Regarding to seaweed extract, Baneen and Al-Abbasi [20] on citrus, Alebidi *et al.* [5] on date palm and Belal *et al.* [21] on Early Sweet grapes, they reported that seaweed extract improved berry physical characteristics.

Chemical Properties of Berries: The data provided in Table 7 reported that both EM and seaweed extract application at the two doses (10 or 20cm^3/vine) either in solely or in combined form significantly improved the chemical properties of berries, which include TSS, acidity, TSS/acid ratio and total anthocyanin in comparison to control in both seasons. Foliar spraying of EM and seaweed extract at the high dose (20cm^3/vine) was significantly superior to the low dose (10cm^3/vine). Highest significant TSS, TSS/acid ratio and anthocyanin content of berry skin and least significant acidity of berry juice were obtained by foliar spraying of EM and seaweed

Table 6: Effect of EM + seaweed extract on physical properties of berries of Crimson Seedless grapevines during 2019 and 2020 seasons

Treatments	Berry weight (g)		Berry size (cm^3)		Berry length (cm)		Berry diameter (cm)	
	2019	2020	2019	2020	2019	2020	2019	2020
EM at 10cm/vine (EM1)	4.04	4.14	3.92	4.01	2.45	2.48	1.73	1.76
EM at 20cm/vine (EM2)	4.12	4.20	4.01	4.09	2.47	2.49	1.74	1.78
Seaweed extract at 10cm/vine (S1)	4.02	4.11	3.89	3.98	2.44	2.47	1.72	1.76
Seaweed extract at 20cm/vine (S2)	4.07	4.16	3.95	4.04	2.45	2.49	1.73	1.77
EM1 + S1	4.16	4.25	4.06	4.14	2.48	2.50	1.75	1.78
EM1 + S2	4.19	4.29	4.09	4.18	2.50	2.53	1.76	1.80
EM2 + S1	4.21	4.32	4.12	4.23	2.52	2.54	1.77	1.82
EM2 + S2	4.26	4.36	4.16	4.26	2.55	2.56	1.79	1.85
Control	3.99	4.06	3.85	3.92	2.43	2.45	1.71	1.75
New LSD at 0.05	0.04	0.03	0.03	0.02	0.02	0.01	0.01	0.02

Table 7: Effect of EM + seaweed extract on chemical properties of berries of Crimson Seedless grapevines during 2019 and 2020 seasons

Treatments	TSS (%)		Acidity (%)		TSS/acid ratio		Total anthocyanin (mg/g F.W)	
	2019	2020	2019	2020	2019	2020	2019	2020
EM at 10cm/vine (EM1)	16.5	16.8	0.58	0.61	28.4	27.5	32.0	32.8
EM at 20cm/vine (EM2)	16.7	16.9	0.56	0.59	29.8	28.6	32.4	33.5
Seaweed extract at 10cm/vine (S1)	16.4	16.7	0.60	0.62	27.3	26.9	31.9	32.6
Seaweed extract at 20cm/vine (S2)	16.6	16.8	0.57	0.59	29.1	28.5	32.3	33.2
EM1 + S1	16.7	17.0	0.55	0.58	30.4	29.3	32.7	33.6
EM1 + S2	16.8	17.1	0.55	0.56	30.5	30.5	32.9	34.0
EM2 + S1	17.1	17.3	0.54	0.56	31.7	30.9	33.4	34.6
EM2 + S2	17.5	17.6	0.53	0.54	33.0	32.6	33.9	35.2
Control	16.3	16.5	0.61	0.64	26.7	25.8	31.5	32.1
New LSD at 0.05	0.3	0.2	0.02	0.01	0.3	0.2	0.4	0.3

extract at the high dose (20cm^3/vine), whereas the least values of TSS, TSS/acid ratio and anthocyanin content of berry skin and highest values acidity of berry juice was attributed to the control in both seasons.

The positive effects of EM on soil fertility and the availability of the majority of nutrients may result in higher growth, which unquestionably has an impact on the berry quality [16]. Applying seaweed extract increases nutritional and water intake, which in turn increases and activates the metabolism of carbohydrates, as evidenced by the enhanced berry chemical characteristics [23 , 4].The obtained results are in harmony with Sabry *et al.* [16] on Red Globe grape, Abd El-Aal *et al.* [17] on Superior grape, El-Mogy [18] on Crimson Seedless grape and Ahmed [19] on Ruby Seedless grape; they reported that the chemical properties of berries were enhanced by EM application. Regarding to seaweed extract, Baneen and Al-Abbasi [20] on citrus, Alebidi *et al.* [5] on date palm and Belal *et al.* [21] on Early Sweet grapes, they reported that seaweed extract enhanced berry chemical properties.

From the obtained results, it can be concluded that EM + seaweed extract at the high dose (20cm^3/vine) is the most effective among all treatments in obtaining the best vegetative growth traits *i.e.* number of leaves/shoot, leaf area, weight of prunings and leaf content of total chlorophyll as well as increasing yield and improving the physical characterizes of clusters and the physical and chemical properties of berries in both seasons.

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