



The Application of Some Biostimulant-Based Substances to Improve the Quality and Productivity of "Ruby Seedless" Grapevines c.v

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ABSTRACT

Innovative and sustainable tools such as biostimulant-based substances play an important key role in the development of environment friendly viticultural strategies to improve yields and fruit quality. In this study, we investigated the effect of foliar spray application of two biostimulants, seaweed extracts made from *Ascophyllum nodosum* and effective microorganisms, alone or combined with each other, on increasing the vegetative growth of *Vitis vinifera* cv. Ruby Seedless. Two concentrations of (1g/L and 2g/L) were used for each substance to assess their impact on yield, chemical characteristics of berries, leaf area shoot length. All treatments showed a stimulatory effect for studied parameters. The results indicate that the vines treated with the lower levels of EM 1g/L combined with higher levels of seaweed *A. nodosum* 2g/L produced the highest absolute yield and least shot berries % relative to the untreated vines.

Keywords: *Ascophyllum nodosum*, Effective microorganisms, Ruby Seedless grapevine, Leaf area, Shot berries %.

1. Introduction

Ruby Seedless is a mid to late-season red, seedless table grape. The cultivar resulted from a cross of I.P 75 (Thompson Seedless x Muscat of Alexandria) and Emperor, made in 1939. Ruby Seedless is known for its ability to mature extremely large crops due to its high bud fertility, which is reflected on the occurrence of the so-called overcropping phenomenon (Harry *et al.*, 1991). The effects of overcropping have been reported to reduce vine vigor, increase shot berries and change the berry composition. The low vine vigor is controlled by using some biostimulants such as seaweeds and effective microorganisms, which have the intention to stimulate the plants natural processes in order to enhancing their nutrient use efficiency and increasing vine vigor (Du Jardin, 2015). Plant biostimulants offer a potential alternative to traditional, agro-chemical inputs, additionally; they can reduce the application rates of mineral fertilizers by enhancing their efficacy (Yakhin *et al.*, 2017).

Foliar applications of seaweed extract have a great effect on plant growth, productivity and fruit quality of grapevines, (Abd Elmoniem *et al.*, 2008). Seaweed extract *Ascophyllum nodosum* brown algae is considered as an important source of nutrition for sustainable agriculture as they contain various trace elements (Fe, Cu, Zn, Co, Mo, Mn and Ni), vitamins, amino acids and plant growth hormones which cause many beneficial effects on plant growth and development (Abdel-Mawgoud *et al.*, 2010).

Moreover, Ruby seedless grapevine cv. suffers from higher shot berries and lower fruit quality. The possibility of using seaweed extract for alleviating such undesirable phenomena could be the result of its promoting effect on supplying the vines with their requirements from different nutrients and natural hormones as well as their positive action on increasing the vines tolerance to various stresses (Sharma *et al.*, 2014).

It was found that Effective Microorganisms (EM) are biological extracts containing naturally selected microbes such as, *Lactobacillus casei*, *Streptomyces albus*, *Rhodopseudomonas palustris*, *Aspergillus oryzae* and *Saccharomyces albus*, (Hu and Qi, 2013). Vaid *et al.* (2017) concluded that when bacterial isolates are used in combination they perform better in comparison to their individual

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use. Moreover, an experiment with the EM cultures have shown that foliar applications of EM significantly increase, yield and quality of over the control according to Olle and Williams (2013). Moreover, Bzdyk *et al.* (2018) showed that foliar application of EM can increase the growth and yield of vegetable crops in are relatively short time.

Based on the aforementioned, the aim of this study was to induce new different strategies to improve the vegetative parameters in order to obtain higher crop quality, increase the efficiency of nutrient use and resist biotic abiotic stress.

2. Material and Methods

This experiment was carried out during the two successive seasons 2019 and 2020 in a vineyard located at Cairo-Alexandria desert road 30 15' 11" N. and 30 39' 43" E. Ten-year-old table grape "Ruby Seedless" spaced 2 x 3 m grown in a sandy soil were used in this investigation.

The chosen vines were irrigated by drip irrigation system and trained to the bilateral cordon system. The vines were pruned to spurs during the last week of January with bud load of (30 buds/vine) and trellised by Spanish parron system.

One hundred thirty-five uniform vines were chosen for this study (9 treatments x 3 replicates x 5 vines/replicate). The experiment was carried out on the same vines for both seasons, and received common horticultural practices and mineral fertilizers doses. All vines were adjusted to 35 clusters/vine after berry set.

Seaweed (*Ascophyllum nodosum*) and Effective microorganisms (EM) were foliar sprayed as supplement at 3 doses, the 1st dose two weeks after bud burst, the 2nd dose one week after fruit set, the 3rd dose at veraison. They received a direct application to the leaves in two concentrations 1 and 2 mg/L each, at a rate of approximately 2L/vine as follow:

1. Control
2. 1g/L EM
3. 2g/L EM
4. 1g/L *Ascophyllum nodosum*
5. 2g/L *Ascophyllum nodosum*
6. 1g/L EM + 1g/L *Ascophyllum nodosum*
7. 1g/L EM + 2g/L *Ascophyllum nodosum*
8. 2g/L EM + 1g/L *Ascophyllum nodosum*
9. 2g/L EM + 2g/L *Ascophyllum nodosum*

The following measurements were taken to evaluate the effect of the different treatments:

2.1. Yield

Samples of 15 clusters were collected randomly for each treatment, when clusters reached their full color and total soluble solids reached about 15-18%, according to Badr and Ramming (1994).

- a. Yield per vine (kg): to calculate the average yield/vine, the random samples were collected and weighed then the mean of cluster weight was multiplied by the number of clusters / vine after being adjusted to 35 clusters per vine after fruit set.
- b. Average cluster weight (g)
- c. Average berry weight (g): weight of 10 berries /10
- d. Average berry size (cm³): by using the measuring cylinder, volume of 10 berries were taken then divided by 10
- e. The percentage of shot berries /cluster (%): At harvest clusters were harvested and the number of shot berries in each cluster were counted and divided by the total number of berries in each treatment to calculate the percentage of shot berries as follow:

The percentage of shot berries = (Number of shot berries / Total number of berries per cluster) x 100.

- f. The net yield (Kg): it was calculated for each treatment after eliminating the shot berries.

2.2. Chemical characteristics of berries

The following determinations were carried out:

- a. Refractometric total soluble solids (TSS %), titratable acidity % (was determined by titration of a water extract of juice with 0.1 N NaOH to an end point of pH 8.1.) and TSS/acid ratio were determined according to A.O.A.C., (2006).
- b. Total anthocyanin in berry skin (mg/100g): the spectrophotometer is used at 250 nm according to Yilidiz and Dikmen (1990).

2.3. Vegetative growth parameters

- a. Leaf area (cm²): Samples of 20 leaves were randomly collected from each treatment for leaf area determination at harvest time (using leaf area meter, Model CI 203, U.S.A.).
- b. Number of leaves/vine: it was calculated by counting the number of leaves / shoot multiplying by the average number of shoots for each vine.
- c. Total leaf area/vine (m²): The mean leaf area (cm²) of the basal [5th to 7th] from the shoot tip was multiplied by the average number of leaves / shoot and average number of shoots for each vine at harvest time.
- d. Shoot length (cm): it was determined by measuring the fruiting shoots before harvest.
- e. Total chlorophyll content of leaves (SPAD): were measured at harvest time in the mature basal leaves of the sixth and seventh nodes by using the nondestructive Minolta chlorophyll meter model SPAD 502.
- f. Total carbohydrates in leaves (%): it was determined, following the standard methods of association of official analytical chemists (A.O.A.C., 2006).

2.4. Statistical analysis

The statistical analysis of the present data was carried out according to Snedecor and Cochran (1980). Averages were compared using the new L.S.D. values at 5% level using a randomized complete block.

3. Results and Discussion

3.1. Yield

3.1.1. Yield per vine (kg), average cluster weight (g), average berry weight (g) and average berry size (cm³)

The yield was significantly increased by the application of effective microorganisms and seaweed *Ascophyllum nodosum* in both concentrations either in the single or in the combined treatments (Table, 2). However, the combined application at the lower concentration 1g/L of EM and the higher concentration of *A. nodosum* 2g/L, resulted in the highest values followed by higher concentration of both, whereas, the lowest values were given by the single treatments and control respectively. These results are linear with those obtained from Olle and Williams, (2013) who concluded that EM can improve yield. Moreover, it was found in another trial that using EM and amino acids as substitutes of inorganic N fertilizers significantly was very effective in improving quality of the berries in terms of increasing berry weight and dimensions (Ahmed *et al.*, 2017).

Seaweed extracts *Ascophyllum nodosum* are a source of natural plant growth regulators which have greater importance as biostimulant for various plants. The growth regulators present within seaweed extracts are believed to be involved in promoting plant growth and productivity and enhance crop qualities when applied exogenously. Seaweed liquid fertilizers are beneficial because they contain growth promoting hormones such as GA3, Auxins, Cytokinin, ABA, Ethylene, Betaine and Polyamines which enhance cell division and enlargement resulting in an appreciable increment in berry weight and size along with vitamins, amino acids, antibiotics and micronutrients (Panda *et al.*, 2012). In addition, the positive effect of effective microorganisms and Seaweed extract *Ascophyllum nodosum* on the yield could be attributed to the enhancing effect on berry weight; in addition, EM contains primarily photosynthetic and lactic acid bacteria, yeast, actinomycetes fermenting fungi increase the microbial diversity, which in turn enhances yield berry weight and size, and improve crops quality (Higa and Kinjo, 1991). Thus, Supplementary foliar sprays with *Ascophyllum nodosum* seaweed extract improve berry size, yield and grape quality (Norrie *et al.*, 2002; Norrie & Keathley, 2006).

3.1.2. The percentage of shot berries /cluster (%)

Data in Table (1) showed that the percentage of shot berries was significantly affected by varying biostimulant treatments. It is obvious that using a combination of effective microorganisms and seaweed *Ascophyllum nodosum* at 1g/L of EM and 2g/L *A. nodosum*, significantly was accompanied with reducing shot berries % relative to the control treatment. Similar results were observed during an experiment using EM, which found had a reducing effect on these undesirable phenomenon, and the highest values were recorded on untreated vines (Abdelaziz *et al.*, 2017).

Regarding to seaweed extract *Ascophyllum nodosum* Ali and Mohamed, (2016) reported that there was a gradual and significant reduction on the percentage of shot berries with seaweed extract proportional to its concentrations comparing to the check treatment. The reduction on such parameter was meaningless in the higher two concentrations namely 0.2 and 0.4%, which ensure our results using a lower concentration of 1g/L or 0.1%.

3.1.3. The net yield (Kg)

Shot berries effect leads to significant commercial losses in the grape production by decreasing yield quality. By calculating the overall loss of yield due to the percentage of shot berries per cluster (Table 1), we can notice that the higher net yield, after eliminating the shot berries was obtained from the vines treated with a combination of EM and seaweed extract *Ascophyllum nodosum*, (Ali and Mohamed, 2016; Abdelaziz *et al.*, 2017).

Table 1: The effect of applying some biostimulant-based substances on yield of Ruby Seedless" grapevines c.v during the two successive seasons 2019 and 2020

Treatments	Yield/vine (kg)		Cluster weight (g)		Berry weight (g)		Berry size (cm ³)		Shot berries (%)		Net yield/vine(kg)	
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
Control	8.1	8.4	280.1	295.3	1.61	1.48	1.48	1.34	15.2	14.5	6.9	7.2
1g/L EM	13.7	14.3	489.6	512.8	2.37	2.25	2.24	1.12	10.5	9.2	12.3	12.9
2g/L EM	14.0	15.1	500.2	587.0	2.50	2.06	2.38	1.92	11.1	10.7	12.4	13.5
1g/L <i>A. nodosum</i>	12.9	13.4	462.9	479.2	2.21	2.45	2.09	2.31	11.3	10.1	11.4	12.0
2g/L <i>A. nodosum</i>	11.2	11.8	401.5	422.7	2.03	2.11	1.98	1.97	8.3	7.8	10.2	10.8
1g/LEM + 1g/L <i>A. nodosum</i>	18.1	18.3	645.4	653.8	2.97	2.87	2.84	2.74	6.1	5.9	16.8	17.2
1g/LEM + 2g/L <i>A. nodosum</i>	19.4	19.7	685.3	704.0	3.21	3.17	3.10	3.06	4.1	3.6	18.6	18.9
2g/LEM + 1g/L <i>A. nodosum</i>	17.7	18.1	632.7	645.2	2.85	2.74	2.81	2.61	6.5	6.7	16.5	16.8
2g/LEM + 2g/L <i>A. nodosum</i>	18.7	19.0	668.2	680.4	3.16	3.12	3.05	3.01	5.8	5.2	17.6	18.0
New L.S.D. at 0.05	0.5	0.5	11.3	13.2	0.02	0.02	0.02	0.01	0.6	0.05	0.04	0.03

3.2. Chemical characteristics of berries

3.2.1. Refractometric total soluble solids (TSS %), titratable acidity (TA) % and TSS / acid ratio

Statistical analysis demonstrated that the performed treatments had significant effects on TSS% titratable acidity % and TSS / acid ratio (Table 2). The average TSS% content in the berries ranged from 15.6°Bx to 17.8°Bx, with total acidity ranging from 0.46 to 0.69 % in the first season. Results showed that biostimulant-based substances, EM and seaweed extract treatments, had an effect on berry juice quality at harvest. However, the combined treatment 1g/L of EM and 2g/L *A. nodosum* significantly was accompanied with increased total soluble solids of the berries recording the highest values followed by the other combined treatments. Similarly, Ahmed *et al.* (2017) stated that using N with EM and as substitutes of inorganic N fertilizers significantly was very effective in improving quality of the berries in terms of T.S.S, T.S.S./acid ratio and reducing total acidity in the berries relative to using N completely.

The best results with regard to quality of the berries were also recorded on the vines that received seaweed sprays. These results may be attributed to its effect in increasing the total leaf area/vine, which in turn increase the TSS % and decreasing the acidity % as mentioned before by Almanza-Merchán *et al.* (2011). They found that vines without leaf removal and higher total leaf area exhibited the highest total soluble solids content.

Table 2: The effect of applying some biostimulant-based substances on chemical characteristics of berries of Ruby Seedless" grapevines c.v during the two successive seasons 2019 and 2020.

Treatments	Total soluble solids (TSS %)		Titratable acidity (%)		TSS / acid ratio.		Total anthocyanin (mg/100g fw)	
	2019	2020	2019	2020	2019	2020	2019	2020
Control	15.6	15.4	0.69	0.67	22.6	23.0	23.4	25.7
1g/L EM	16.2	16.3	0.54	0.56	30.0	29.1	33.5	32.9
2g/L EM	16.0	16.1	0.58	0.59	27.9	27.3	28.7	29.4
1g/L <i>A. nodosum</i>	16.1	16.0	0.55	0.58	29.3	27.3	30.8	31.0
2g/L <i>A. nodosum</i>	16.0	15.9	0.53	0.53	30.0	30.0	34.9	34.0
1g/LEM + 1g/L <i>A. nodosum</i>	16.8	16.5	0.50	0.51	33.6	32.4	37.3	38.0
1g/LEM + 2g/L <i>A. nodosum</i>	17.8	18.1	0.46	0.44	38.7	41.1	39.6	40.2
2g/L EM + 1g/L <i>A. nodosum</i>	16.2	16.3	0.51	0.52	31.8	31.3	36.4	37.1
2g/L EM + 2g/L <i>A. nodosum</i>	17.2	17.4	0.48	0.49	35.8	35.5	38.2	38.9
New L.S.D. at 0.05	0.1	0.2	0.02	0.01	1.5	1.9	0.7	0.5

3.2.2. Total anthocyanin in berry skin (mg/100g)

Data in Table (4) revealed that using biostimulants as supplement of mineral fertilizers affected the accumulation of anthocyanin in both seasons. Maximum anthocyanin content was gained by the seventh treatment 1g/L EM + 2g/L *Ascophyllum nodosum*. Similarly, Kok *et al.* (2010) found that *Ascophyllum nodosum* seaweed extract applied pre-bloom and at pea berry size, improved the brightness and redness of Trakya Ilkeren grapes. It is also in linear with a study where foliar applications of seaweed extract to Flame Seedless increased anthocyanin concentration (Strydom, 2013). At the concentration of 1.0 g/L of *Ascophyllum nodosum*, treatment significantly increased the total phenolics and flavonoids content, which are the key role of increasing anthocyanin (Popescu and Popescu, 2014). *A. nodosum* applications delayed berry ripening, leading to a higher anthocyanin content in treated berry skins (Salvi *et al.*, 2019).

3.3. Vegetative growth parameters

3.3.1. Leaf area (cm²)

The leaf area was significantly increased by the application of effective microorganisms and seaweed *Ascophyllum nodosum* in both concentrations either in the single or in the combined treatments (Table, 3). However, the combined application at the lower concentration 1g/L of EM and the higher concentration of *A. nodosum* 2g/L, resulted in the highest values followed by higher concentration of both, whereas, the lowest values were given by the single treatments and control respectively. These results are linear with those obtained by Khan *et al.* (2012) who stated that grapevines subjected to foliar spray applications of seaweed extract (*A. nodosum*) exhibited a higher increase in leaf size than untreated grapevines. This increase in leaf size may be ascribed to the hormonal action of seaweed extract, which increased the endogenous hormonal level of treated grapevines.

The positive effects of effective microorganisms were proved by Abdelaziz *et al.* (2017) who found that the highest values of leaf area were observed with using EM.

3.3.2. Number of leaves/vine and Total leaf area/vine (m²)

Number of leaves per vine and leaf area are the main factors that determine the total leaf area per vine. The combined application at the lower concentration 1g/L of EM and the higher concentration of *A. nodosum* 2g/L resulted in the highest values in the mentioned parameter (Table 3). The results may be explained according to the dual effect of the biostimulants, Seaweed extract *A. nodosum* which produce growth regulators and contain various trace elements (Fe, Cu, Zn, Co, Mo, Mn and Ni), vitamins, amino acids which cause many beneficial effects on the vegetative growth and development (Abdel-Mawgoud *et al.*, 2010).

In addition, Rayorath *et al.* (2008) have showed that extracts of *A. nodosum* the number of leaves were affected positively at concentration of 1 g/L, which in turn increased the total leaf area per vine.

3.3.3. Shoot length (cm)

It is obvious from data in Table (4) that using EM and seaweed extract treatments significantly were accompanied with enhancing shoot length. The highest values were observed with using the combined application at 1g/L of EM and 2g/L seaweed. Similarly, the maximum values of shoot length were obtained under EM conditions (Wassel *et al.*, 2014). However, in another study, regarding the influence of seaweed concentrate on shoot length, it was found that there were significant differences between different concentrations where the effect of *Ascophyllum nodosum* at a concentration of 0.17% gave a significant increase in shoot length than vines treated with lower concentration (Popescu and Popescu, 2014).

3.4. Total chlorophyll content of leaves (SPAD)

The achieved results of total chlorophyll content of leaves varied between 30.2 and 41.5 SPAD in the second season (Table 3). It was clear that all the seaweed and EM treatments increased total chlorophyll content with a superiority of the combined treatment 1g/L of EM and 2g/L seaweed. Regarding to the application of *Ascophyllum nodosum* extract, it caused an increase in chlorophyll content in the leaves. Similar results were obtained by Khan *et al.* (2009) presents the possible mechanisms by which the extracts from seaweed have beneficial effect on agriculture: increased photosynthetic efficiency and carbon assimilation and nutrient uptake. Moreover, Spinelli *et al.* (2010) in a trial done on a strawberry crop noticing that the application of seaweed extract (*Ascophyllum nodosum*) in a concentration of 2% increased the leaf chlorophyll content by 11 %. Shehata *et al.* (2011) ascribed this increase to the decrease in degradation of chlorophyll, due to the betains found in the liquid fertilizers made of seaweed extracts.

In addition, biofertilization with EM significantly stimulated plant pigments namely chlorophylls relative to the check treatment. These results were true during both seasons (Abdelaziz, *et al.*, 2017).

Table 3: The effect of applying some biostimulant-based substances on the vegetative growth parameters of Ruby Seedless" grapevines c.v during the two successive seasons 2019 and 2020

Treatments	Leaf area (cm ²)		Total leaf area (m ²)		Shoot length (cm)		Total chlorophyll SPAD		Total Carbohydrates (%)	
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
Control	120.4	109.5	12.6	11.4	119.2	125.3	29.7	30.2	19.6	19.3
1g/L EM	146.0	159.1	15.4	16.7	141.6	150.2	35.2	36.7	24.4	25.4
2g/L EM	138.6	168.4	14.6	17.6	139.1	141.4	33.0	32.9	22.1	23.1
1g/L A. nodosum	144.8	151.0	15.2	15.9	135.6	137.0	34.6	36.5	23.2	24.3
2g/L A. nodosum	150.7	175.4	15.8	18.4	147.4	157.6	36.4	37.6	25.5	26.6
1g/L EM + 1g/L A. nodosum	180.4	194.2	18.9	20.3	162.5	165.0	37.5	38.4	27.8	28.9
1g/L EM + 2g/L A. nodosum	208.0	220.5	22.1	23.2	180.5	189.1	40.2	41.5	30.1	31.2
2g/L EM + 1g/L A. nodosum	171.3	183.0	18.0	19.2	157.0	160.7	37.0	38.1	26.6	27.7
2g/L EM + 2g/L A. nodosum	197.4	208.6	20.6	21.8	167.2	174.5	38.7	39.0	28.9	30.0
New L.S.D. at 0.05	5.4	6.2	0.7	0.6	11.8	10.5	0.9	0.8	0.9	1.1

3.5. Total carbohydrates in leaves (%)

The effect of seaweed extracts treatments and effective microorganisms with different concentrations on leaves total carbohydrates is shown in Table (3). The plants treated with the higher levels of seaweed concentrate combined with the lower levels of *A. nodosum* produced the highest values relative to untreated vines or the single applications. Similarly, Abbas (2013) found that seaweed extracts increase protein content which is always associated with an increase of carbohydrate concentration in leaves. In addition, Bzdyk *et al.* (2018) stated that foliar application of EM on the vegetative growth resulted in a large number of beneficial microorganisms including photosynthetic bacteria and N-fixing bacteria, at the leaf surface. These microorganisms in the EM culture enhance the plant's photosynthetic rate and efficiency as well, besides their N-fixing capacity.

4. Conclusion

In conclusion, our findings revealed that seaweed extracts (*Ascophyllum nodosum*) and effective microorganisms, alone or combined with each other, increased all growth parameters of *Vitis vinifera* cv. Ruby Seedless. The results indicate that the vines treated with the lower levels of EM 1g/L combined with higher levels of seaweed *A. nodosum* 2g/L produced the highest absolute yield and least shot berries % relative to the untreated vines.

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