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Influence of Effective Micro-Organisms (EM) and Ascorbic Acid on Growth, Yield, Fruit Quality and Storability of Flame Seedless Grape Cultivar

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Abstract: This experiment was conducted on six-year-old Flame Seedless grapevines investigation two consecutive growing seasons (2021 and 2022) at the El-Baramon farm in Mansoura, Dakahlia Governorate, Egypt to study the influence of effective micro-organisms (EM) and ascorbic acid on vegetative growth, yield and fruit quality as well as storability of Flame Seedless grape cultivar. The selected vines were six-year-old, irrigated using a flood irrigation method and cultivated in clay soil. The grapevines were planted at 2 x 3 m intervals, spur-pruned during the 2nd week of January, loaded with 68 buds per vine, and trellised using the Spanish baron system. Eight treatments were conducted as follow: soil inoculation with effective micro-organisms (EM) at 20cm3/vine and foliar spray with three doses of ascorbic acid (200, 400 and 600 ppm) were applied either solely or in combination with each other, in addition to the control treatment (untreated vines). All applied treatments were conducted at three times: the 1st date (after bud burst phase), the 2nd date (at berry set phase), and the 3rd date (at veraison phase). Clusters of all treatments were stored for 4 weeks at ± 0ºC and 90-95% RH and fruit quality was assessed via harvest and cold storage.

The study demonstrated that the combined application of effective micro-organisms (EM) at $20cm^3$/vine plus ascorbic acid at 600 ppm had the superior outcomes in terms of obtaining the best aspects of vegetative growth and the highest nutritional content of the vine, raising fruit production and enhancing fruit quality characteristics as well as enhancing the storability of Flame Seedless grape by decreasing physiological loss in weight, decay and shattering, maintaining firmness and delaying the changes in total soluble solids, acidity and total sugars compared to control during extended cold storage in both seasons.

Key words: Grape, Flame Seedless, yield, fruit quality, vegetative growth, EM, ascorbic acid and storability.

INTRODUCTION

Effective micro-organisms (EM) bio-fertilizer mainly consists of approximately 80 different types of micro-organisms that are able to decompose organic matter, and beneficial micro-organisms are regarded as an essential bio fertilizer. It is in charge of fixing nitrogen via larger concentrations of minerals, hormones, and Vitamines B, **(Olle and Williams, 2013).** Moreover, microorganisms create bioactive substances that are advantageous to vine, such as hormones and growth factors that cause cells multiply. More and more efficient microorganisms (EM) are being used to promote nutrient cycling and plant growth. (**Sabeti *et al.*, 2017)**. Effective micro-organisms also are additional benefits of their enhancement of soil composition via fertility, acceleration of organic compound mineralization, elimination of putrefactive processes, and enhancement of the availability of such nutritional content of the vine (**Lack *et al.*, 2013)**. Moreover, using of Effective micro-organisms can increase the growth parameters, production and standard quality of grapes (**Sabry *et al.*, 2009, Abd El-Hameed *et al.*, 2010, Ahmed *et al.*, 2011, Abd El-Aal *et al.*, 2013, El-Mogy 2017 and Ahmed, 2022).**

Antioxidants are crucial for both preventing senescence in the cell and improve productivity of organic fruits. Antioxidants are safe to use around humans and stop the oxidation of lipids, which are part of the plasma membrane, by free radicals created during plant metabolism, which results in a loss of permeability and cell death. **(Fayek *et al.*, 2014).** Ascorbic acid is a strange antioxidant; it plays a variety of crucial roles in defense, photosynthetic regulation and growth regulation **(Blokhina *et al.*, 2003).** Maintaining the quality attributes of fruits treated with effective micro-organisms (EM) and ascorbic acid during cold storage may be due to their influence in inhibiting the activity of enzymes that break down pectin, which can cause the fruit to soften and deteriorate (**Kilic *et al.*, 2015 and Abd El-Wahab *et al.*, 2020).** The goal of the investigation is to study the effect of effective microorganisms and ascorbic acid on growth, production, fruit quality, and storability of the Flame Seedless grape variety.

MATERIALS AND METHODS

This experiment was executed out on six-year-old Flame Seedless grapevines grafted on Freedom rootstock investigation two consecutive growing seasons (2021 and 2022) at the El-Baramon farm in Mansoura, Dakahlia Governorate, Egypt. The grapevines were as regular as possible and grown in clay soil using the approach described by **Chapman and Pratt (1987)** as display in **(**Table, 1**)** under flood irrigation system. In each season, the 2nd week of January, and the grapevines were spur-pruned and loaded with 68 buds per vine with the Spanish baron system and trained using the quadrilateral cordon system. The vines were planted at 2 x 3 m intervals.

Table (1). Physical and chemical analysis of the experimental soil

Characters	Values
Sand %	**26.43**
Silt %	**23.22**
Clay %	**50.33**
Texture	**Clay**
PH (1:2.5)	**7.82**
Organic carbon %	**1.88**
$CaCO_3$ %	**1.83**
E.C. (1:5 extract) (mmhos/1 cm)	**0.67**
N (%)	**0.28**
P (%)	**0.16**
K (%)	**0.32**

Ninety-six regular vines were selected on based on their development depending on pruning wood weight and vine trunk diameter which served as supplemental measures of vine force. Each four vines acted as a replicate and each three replicates acted as a treatment.

Eight treatments were conducted as follows:

- Control (untreated vines)
- Soil inoculation with effective micro-organisms (EM) at 20cm^3/vine
- Foliar spray with ascorbic acid at 200 ppm
- Foliar spray with ascorbic acid at 400 ppm
- Foliar spray with ascorbic acid at 600 ppm
- EM + Ascorbic acid at 200 ppm
- EM + Ascorbic acid at 400 ppm
- EM + Ascorbic acid at 600 ppm

Effective micro-organisms (EM) were used as bio-fertilizer constitute of mixed culture of primarily photosynthetic beneficial microorganisms, lactic acid bacteria, yeast and streptomycetes as shown in (Table, 2).

Table (2). Components of Effective micro-organisms (EM)

Total bacterial	Lactic acid bacteria	Yeast	Streptomycetes
2.4 – 9.3 X 10^4 cfu/ml	6.2 – 9.8 X 10^6 cfu/ml	10^5 - 10^6 cfu/ml	8.3 X 10^3 cfu/ml

Effective micro-organisms (EM) were applied as a soil inoculation with at 20cm^3/vine in pits beside each vine roots at spacing of 50 cm far the vine stem and 50 cm depth.

Foliar spray with ascorbic acid was applied at three doses (200, 400 and 600 ppm). All foliar solutions had Triton B added as a wetting agent at a rate of 0.1%, and spraying continued until runoff.

All applied treatments were conducted at three times: the 1st date (after bud burst phase), the 2nd date (at berry set phase), and the 3rd date (at veraison phase).

The following criteria were embraced to assess the tested treatments:

1- Vegetative growth parameters

At the second week of July, vegetative growth parameters were established on four non-fruiting shoots as follows:

-Average shoots length (cm).

-Average shoot diameter (cm)

-Average number of leaves per shoot

-Average leaf surface area (cm^2): The 6th and 7th leaves were taken from the end of the growing branch were employed to measure leaf area, according to **Montero *et al.* (2000).**

2- Leaf content of total chlorophyll and mineral elements

Leaf samples were taken at the second week of July from the 6th and 7th leaves from the developing shoot end s for the estimating of leaf content of total chlorophyll and mineral elements: Total chlorophyll content (mg/g F.W.) was predestined according the method described by **Mackinny (1941),** total nitrogen (%) was predestined according to **Pregl (1945)** using the modified micro-Kjeldahl method, phosphorus (%) was calorimetrically measured as described by **Snell and Snell (1967)** and potassium (%) was measured according to **Jackson (1967)** using Flame photometry instrument.

3- Yield and physical properties of clusters and berries

According to **Tourky *et al*. (1995)**, representative random samples consisting of six clusters/vine collected at repining when total soluble solids (TSS) exceeded roughly 16 percent for the following assessment:

- Yield/vine (kg) which was calculation by multiplied average cluster weight by number of clusters per vine
- Average cluster weight (g)
- Average berry weight (g)
- Average berry size (cm^3)
- Average berry firmness (g/cm^2).

4- Chemical properties of berries

- Total soluble solids (TSS) was measured as a percentage by using a hand refractometer.
- Total acidity as (g tartaric acid/100 ml juice) was established according to **A.O.A.C. (1995)**.
- Total sugars (%) were predestined according to **Sadasivam and Manickam (2004).**
- Total anthocyanin (mg/100 g fresh weight) was measured according to **Husia *et al.* (1965).**

5- Fruit quality assessments during cold storage conditions

During the stage of ripeness, when TSS exceeded 16% as expressed by **Tourky *et al*. (1995)**, clusters of all treatments were collected and packed in perforating containers. polyethylene bags; each bag included 550 – 650 g, and then placed in carton boxes and each box contained three bags. All treatments were packed into 48 carton boxes (1.5 - 2 Kg/box). Each two carton boxes doing as a replicate and each three replicates were represented one treatment. All treatments were cold stored at ± 0°C and 90-95% RH for 4 weeks. Samples were taken at 0, 1, 2, 3, 4 weeks after cold storage and the following of the observing modifications in both chemical and physical aspects attributes of grape fruits were assessed as following:

Fruit physical properties

- Weight loss (%) per box was established using the formula (weight loss X 100 / the initial weight of box).

- Decay (%) per box was established using the formula (weight of decayed X 100 / the initial weight of box).

- Shattering (%) per box was established using the formula (weight of the shattered berries X 100 / the initial weight of box).

- Berry firmness (g/cm^2) was calculated during the use of texture analyzer instrument utilizing a punctured Cylinder of 1mm of diameter to a constant space 1 mm into the fruit skin by a constant speed 2mm per sec. via the peak of resisting strength of the outer layer.

Fruit chemical properties

- Total soluble solids (TSS %) has been measured using a hand refractometer.
- Total acidity as tartaric acid (%)has been predestined (**A.O.A.C., 1995**).
- Total sugars (%) were predestined according to **Sadasivam and Manickam (2004)**.

Experimental design and statistical analysis

A completely randomized design was performed for the experiment. Data were statistically analyzed in accordance to **Snedecor and Cochran (1980)**. Averages were compared using the new LSD values at 5% level (**Steel and Torrie, 1980**).

RESULTS AND DISCUSSION

1- Vegetative growth parameters

The data in Table 3 showed that, in both seasons, the application of effective microorganisms (EM), either alone or in combination with all doses of ascorbic acid, significantly improved all growth parameters, including shoot length, shoot diameter, number of leaves /shoot, and leaf area. The foliar spray with ascorbic acid at high dose (600ppm) was significantly superior than the use of other doses (400 or 200ppm).

Highest significant all vegetative growth characteristics were obtained by applying effective micro-organisms (EM) at 20cm^3 /vine plus ascorbic acid at 600 ppm, whereas the least values of these ones was attributed to the control in two seasons.

The enhancement of vine growth as a result of the use of active microorganisms (EM) may be attributed to their profound effect in the production of substances that regulate plant growth or increase the soil's availability and uptake of nutrients including Mg, k, N, and Fe, which participate in the structure of chlorophyll and improve vine growth **(Martin *et al.*, 1989).**

The beneficial impact of ascorbic acid on vegetative growth parameters per shoot could be due to that ascorbic acid has numerous growth-stimulating impacts on a variety of plants and trigger physiological operations such cell division, elongation, and respiration, which significantly affect shoot length and leaf area. **(Blokhina *et al.*, 2003).**

The obtained data are in agreement with the findings of **Sabry *et al.* (2009)** on Red Globe grape**, Abd El-Hameed *et al.* (2010) and Ahmed *et al.* (2011)** on table grape**, Abd El-Aal *et al.* (2013)** on Superior grape, **El-Mogy (2017)** on Crimson Seedless grape and **Ahmed (2022)** on Ruby Seedless grape; they reported that vegetative parameters were improved by EM application. Regarding to antioxidants, **Belal (2015)** on King Ruby grape and **El-kenawy (2017)** on Thompson Seedless grape; they demonstrated the efficacy of antioxidants in improving vegetative growth parameters.

Table (3). Influence of effective micro-organisms (EM) and ascorbic acid on vegetative growth parameters of Flame Seedless grapevines in 2021 and 2022 seasons

Measurements / Treatments	Shoot length (cm)		Shoot diameter (cm)		Number of leaves/Shoot		Leaf area (cm^2)	
	2021	2022	2021	2022	2021	2022	2021	2022
Control	148.6	151.6	1.16	1.18	22.9	23.2	133.6	140.6
Effective micro-organisms (EM)	161.4	166.8	1.24	1.27	25.8	25.6	144.7	149.2
Ascorbic acid at 200 ppm	150.3	155.3	1.17	1.19	23.2	23.8	136.8	142.1
Ascorbic acid at 400 ppm	154.1	159.2	1.19	1.21	23.7	24.1	136.1	143.4
Ascorbic acid at 600 ppm	160.2	164.4	1.20	1.24	24.6	25.4	141.9	146.7
EM + Ascorbic acid at 200 ppm	168.2	174.3	1.29	1.31	26.0	27.1	149.2	155.3
EM + Ascorbic acid at 400 ppm	169.6	176.6	1.32	1.34	26.2	27.4	152.3	156.1
EM + Ascorbic acid at 600 ppm	173.3	180.1	1.35	1.32	26.8	28.0	153.7	157.4
New L.S.D at 5%	4.7	4.2	0.02	0.01	0.7	0.6	3.4	3.3

2- Leaf content of total chlorophyll and mineral elements

Referring to Table (4), it is mentioned that the leaf content of total chlorophyll, total nitrogen, phosphors and potassium elements were significantly influenced by application of effective micro-organisms (EM) either solely or in combined with all different doses of ascorbic acid as compared to control in both seasons. The foliar spray with ascorbic acid at high dose (600ppm) had the best results as compared to the other doses (400 or 200ppm).

The combined application of effective micro-organisms (EM) at 20cm^3 /vine plus ascorbic acid at 600 ppm significantly on record the greatest values of these estimations. Contrarily, control in two seasons had lowest levels of these. A more effective absorption of nutrients is produced by the positive effective micro-organisms (EM), which increases nutrient availability and changes the morphology or physiology of root growth through the hormonal exudates of bio-fertilizer bacteria **(Mohamed *et al.*, 2007).** Moreover, antioxidants may have a good effect on root growth, which in turn increases nitrate absorption, which accounts for the improved in N, P, and K levels. Additionally, it could be influencing metabolic and physiological systems **(Fayed, 2010).**

These outcomes are consistent with those mentioned by **Sabry *et al.* (2009)** on Red Globe grape**, Abd El-Hameed *et al.* (2010) and Ahmed *et al.* (2011)** on Thompson grape**, Abd El-Aal *et al.* (2013)** on Superior grape, **El-Mogy (2017)** on Crimson Seedless grape and **Ahmed (2022)** on Ruby Seedless grape; they showed that leaf content of mineral nutrition and chlorophyll were improved by EM application.

On the other hand, **Belal (2015)** on King Ruby grape and **El-kenawy (2017)** on Thompson Seedless grape; they demonstrated the efficacy of antioxidants in improving vine nutrition status.

Table (4). Influence of effective micro-organisms (EM) and ascorbic acid on leaf content of total chlorophyll and mineral elements of Flame Seedless grapevines in 2021and 2022 seasons

Measurements / Treatments	Total chlorophyll (mg/g F.W)		Total nitrogen (%)		Phosphors (%)		Potassium (%)	
	2021	2022	2021	2022	2021	2022	2021	2022
Control	13.96	15.07	1.93	2.01	0.20	0.24	1.44	1.61
Effective micro-organisms (EM)	14.97	16.11	2.67	2.79	0.28	0.31	1.74	1.87
Ascorbic acid at 200 ppm	14.23	15.40	2.07	2.17	0.22	0.26	1.51	1.65
Ascorbic acid at 400 ppm	14.48	15.65	2.25	2.33	0.25	0. 26	1.56	1.72
Ascorbic acid at 600 ppm	14.85	16.02	2.38	2.48	0.26	0.27	1.65	1.83
EM + Ascorbic acid at 200 ppm	15.39	16.40	2.78	2.92	0.29	0.33	1.77	1.89
EM + Ascorbic acid at 400 ppm	15.43	16.56	2.83	2.96	0.34	0.39	1.79	1.92
EM + Ascorbic acid at 600 ppm	15.71	16.82	2.88	3.02	0.36	0.41	1.86	2.01
New L.S.D at 5%	0.13	0.11	0.08	0.05	0.02	0.01	0.07	0.05

3- Yield and physical properties of clusters and berries

Findings in table (5) showed that, the application of effective microorganisms (EM), either alone or in combination with all doses of ascorbic acid significantly increased yield per vine, cluster weight, weight of berry, average berry size, and average berry firmness when compared to control. The foliar spray with ascorbic acid at high dose (600ppm) was significantly superior than the use of other doses (400 or 200ppm). Highest significant yield and physical properties of clusters and berries were obtained by applying effective micro-organisms (EM) at 20cm^3 /vine plus ascorbic acid at 600 ppm, whereas the least values of these ones was attributed to the control in both seasons.

Effective micro-organisms (EM) bio-stimulants mainly consist of more than 80 different types of micro-organisms that are able to decompose organic matter, and beneficial micro-organisms are considered an important bio-fertilizer which leads to increase yield and enhance the quality of clusters and berries **(Olle and Williams, 2013).** The auxin impact of ascorbic acid on increasing cell enlargement via cell division, which positively reflected on growth parameters and reflected on the yield and quality of clusters and berries, could be responsible for the favourable response of antioxidants on the crop and quality of clusters. **(Omar, 1999).**

The outcomes obtained are consistent with those of **Sabry *et al.* (2009)** on Red Globe grape**, Abd El-Hameed *et al.* (2010), Ahmed *et al.* (2011)** on Thompson grape**, Abd El-Aal *et al.* (2013)** on Superior grape, **El-Mogy (2017)** on Crimson Seedless grape and **Ahmed (2022)** on Ruby Seedless grape; they found that yield and its attributes were improved by EM application. On the other hand, **Belal (2015)** on King Ruby grape and **El-kenawy (2017)** on Thompson Seedless grape; they demonstrated the efficacy of antioxidants in improving yield and fruit physical characteristics.

Table (5). Influence of effective micro-organisms (EM) and ascorbic acid on yield and physical properties of clusters and berries of Flame seedless grapevines in 2021 and 2022 seasons

Measurements / Treatments	Yield/vine (Kg)		Average cluster weight (g)		Average berry weight (g)		Average berry size (cm^3)		Average berry firmness (g/cm^2)	
	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
Control	12.28	12.83	472.5	493.6	3.07	3.15	2.92	2.97	41.3	42.7
Effective micro-organisms (EM)	13.60	14.25	523.3	548.4	3.36	3. 47	3.21	3.28	46.2	47.6
Ascorbic acid at 200 ppm	12.60	13.06	484.7	502.5	3.12	3.21	2.97	3.03	43.8	44.5
Ascorbic acid at 400 ppm	12.81	13.27	492.4	510.6	3.17	3.27	2.99	3.06	44.9	45.2
Ascorbic acid at 600 ppm	13.16	13.69	506.3	526.6	3.24	3.33	3.08	3.17	45.1	46.8
EM + Ascorbic acid at 200 ppm	14.28	14.93	549.1	574.3	3.48	3.60	3.28	3.34	47.4	48.3
EM + Ascorbic acid at 400 ppm	14.64	15.38	563.3	591.6	3.52	3.63	3.33	3.39	48.1	48.9
EM + Ascorbic acid at 600 ppm	15.08	15.86	580.2	610.1	3.61	3.73	3.39	3.45	48.9	49.4
New L.S.D at 5%	0.35	0.38	13.5	14.8	0.08	0.09	0.07	0.06	0.7	0.4

4- Chemical properties of berries

Referring to Table (6), it is mentioned that all chemical properties of berries including total soluble solids (TSS), total acidity, total sugars and berry skin content of total anthocyanin were significantly influenced by application of effective micro-organisms (EM) either solely or in combined with all different doses of ascorbic acid as compared to control in two seasons. The foliar spray with ascorbic acid at high dose (600ppm) had the best results as compared to the other doses (400 or 200ppm). The combined application of effective micro-organisms (EM) at 20cm^3 /vine plus ascorbic acid at 600 ppm significantly achieved the greatest values of TSS, TSS/acid ratio and anthocyanin of berry skin as well as the least values acidity of fruit juice, whereas, control had the least values of TSS, TSS/acid ratio and anthocyanin of berry skin as well as the greatest values acidity of berry juice in both seasons. The encouraging impact of EM on the availability of and soil fertility of the majority of nutrients may lead to increased growth, which undoubtedly reflects on the quality of the berries **(Sabry *et al.* 2009)**.

Regarding ascorbic acid benefits, they might be brought on by how it enhances chlorophyll, which was reflected in the photosynthesis operation and thus led to the improvement of the chemical properties of the berries **(Belal, 2015).**

All of these results correspond with those reported by **El-Mogy (2017)** on Crimson Seedless grape and **Ahmed (2022)** on Ruby Seedless grape; they found that SSC, total sugars and total anthocyanin in the fruit skin were improved by EM application. On the other hand, **Belal (2015)** on King Ruby grape and **El-kenawy (2017)** on Thompson Seedless grape; they demonstrated the efficacy of antioxidants in improving berry chemical properties.

Table (6). Influence of effective micro-organisms (EM) and ascorbic acid on berry chemical properties of Flame Seedless grapevines in 2021 and 2022 seasons

Treatments \ Measurements	TSS (%)		Total acidity (%)		Total sugars (%)		Total anthocyanin (mg/100g F.W)	
	2021	2022	2021	2022	2021	2022	2021	2022
Control	16.14	16.31	0.66	0.62	13.44	13.83	35.31	36.23
Effective micro-organisms (EM)	16.42	16.54	0.63	0.60	13.74	14.26	37.42	38.35
Ascorbic acid at 200 ppm	16.21	16.42	0.65	0.61	13.52	13.91	35.83	36.82
Ascorbic acid at 400 ppm	16.23	16.51	0.64	0.61	13.63	14.12	36.34	37.23
Ascorbic acid at 600 ppm	16.32	16.53	0.64	0.60	13.71	14.13	36.63	37.61
EM + Ascorbic acid at 200 ppm	16.51	16.62	0.63	0.59	13.82	14.31	36.82	38.63
EM + Ascorbic acid at 400 ppm	16.53	16.74	0.62	0.59	13.91	14.34	37.16	38.84
EM + Ascorbic acid at 600 ppm	16.54	16.91	0.60	0.58	14.13	14.42	37.83	39.41
New L.S.D at 5%	0.21	0.17	0.02	0.01	0.26	0.19	0.64	0.53

5- Fruit quality assessments during cold storage conditions

Fruit physical properties

Weight loss

The data in Table (7) showed that fruit weight loss percentage gradually increased with the prolongation of the cold storage period for all treatments in both seasons. It can be seen that the use of EM either individual or in conjunction with all the different doses of ascorbic acid significantly reduced the fruit weight loss than the control under the cold storage conditions in two seasons. The least significant percentage of fruit weight loss was obtained by applying effective microorganisms (EM) at 20 cm3/vine plus ascorbic acid at 600 ppm, while the greatest values of this one was significantly attained by control after four weeks in two seasons of cold storage. The increase in the fruit weight loss during cold storage possibly as a result of respiration and perspiration (**Wolucka *et al*., 2005**).

The findings obtained are comparable to those realised by **Hassan & Imam (2015)** on strawberry fruits, **Kilic *et al.* (2015)** on kiwi fruits and **Aboryia (2020)** on Mandarin fruits; they mentioned that application with effective microorganisms (EM) greatly decreased the rise in the weight loss percentage compared to control during cold storage.

As for the effect of ascorbic acid, **Al-Obeed (2011)** on Flame table grapes and **Abd El-Wahab *et al.* (2020)** on Superior Seedless grapes reported that foliar spraying with ascorbic acid significantly decreased the rise in the weight loss percentage compared to control during cold storage.

Decay

As evident in Table (8), a significant discernible steady rise in fruit decay percentage was observed with prolongation of the cold storage period for all treatments in two seasons. It can be observed that the application of EM either solo or in combination with all the various dosages of ascorbic acid leading to a significant decrease in the percent of fruit decay compared to control under cold storage conditions in both seasons. Control fruits exhibited the highest significant fruit decay percentage after four weeks of cold storage. On the other hand, the combined application of effective micro-organisms (EM) at 20cm^3 /vine plus ascorbic acid at 600 ppm significantly achieved the least fruit decay percentage after four weeks of the cold storage in two seasons. Decay is among the most crucial postharvest factors in reduction of quality horticultural crops. Fruits are affected by post-harvest diseases and disturbances due to physiological changes brought on by ageing during storage (**Prusky & Keen, 1993**).

These findings are consistent with those obtained by **Hassan & Imam (2015)** on strawberry fruits, **Kilic *et al.* (2015)** on kiwi fruits and **Aboryia (2020)** on Mandarin fruits; they found that application with effective microorganisms (EM) significantly decreased the rise in the decay percentage compared to control during cold storage. Regarding the effect of ascorbic acid, **Al-Obeed (2011)** on Flame table grapes and **Abd El-Wahab *et al.* (2020)** on Superior Seedless grapes reported foliar spraying with ascorbic acid significantly low the rise in decay percentage during cold storage compared to control.

Table (7). Influence of effective micro-organisms (EM) and ascorbic acid on weight loss (%) of Flame Seedless grapes during cold storage in 2021 and 2022 seasons

Treatment (T) \ Date (D)	2021, season					
	Storage period (week)					
	0	1	2	3	4	Means (T)
Control	0.00	1.07	1.45	2.38	6.13	2.21
Effective micro-organisms (EM)	0.00	0.98	1.30	2.16	5.87	2.06
Ascorbic acid at 200 ppm	0.00	1.03	1.39	2.31	6.01	2.15
Ascorbic acid at 400 ppm	0.00	1.01	1.35	2.25	5.95	2.11
Ascorbic acid at 600 ppm	0.00	1.00	1.32	2.19	5.92	2.09
EM + Ascorbic acid at 200 ppm	0.00	0.95	1.26	2.11	5.81	2.03
EM + Ascorbic acid at 400 ppm	0.00	0.93	1.23	2.09	5.78	2.01
EM + Ascorbic acid at 600 ppm	0.00	0.90	1.20	2.03	5.67	1.96
Means (D)	0.00	0.98	1.31	2.19	5.89	
New LSD at 5% (T) =	0.07					
New LSD at 5% (D) =	0.05					
New LSD at 5% (TXD) =	0.16					
Treatment (T) \ Date (D)	**2022, season**					
	Storage period (week)					
	0	**1**	**2**	**3**	**4**	**Means (T)**
Control	0.00	1.17	1.6	2.68	7.07	2.50
Effective micro-organisms (EM)	0.00	1.03	1.39	2.38	6.77	2.31
Ascorbic acid at 200 ppm	0.00	1.12	1.51	2.57	6.95	2.43
Ascorbic acid at 400 ppm	0.00	1.09	1.46	2.5	6.89	2.39
Ascorbic acid at 600 ppm	0.00	1.07	1.43	2.44	6.84	2.36
EM + Ascorbic acid at 200 ppm	0.00	1.01	1.36	2.35	6.71	2.29
EM + Ascorbic acid at 400 ppm	0.00	0.97	1.32	2.31	6.66	2.25
EM + Ascorbic acid at 600 ppm	0.00	0.94	1.27	2.24	6.63	2.22
Means (D)	0.00	1.05	1.42	2.43	6.82	
New LSD at 5% (T) =	0.04					
New LSD at 5% (D) =	0.03					
New LSD at 5% (TXD) =	0.09					

Table (8). Influence of effective micro-organisms (EM) and ascorbic acid on decay (%) of Flame Seedless grapes during cold storage in 2021 and 2022 seasons

Date (D) / Treatment (T)	2021, season					
	Storage period (week)					
	0	1	2	3	4	Means (T)
Control	0.00	0.12	0.17	0.26	0.61	0.23
Effective micro-organisms (EM)	0.00	0.08	0.12	0.19	0.53	0.18
Ascorbic acid at 200 ppm	0.00	0.11	0.14	0.25	0.58	0.22
Ascorbic acid at 400 ppm	0.00	0.09	0.13	0.23	0.58	0.21
Ascorbic acid at 600 ppm	0.00	0.09	0.12	0.20	0.55	0.19
EM + Ascorbic acid at 200 ppm	0.00	0.07	0.11	0.17	0.50	0.17
EM + Ascorbic acid at 400 ppm	0.00	0.07	0.09	0.16	0.49	0.16
EM + Ascorbic acid at 600 ppm	0.00	0.05	0.08	0.13	0.45	0.14
Means (D)	0.00	0.09	0.12	0.20	0.54	
New LSD at 5% (T) =	0.06					
New LSD at 5% (D) =	0.05					
New LSD at 5% (TXD) =	0.14					
Date (D) / Treatment (T)	**2022, season**					
	Storage period (week)					
	0	**1**	**2**	**3**	**4**	**Means (T)**
Control	0.00	0.17	0.23	0.31	0.68	0.28
Effective micro-organisms (EM)	0.00	0.12	0.18	0.24	0.61	0.23
Ascorbic acid at 200 ppm	0.00	0.15	0.21	0.28	0.66	0.26
Ascorbic acid at 400 ppm	0.00	0.14	0.19	0.28	0.63	0.25
Ascorbic acid at 600 ppm	0.00	0.12	0.19	0.25	0.63	0.24
EM + Ascorbic acid at 200 ppm	0.00	0.10	0.16	0.22	0.59	0.21
EM + Ascorbic acid at 400 ppm	0.00	0.09	0.13	0.19	0.56	0.19
EM + Ascorbic acid at 600 ppm	0.00	0.07	0.12	0.16	0.55	0.18
Means (D)	0.00	0.12	0.18	0.24	0.61	
New LSD at 5% (T) =	0.05					
New LSD at 5% (D) =	0.04					
New LSD at 5% (TXD) =	0.11					

Shattering

The data in Table (9) demonstrated that, for all treatments in both seasons, the shattering percentage increased gradually with the extension of the cold storage period. It can be seen that the use of EM either single or in combination with all the various dosages of ascorbic acid resulting in a significant decrease in the percentage of shattering compared to control under cold storage conditions in both seasons. The least significant percentage of shattering was obtained by applying effective microorganisms (EM) at 20 cm3/vine plus ascorbic acid at 600 ppm, while the greatest values of this one was significantly attained by control after 28 days of cold storage in two seasons. These findings are consistent with those acquired by **Al-Obeed (2011)** on Flame grapes and **Abd El-Wahab *et al.* (2020)** on Superior Seedless grapes mentioned that foliar spraying with ascorbic acid significantly resulted in the highest berry adherence strength compared to control during cold storage.

Firmness

As shown in Table (10), a significant gradual reduce in the fruit firmness was along with prolongation of the cold storage period for all treatments in two seasons. It can be observed that the treatment of EM either single or in combination with all the various dosages of ascorbic acid resulted in a significant improve in the fruit firmness compared with control under cold storage conditions in both seasons. Control fruits exhibited the least significant fruit firmness after four weeks of cold storage. On the other hand, the combined application of effective micro-organisms (EM) at 20 cm^3 /vine plus ascorbic acid at 600 ppm significantly achieved the highest fruit firmness after 28 days of cold storage in two seasons.

Fruit ripening causes loss of firmness throughout the course of storage because it increases the activity of cell wall hydrolysis enzymes like pectinesterase, polygalacturonase, pectin methylesterase, via pectatelyases during harvest and cold storage. (**Ali *et al.*, 2004**). The maintenance of firmness in the fruits applied with effective micro-organisms (EM) and ascorbic acid during storage is due to their influence in inhibiting the activity of enzymes that break down pectin, which can cause the fruit to soften and deteriorate (**Kilic *et al.*, 2015 and Abd El-Wahab *et al.*, 2020)**. Findings were obtained that are comparable to those achieved by **Hassan and Imam (2015)** on strawberry fruits and **Kilic *et al.* (2015)** on kiwi fruits; they found that application with effective microorganisms (EM) significantly decreased the low in the fruit firmness during cold storage. As for the effect of ascorbic acid, are supported by **Al-Obeed (2011)** on Flame Seedless grapes and **Abd El-Wahab *et al.* (2020)** on Superior Seedless grapes, ascorbic acid foliar spraying considerably slowed the loss of fruit firmness during cold storage compared to control.

Table (9). Influence of effective micro-organisms (EM) and ascorbic acid on shattering (%) of Flame Seedless grapes during cold storage in 2021 and 2022 seasons

Date (D) / Treatment (T)	2021, season					
	Storage period (week)					
	0	**1**	**2**	**3**	**4**	**Means (T)**
Control	0.00	3.32	3.82	4.59	5.67	3.48
Effective micro-organisms (EM)	0.00	0.97	1.65	1.56	2.05	1.25
Ascorbic acid at 200 ppm	0.00	1.24	1.83	1.91	2.47	1.49
Ascorbic acid at 400 ppm	0.00	1.17	1.77	1.81	2.36	1.42
Ascorbic acid at 600 ppm	0.00	1.11	1.73	1.74	2.27	1.37
EM + Ascorbic acid at 200 ppm	0.00	0.91	1.53	1.48	1.97	1.18
EM + Ascorbic acid at 400 ppm	0.00	0.82	1.45	1.37	1.88	1.10
EM + Ascorbic acid at 600 ppm	0.00	0.72	1.37	1.24	1.76	1.02
Means (D)	0.00	1.28	1.89	1.96	2.55	
New LSD at 5% (T) =	0.11					
New LSD at 5% (D) =	0.08					
New LSD at 5% (TXD) =	0.25					
Date (D) / Treatment (T)	**2022, season**					
	Storage period (week)					
	0	**1**	**2**	**3**	**4**	**Means (T)**
Control	0.00	4.76	5.21	5.32	6.49	4.36
Effective micro-organisms (EM)	0.00	1.74	1.98	2.01	2.56	1.66
Ascorbic acid at 200 ppm	0.00	2.04	2.37	2.35	2.98	1.95
Ascorbic acid at 400 ppm	0.00	1.96	2.22	2.26	2.87	1.86
Ascorbic acid at 600 ppm	0.00	1.90	2.16	2.19	2.79	1.81
EM + Ascorbic acid at 200 ppm	0.00	1.67	1.90	1.94	2.44	1.59
EM + Ascorbic acid at 400 ppm	0.00	1.58	1.81	1.84	2.38	1.52
EM + Ascorbic acid at 600 ppm	0.00	1.46	1.74	1.71	2.23	1.43
Means (D)	0.00	2.14	2.42	2.45	3.09	
New LSD at 5% (T) =	0.08					
New LSD at 5% (D) =	0.06					
New LSD at 5% (TXD) =	0.18					

Table (10). Influence of effective micro-organisms (EM) and ascorbic acid on berry firmness (g/cm^2) of Flame Seedless grapes during cold storage in 2021 and 2022 seasons

Treatment (T) / Date (D)	2021, season					
	Storage period (week)					
	0	**1**	**2**	**3**	**4**	**Means (T)**
Control	41.3	36.1	33.7	29.3	26.9	33.5
Effective micro-organisms (EM)	46.2	42.0	39.2	35.1	33.0	39.1
Ascorbic acid at 200 ppm	43.8	40.6	37.9	33.7	29.2	37.1
Ascorbic acid at 400 ppm	44.9	40.9	38.2	34.2	31.6	38.0
Ascorbic acid at 600 ppm	45.1	41.2	38.8	34.6	32.3	38.4
EM + Ascorbic acid at 200 ppm	47.4	42.3	39.9	35.7	33.4	39.8
EM + Ascorbic acid at 400 ppm	48.1	42.7	40.2	36.2	34.3	40.3
EM + Ascorbic acid at 600 ppm	48.9	43.3	40.7	36.5	34.8	40.9
Means (D)	45.7	41.2	38.6	34.5	32.0	
New LSD at 5% (T) =	3.2					
New LSD at 5% (D) =	2.4					
New LSD at 5% (TXD) =	7.2					
Treatment (T) / Date (D)	**2022, season**					
	Storage period (week)					
	0	**1**	**2**	**3**	**4**	**Means (T)**
Control	42.7	34.9	33.0	28.4	26.2	33.0
Effective micro-organisms (EM)	47.6	40.5	37.9	34.0	32.0	38.4
Ascorbic acid at 200 ppm	44.5	39.2	36.6	32.6	28.4	36.3
Ascorbic acid at 400 ppm	45.2	39.5	37.7	33.1	30.7	37.2
Ascorbic acid at 600 ppm	46.8	39.8	37.5	33.8	31.3	37.8
EM + Ascorbic acid at 200 ppm	48.3	40.8	38.6	34.6	32.4	38.9
EM + Ascorbic acid at 400 ppm	48.9	41.3	38.5	35.4	33.2	39.5
EM + Ascorbic acid at 600 ppm	49.4	41.8	39.3	35.3	33.9	39.9
Means (D)	46.7	39.7	37.4	33.4	31.0	
New LSD at 5% (T) =	2.4					
New LSD at 5% (D) =	1.8					
New LSD at 5% (TXD) =	5.4					

Fruit chemical properties

Total soluble solids (TSS)

The data in Table (11) cleared that total soluble solids percentage gradually rises with the extension of the cold storage period for all treatments in two seasons. It can be seen that the use of EM either single or in combination with all the various dosages of ascorbic acid resulted in a significant decrease in percentage of TSS compared to the control under cold storage conditions in both seasons. The lowest significant percentage of total soluble solids was obtained by applying effective microorganisms (EM) at 20 cm3/vine plus ascorbic acid at 600 ppm, while the greatest values of this one was significantly attained by control after four weeks in two seasons of cold storage. The rise in the percentage of dry matter owing to respiration and metabolic activity as well as the loss of humidity from the fruit during transpiration may be the cause of the greatest fruit TSS with increasing storage time. (**Nandaniya *et al.*, 2017**).

These findings are in agree with those achieved by **Aboryia (2020)** on Mandarin fruits who found that application with effective microorganisms (EM) significantly decreased the rise in the TSS percentage compared to untreated during cold storage. Regarding the effect of ascorbic acid, **Al-Obeed (2011)** on Flame grapes and **Abd El-Wahab *et al.* (2020)** on Superior Seedless grapes reported foliar spraying with ascorbic acid significantly decreased the rise in TSS percentage compared with control during cold storage.

Total acidity

As shown in Table (12), a significant gradual decrease in the total acidity was noticed with the prolongation of the cold storage period for all treatments in both seasons. It can be observed that the application of EM either single or in combination with all the various dosages of ascorbic acid resulted in a significant rise in total acidity compared with the control under cold storage conditions in both seasons. Control fruits exhibited the least significant total acidity after four weeks of cold storage. On the other hand, the combined application of effective micro-organisms (EM) at 20cm3 /vine plus ascorbic acid at 600 ppm significantly achieved the highest total acidity after 28 days of cold storage in two seasons. Fruit acidity gradually decreases when being stored at low temperatures, which may be caused by through the oxidation of acids inside sugars and the quick utilization of organic acids during respiration. (**Chulaki *et al.*, 2017**).

These findings are in agree with those achieved by **Hassan & Imam (2015)** on strawberry fruits and **Aboryia (2020)** on Mandarin fruits; they found that application with effective microorganisms (EM) significantly proved the fruit acidity compared to control during cold storage. As for the effect of ascorbic acid, **Al-Obeed (2011)** on Flame grapes and **Abd El-Wahab *et al.* (2020)** on Superior Seedless grapes mentioned that foliar spraying with ascorbic acid significantly enhancement the fruit acidity compared to control during cold storage.

Table (11). Influence of effective micro-organisms (EM) and ascorbic acid on TSS (%) of Flame Seedless grapes during cold storage in 2021 and 2022 seasons

Date (D) / Treatment (T)	2021, season					
	Storage period (week)					
	0	**1**	**2**	**3**	**4**	**Means (T)**
Control	16.14	16.94	17.53	18.01	18.59	17.44
Effective micro-organisms (EM)	16.42	16.67	17.08	17.63	18.16	17.19
Ascorbic acid at 200 ppm	16.21	16.93	17.46	17.97	18.41	17.40
Ascorbic acid at 400 ppm	16.23	16.84	17.37	17.89	18.32	17.33
Ascorbic acid at 600 ppm	16.32	16.81	17.21	17.74	18.21	17.26
EM + Ascorbic acid at 200 ppm	16.51	16.74	17.01	17.58	18.07	17.18
EM + Ascorbic acid at 400 ppm	16.53	16.62	16.94	17.42	17.94	17.09
EM + Ascorbic acid at 600 ppm	16.54	16.59	16.87	17.31	17.85	17.03
Means (D)	16.36	16.77	17.18	17.69	18.19	
New LSD at 5% (T) =	0.25					
New LSD at 5% (D) =	0.19					
New LSD at 5% (TXD) =	0.56					
Date (D) / Treatment (T)	**2022, season**					
	Storage period (week)					
	0	**1**	**2**	**3**	**4**	**Means (T)**
Control	16.31	17.23	17.86	18.38	18.97	17.75
Effective micro-organisms (EM)	16.54	16.83	17.29	17.84	18.31	17.36
Ascorbic acid at 200 ppm	16.42	17.18	17.75	18.21	18.74	17.66
Ascorbic acid at 400 ppm	16.51	17.09	17.66	18.07	18.51	17.57
Ascorbic acid at 600 ppm	16.53	16.94	17.41	17.98	18.42	17.46
EM + Ascorbic acid at 200 ppm	16.62	16.79	17.13	17.69	18.24	17.29
EM + Ascorbic acid at 400 ppm	16.74	16.77	17.05	17.53	18.11	17.24
EM + Ascorbic acid at 600 ppm	16.91	16.83	16.92	17.39	18.02	17.21
Means (D)	16.57	16.96	17.38	17.89	18.42	
New LSD at 5% (T) =	0.21					
New LSD at 5% (D) =	0.16					
New LSD at 5% (TXD) =	0.47					

Table (12). Influence of effective micro-organisms (EM) and ascorbic acid on acidity (%) of Flame Seedless grapes during cold storage in 2021 and 2022 seasons

Treatment (T) / Date (D)	2021, season					
	Storage period (week)					
	0	1	2	3	4	Means (T)
Control	0.66	0.59	0.51	0.47	0.42	0.53
Effective micro-organisms (EM)	0.63	0.62	0.53	0.50	0.49	0.55
Ascorbic acid at 200 ppm	0.65	0.59	0.50	0.48	0.46	0.54
Ascorbic acid at 400 ppm	0.64	0.60	0.51	0.48	0.47	0.54
Ascorbic acid at 600 ppm	0.64	0.61	0.52	0.49	0.48	0.55
EM + Ascorbic acid at 200 ppm	0.63	0.62	0.54	0.51	0.50	0.56
EM + Ascorbic acid at 400 ppm	0.62	0.60	0.55	0.54	0.52	0.57
EM + Ascorbic acid at 600 ppm	0.60	0.59	0.59	0.57	0.56	0.58
Means (D)	0.63	0.60	0.53	0.51	0.49	
New LSD at 5% (T) =	0.03					
New LSD at 5% (D) =	0.02					
New LSD at 5% (TXD) =	0.07					

Treatment (T) / Date (D)	2022, season					
	Storage period (week)					
	0	1	2	3	4	Means (T)
Control	0.62	0.55	0.49	0.44	0.41	0.50
Effective micro-organisms (EM)	0.60	0.57	0.52	0.51	0.49	0.54
Ascorbic acid at 200 ppm	0.61	0.54	0.50	0.48	0.47	0.52
Ascorbic acid at 400 ppm	0.61	0.55	0.50	0.49	0.47	0.52
Ascorbic acid at 600 ppm	0.60	0.56	0.51	0.50	0.48	0.53
EM + Ascorbic acid at 200 ppm	0.59	0.58	0.53	0.52	0.50	0.54
EM + Ascorbic acid at 400 ppm	0.59	0.59	0.56	0.54	0.51	0.56
EM + Ascorbic acid at 600 ppm	0.58	0.63	0.59	0.58	0.53	0.58
Means (D)	0.60	0.57	0.53	0.51	0.48	
New LSD at 5% (T) =	0.02					
New LSD at 5% (D) =	0.02					
New LSD at 5% (TXD) =	0.05					

Total sugars

The data in Table (13) showed that total sugars percentage gradually increased during the cold storage period for all application in both seasons. It can be seen that the use of EM either single or in combination with all the various dosages of ascorbic acid resulted in a significant decrease in the percentage of total sugars compared to the untreated under cold storage conditions in both seasons. The lowest significant percentage of total sugars was obtained by applying effective microorganisms (EM) at 20 cm3/vine plus ascorbic acid at 600 ppm, while the highest values of this one was significantly attained by control after 28 days of cold storage in two seasons.

The findings align with those discovered by **Al-Obeed (2011)** on table grapes and **Abd El-Wahab *et al.* (2020)** on Superior grapes mentioned that foliar spraying with ascorbic acid significantly low the rise in the total sugars percentage compared with control during cold storage.

Table (13). Influence of effective micro-organisms (EM) and ascorbic acid on total sugars (%) of Flame Seedless grapes during cold storage in 2021 and 2022 seasons

Treatment (T) \ Date (D)	2021, season					
	Storage period (week)					
	0	1	2	3	4	Means (T)
Control	13.44	14.67	14.82	15.36	15.76	14.81
Effective micro-organisms (EM)	13.74	14.41	14.48	14.85	15.21	14.54
Ascorbic acid at 200 ppm	13.52	14.53	14.69	15.17	15.53	14.69
Ascorbic acid at 400 ppm	13.63	14.49	14.57	15.03	15.41	14.63
Ascorbic acid at 600 ppm	13.71	14.43	14.52	14.91	15.37	14.59
EM + Ascorbic acid at 200 ppm	13.82	14.37	14.43	14.72	15.19	14.51
EM + Ascorbic acid at 400 ppm	13.91	14.23	14.31	14.67	15.01	14.43
EM + Ascorbic acid at 600 ppm	14.13	14.19	14.24	14.43	14.96	14.39
Means (D)	13.74	14.42	14.51	14.89	15.31	
New LSD at 5% (T) =	0.19					
New LSD at 5% (D) =	0.14					
New LSD at 5% (TXD) =	0.43					
Treatment (T) \ Date (D)	**2022, season**					
	Storage period (week)					
	0	**1**	**2**	**3**	**4**	**Means (T)**
Control	13.83	14.86	15.07	15.45	15.86	15.01
Effective micro-organisms (EM)	14.26	14.52	14.64	14.87	15.31	14.72
Ascorbic acid at 200 ppm	13.91	14.73	14.91	15.26	15.63	14.89
Ascorbic acid at 400 ppm	14.12	14.61	14.83	15.12	15.51	14.84
Ascorbic acid at 600 ppm	14.13	14.56	14.72	14.93	15.47	14.76
EM + Ascorbic acid at 200 ppm	14.31	14.47	14.51	14.81	15.29	14.68
EM + Ascorbic acid at 400 ppm	14.34	14.35	14.46	14.76	15.11	14.60
EM + Ascorbic acid at 600 ppm	14.42	14.28	14.39	14.52	15.06	14.53
Means (D)	14.17	14.55	14.69	14.97	15.41	
New LSD at 5% (T) =	0.16					
New LSD at 5% (D) =	0.12					
New LSD at 5% (TXD) =	0.36					

Conclusion

From the above results, soil inoculation of effective micro-organisms (EM) plus foliar spraying with ascorbic acid at 600 ppm had the superior results in terms of guarantee the superior vegetative growth aspects, improving yield and improving fruit quality attributes as well as enhancing the storability via decreasing the rate of deterioration of physical and chemical characteristics of grapes by decreasing physiological loss in weight, decay and shattering, maintaining firmness and delaying the changes in total soluble solids, total acidity and total sugars compared to control of Flame Seedless grapes during extended cold storage in both seasons.

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