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Response of Early Sweet and Thompson Seedless Grapevines cvs. to some Biofertilization Treatments

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Abstract: This investigation was carried out during 2010 and 2021 seasons on Early Sweet and Thompson Seedless grapevines *cvs.*, cultivated in sandy soil located at Malawi district - El-Minia Governorate, Egypt. The two examined *cvs.* were irrigated by using drip irrigation system. This study was focused on the response of Early Sweet and Thompson Seedless *cvs.* to inoculation with Arbuscular Mycrrohiza Fungi (AMF), Azospirillum bacteria and Azotobacter bacteria individually or in combination. The obtained shows that inoculated the Early Sweet and Thompson Seedless were very effective in improving the nutritional status, productivity and berry physical and chemical properties. The combined inoculations with any two micro-organisms were more effective than the individual inoculations. However, the best results were obtained when the vines received the three micro-organisms in combination. Then, it is strongly recommended to inoculating the Early Sweet and Thompson Seedless *cvs.* grown in sandy soil with AMF, Azospirillum and Azotobacter in combination three times yearly, to enhancing the nutritional status, productivity and improving berries physical and chemical properties.

Key words: Grapevines, Early Sweet, Thompson Seedless, grapevines, Mycrrohiza, Azospirillum, Azotobacter.

1. Introduction

The grapevine (*Vitis vinifera* L.) considered as one of the oldest fruit crops in the world, it is one of the major horticulture crops all over the world (**Doring *et al.*, 2015**). It has a great adaptability in wide range of climatic and soil conditions (**Delas, 2000; Sluys, 2006 and Ibrahim *et al.*, 2020**). It is growing in wide soil types, from sand to heavy clay, around the temperate bands between 20°C and 50°C Latitude, north or south of the Equator (**Srinivasan and Mullins, 2001**).

Egypt is one of the leading productions in Africa. However, El-Minia region is one of the leading rejoin in grapevines cultivation in Egypt, it's got dozens of cultivars, old or newly introduced (**Ibrahim, 2015**). Two white cultivars were chosen for achieving this study:

Early Sweet cultivar is seedless cultivar, introduced in Egypt recently, and it has been popular with the Egyptian consumer. It characterized by berry medium size, crispy pulp texture and good flavors (**Galet, 2000**). Thompson Seedless also called Banaty grapevines, it is one of the oldest popular cultivars in Egyptian, it characterized by medium size of berry, yellow color, the berry slightly tends, high sugars percintage. But it has a compacted cluster and weakness fruit skin (**Galet, 2000**).

Actually, the main interest of scientists is to find natural ways of improving fruit trees growth and productivity that led to environmentally friendly agriculture fertilizers. Biofertilizers have a great potential to achieve this aim, a little information about their application in grapevines growing in Egypt, especially in the Minia region. The current study was given highlighting the role of three micro-organisms (Extensively used as bio-fertilizers) namely: Mycrrohiza fungi (AMF), Azospirillum and Azotobacter bacteria, on the nutritional status and productivity of main cultivars of grapevines grown under sandy soil in North Upper Egypt (El-Minia Governorate).

2. Material and Methods

The current study was conducted during 2010 and 2021 seasons on two grapevines cultivars namely; Early Sweet and Thompson Seedless. The two cultivars were cultivated in well drained water sandy soil. The farm which this experiment achievement was located at beside the Cairo-Assiut Western desert rood in front of Malawi district El-Minia Governorate – Egypt. The two examined *cvs.* were irrigated by using drip irrigation system, all mineral fertilizers treatments were applied through the irrigation system.

2.1. Plant material

The selected cultivars were 8 years old at the start of experiment. The chosen vines were trained according to cane pruning system using Gable shape supporting system. Vine load was adjusted to 80 eyes per vine (8 fruiting cans × 8 eyes plus 8 renewal spurs × 2 eyes).

2.2. Soil and water analysis

A composite soil and irrigation water samples were collected and subjected to Physical and chemical analysis according to **Wilde *et al.* (1985)**.

Table (1). Analysis of orchard soil and irrigation water

Soil analysis		Water analysis	
Constituents	**Values**	**Constituents**	**Values**
Sand %	79.1	**E.C (mmhos/cm/25C)**	1.7
Silt %	11.1	**Hardness**	17.1
Clay %	9.8	**pH**	7.62
Texture	Sandy	**Ca (mg/L)**	42.1
EC (1:2.5 extract) mmhos/cm/ 25 C	2.8	**Mg (mg/L)**	22.3
Organic matter %	0.09	**K (mg/L)**	6.17
pH (1 : 2.5 extract)	8.42	**Na (mg/L)**	77.6
Active lime ($CaCO_3$ %)	9.13%	**Sum of Cations (mg/L)**	9.16
Total N %	0.09	**Alkalinity (mg/L)**	177
Available Phosphorus (ppm)	2.85	**Chlorides (mg/L)**	119
Available Ca (meq/100g)	20.1	**Nitrate (mg/L)**	11.0
Available Mg (meq/100g)	2.03	**Sulphates (mg/L)**	47.9
Available K (meq/100g)	0.57	**Sum of anions (mg/L)**	8.19
C/N Ratio	17.2	**SAR**	2.96

2.3. Preparation of the three micro-organisms strains

The Fungi and bacterial strains used in this study (*Arbuscular mycorrhizal* fungi "AMF", *Azospirillum brasilense* bacteria, *Azotobacter chrococcum* bacteria) were kindly isolated, purified and propagated in the Laboratory of Microbiology, Minia University, Egypt, according to the methods outlined by **Ranganayaki *et al.* (2006)**. The vines were inoculated three times yearly in terms of 200 ml for Azospirillum and Azotobacter bacteria or 200 g for AMF. Each 1 ml or 1 g contained 10^8 spores or cell of bacteria.

The three micro-organisms or their combinations were mixed with 1 kg of compost immediately before vines inoculation. The first dose was added during burst bud stage, the second one was added during full blooming stage and the third dose was added at one month later. Eight treatments were applied for each cultivar as followed: Control (un-inoculated vines), Inoculation with (AMF), Inoculation with Azospirillum bacteria, Inoculation with Azotobacter bacteria, Inoculation with AMF + Azospirillum, Inoculation with AMF + Azotobacter, Inoculation with Azospirillum + Azotobacter and Inoculation with AMF + Azospirillum + Azotobacter. Each treatment was replicated three times, one vine per each.

2.4. Determination of N, P, K and Mg in leaves

Eight leaves were picked (in the opposite of the basal cluster) for each vine was taken at the middle of April during the two experimental seasons. The petioles washed, dried at oven and grounded. Then, 0.5 g was digested by using H_2SO_4 and H_2O_2 until clear solution was obtained. Thereafter, contents of N. P, K and Mg for each sample were determined as follows: Nitrogen determined by the modified microkejldahl method (**Martin-Préval *et al.*, 1984**). Phosphorus determined by using colorimetric method (**Wild *et al.*, 1985**), by measuring the optical density of phosphor-molibdo-vanadate complex by Spectro-photometrically at wave length 430 nm. Potassium determined by using flam photometrically method (**Martin-Préval *et al.*, 1984**). While, the magnesium was determined by atomic absorption methods.

2.5. Yield and berry physicochemical properties

The yield per vine was recorded in terms of weight (kg) and number of clusters / vine. At harvesting time four clusters were taken random from the yield of each vine and using for determination the following physical and chemical parameters: Average cluster length (cm), average cluster width (cm), average cluster weight (g), average berry weight (g) by using 0.01 sensitivity balance, Berry dimensions (longitudinal and equatorial "cm") were measured by using vernier caliper, juice total soluble solids (TSS%) were achieved by using handy refractometer, percentage of total acidity by titration with 0.1 NaOH (according to **AOAC, 2000**), and percentage of reducing sugar was achieved by using Lane and Eynone volumetric method (**Rangana, 1990**).

2.6. Experimental design and Statistical analysis of data

The treatments were arranged in a complete randomized block design (RCBD). Each treatment was replicated three times, one vine per each replicate. The obtained data were tabulated and subjected for the statistical analysis. Comparisons between means were made by the least significant differences (New L.S.D) at P= 0.05 (**Snedecore and Cochran, 1990**).

3. Results and Discussion

3.1. Mineral status

3.1.1. Leaf Nitrogen and Phosphorus percentages

The impact of inoculation with AMF, Azospirillum and Azotobacter each one individually or in combinations on adult leaves nitrogen and phosphorus contents during 2020 and 2021 seasons of Early Sweet, and Thompson Seedless cultivars are displayed in Table (2).

Nitrogen %: It is noticed from the obtained data that; all treatments were capable to significantly improve in leaves Nitrogen contents during the two experimental seasons for the two examined cultivars.

Except the case of Early Sweet cv in the first season, whereas the individual inoculations (AMF, Azospirillum, or Azotobacter) failed to varied the Nitrogen contents in Early Sweet leaves during the first season only.

Leaves phosphorus %: It is noticed from the obtained data (Table 2) that, the response of the two cultivars to inoculations with the three examined micro-organisms was clearer in the second season rather than those of the first season. During the first season, non-significant differences were observed in phosphorus contents of Early Sweet cultivars. While, Thompson Seedless cultivars, only the combined inoculations with the three micro-organisms (AMF + Azospirillum, AMF + Azotobacter, and AMF + Azospirillum + Azotobacter) was capable to increase leaf phosphorus content significantly, While the individual treatments failed to do this. During the second season, the individual inoculations with AMF, Azospirillum, and Azotobacter treatments were unable to cause any significant effect increase in leaf P contents of two examined cultivars. On the opposite side, all combined inoculations (AMF + Azospirillum, AMF + Azotobacter, and AMF + Azospirillum + Azotobacter) were capable to enhance the leaves phosphorus contents of Early Sweet and Thompson Seedless, during the second season. It is clear from the same table that, the vines received the three examined micro-organisms (AMF + Azospirillum + Azotobacter) in combination produced the highest N and P percentages of Early Sweet, and Thompson Seedless *cvs.* during 2021.

Table (2). Effect of inoculation with AMF, Azospirillum and Azotobacter on Nitrogen and phosphorus percentages, during 2020 and 2021 seasons

Treatments	N %				P %			
	Early Sweet		Thompson Seedless		Early Sweet		Thompson Seedless	
	2020	2021	2020	2021	2020	2021	2020	2021
Control	1.61	1.58	1.60	1.62	0.19	0.19	0.19	0.18
AMF	1.72	1.75	1.81	1.83	0.19	0.22	0.22	0.22
Azospirillum	1.69	1.76	1.78	1.79	0.19	0.20	0.21	0.22
Azotobacter	1.70	1.79	1.83	1.90	0.18	0.21	0.22	0.21
AMF + Azospirillum	1.82	1.84	1.88	1.92	0.20	0.23	0.24	0.25
AMF + Azotobacter	1.79	1.85	1.91	1.93	0.19	0.24	0.22	0.23
Azospirillum + Azotobacter	1.74	1.77	1.90	1.88	0.20	0.22	0.22	0.24
AMF + Azospr. + Azotob.	1.85	1.89	1.97	1.99	0.20	0.26	0.25	0.26
New LSD $_{5\%}$	**0.13**	**0.16**	**0.14**	**0.15**	**NS**	**0.04**	**0.05**	**0.05**

3.1.2. Potassium and Magnesium percentages

Leaves potassium %: In the first season, the results of Thompson Seedless cultivar declare that, all treatments (either individually or in combination) were capable to increase leaves potassium percentage. While, the Early Sweet cultivar was least responsive to inoculation with the three micro-organisms either individually or in combination, as none the treatments led to any significant increase of leaf potassium contents, during the first season. During the second season, all treatments with the three micro-organisms, either individually or in combinations were capable to enhance leaf K % of both cultivars, except the case of inoculated ‘Early sweet’ with Azospirillum bacteria individually.

The obtained data shows also that, any co-inoculation with any two micro-organisms was better than the individual treatment with either of them. Furthermore, the vines inoculated with the three micro-organisms in combination (AMF + Azospirillum + Azotobacter) present the highest level of K, during the two seasons respectively.

Leaves Magnesium %: Data in Table (3) clearly shows that; all treatments with the three micro-organisms either individually or in combination significantly increase the Mg % in the leaves of the two examined cultivars (Early Sweet, and Thompson Seedless). These findings were true during the two experimental seasons. Furthermore, the vines inoculated with three micro-organisms in combination (AMF + Azospirillum + Azotobacter) present the maximized Mg%. In the contrary, un-inoculated vines present the minimized contents of Mg % in their leaves. These data were true during the two seasons respectively.

Table (3). Effect of inoculation with AMF, Azospirillum and Azotobacter on potassium and magnesium percentages, during 2020 and 2021 seasons

Treatments	K %				Mg %			
	Early Sweet		Thompson Seedless		Early Sweet		Thompson Seedless	
	2020	2021	2020	2021	2020	2021	2020	2021
Control	1.49	1.40	1.38	1.39	0.48	0.44	0.47	0.49
AMF	1.50	1.59	1.67	1.69	0.70	0.71	0.66	0.69
Azospirillum	1.54	1.46	1.55	1.57	0.61	0.66	0.58	0.61
Azotobacter	1.55	1.56	1.56	1.59	0.62	0.64	0.55	0.59
AMF + Azospirillum	1.62	1.67	1.72	1.77	0.75	0.77	0.72	0.71
AMF + Azotobacter	1.60	1.62	1.69	1.72	0.71	0.75	0.73	0.72
Azospirillum + Azotobacter	1.55	1.59	1.63	1.70	0.69	0.73	0.70	0.71
AMF + Azospr. + Azotob.	1.60	1.69	1.87	1.90	0.71	0.77	0.79	0.82
New LSD $_{5\%}$	**NS**	**0.16**	**0.15**	**0.16**	**0.05**	**0.06**	**0.05**	**0.07**

Similar results to those obtained in the current study was confirmed on different grapevines cultivar by other researchers such as: **Ibrahim (2015)** on ‘Superior’ and ‘Early Superior’ seedless grapevines; **Abd El-Migeed *et al.*, 2006** on ‘Thompson Seedless’ grapevines; **Shaheen *et al.* (2013)** on ‘Superior Seedless’ grapevines; **Ahmed *et al.*, (2017)** on ‘Superior Seedless’ grapevine; **Mekawy and Abd El-Hafeez (2020)** on Red Globe’.

The promoting effect of AMF inoculation on grapevines macro elements during 2020 and 2021 seasons (i.e. increasing N, P, K and Mg contents) can explained by the positive effect of AMF on increasing mineral elements availability and water absorption and transportation (**Sun *et al.*, 2018** and **Bargaz *et al.*, 2018**), as well as increasing the synthesis of some plant growth regulators (**Rouphail *et al.*, 2015 and Bargaz *et al.*, 2018**). Furthermore, some authors confirmed that inoculation grapevines cultivars with Azospirillum and Azotobacter as a nitrogen fixing bacteria was associated with significantly improvement in essential elements in their leaves (**Abd El-Migeed *et al.*, 2006; Ahmed & Mohamed2018; Abd El-Gani *et al.*, 2020 and Metawie 2020**).

3.2. Yield and its component

The Statistical analysis of vines production (in terms of yield per vine, clusters number per vine and cluster weight) of the two examined cultivars in relation to inoculate the vines with AMF, Azospirillum, and Azotobacter bacteria separately or in-combinations during 2020 and 2021 seasons are displayed in Tables (4 and 5).

3.2.1. Clusters number per vines

Data shows that, during the first season of this study any inoculation (with the three micro-organisms) had any significant effect on any of the two examined cultivars. This seems logical, as the flowering buds initiation and differentiation in grape cultivars takes place during the preceding summer preceding, when the treatments under current study were has not been conducted yet. On the opposite side, during the second experimental season all treatments with AMF, Azospirillum, and Azotobacter either separated or in combination was capable to significantly increase the number of cluster per vine, this finding were true for the two examined cultivars, except the case of inoculated Early Sweet *cv* with Azospirillum or Azotobacter each one individually, which failed to increase the number of clusters per vine.

3.2.2. Cluster weight (g)

The obtained data presented in Table (4) shows that, the response of the two cultivars to inoculations with AMF, Azospirillum and Azotobacter was differed from cultivar to other. Regarding the individual inoculation with each micro-organism, inoculated Early Sweet cultivar with any individual micro-organism was not able to cause a significant increase in the average cluster weight during the two experimental seasons. Contrary, inoculated 'Thompson Seedless' with any micro-organism alone (AMF, Azospirillum, and Azotobacter) was capable to significantly enhance the cluster weight during the two experimental seasons. However, all combined inoculations with AMF, Azospirillum and Azotobacter were capable to increase the average cluster weight (g) significantly of the two examined cultivars during the two experimented seasons. The obtained data shows also that, the vines inoculated with AMF + Azospirillum + Azotobacter in combination produced the best cluster weight. On the other side, untreated vines produced the lowest average cluster weight, during the two experimental seasons.

Table (4). Effect of inoculation with AMF, Azospirillum and Azotobacter on cluster numbers /vine and cluster weight (g), during 2020 and 2021 seasons

Treatments	Cluster Numbers/vine				Cluster weight (g)			
	Early Sweet		Thompson Seedless		Early Sweet		Thompson Seedless	
	2020	2021	2020	2021	2020	2021	2020	2021
Control	15.5	14.9	17.7	16.6	409	411	417	419
AMF	15.2	16.2	17.3	19.1	429	433	512	521
Azospirillum	15.1	15.9	17.5	18.3	411	421	470	479
Azotobacter	16.0	15.7	18.1	18.0	422	428	466	469
AMF + Azospirillum	16.3	17.1	17.6	20.0	466	474	639	647
AMF + Azotobacter	15.8	16.3	18.7	19.5	464	469	648	645
Azospirillum + Azotobacter	16.2	16.0	18.0	18.9	454	451	629	631
AMF + Azospr. + Azotob.	16.0	18.7	18.5	20.3	583	592	629	701
New LSD $_{5\%}$	**NS**	**1.2**	**NS**	**1.6**	**32**	**30**	**42**	**47**

3.2.3. Yield (kg/vine)

Data presented in Table (5) declare that, the response to micro-organisms inoculations were varied from one cultivar to another and from year to year. During the first season, all combined inoculations were capable to increase the yield (kg/vine) of Thompson Seedless *cv.*, while the individual inoculation failed to do this. Concerning the Early Sweet cv., oly the combined inoculation with the three micro-organisms (AMF + Azospirillum + Azotobacter) was able to significantly increase the yield (kg/vine) of Early Sweet *cv.* During the second season, both Early Sweet and Thompson Seedless cultivars, the individual inoculation with AMF or the combined inoculation were able to cause a significant increment in the yield (kg/vine).

Furthermore, it is clear from Table (5) that, concerning the two cultivars, the vines received the combined inoculations with the three examined micro-organisms (AMF + Azospirillum + Azotobacter) produced the highest yield (kg/vine), during the two seasons respectively.

Table (5). Effect of inoculation with AMF, Azospirillum and Azotobacter on yield (kg/vine) and berry weight (g), during 2020 and 2021 seasons

Treatments	Yield (kg/vine)				Berry weight (g)			
	Early Sweet		Thompson Seedless		Early Sweet		Thompson Seedless	
	2020	2021	2020	2021	2020	2021	2020	2021
Control	6.34	6.12	7.38	6.96	3.2	3.3	2.8	2.7
AMF	6.52	7.01	8.85	9.95	3.6	3.6	3.0	3.4
Azospirillum	6.21	6.69	8.22	8.77	3.1	3.4	2.9	3.2
Azotobacter	6.75	6.72	8.43	8.44	3.4	3.4	2.8	3.0
AMF + Azospirillum	7.60	8.11	11.25	12.94	3.6	3.9	3.4	3.6
AMF + Azotobacter	7.33	7.65	12.12	12.58	4.0	4.1	3.2	3.6
Azospirillum + Azotobacter	7.35	7.22	11.32	11.93	3.6	3.9	3.4	3.4
AMF + Azospr. + Azotob.	9.32	11.07	12.38	14.41	4.2	4.5	3.8	4.0
New LSD $_{5\%}$	**1.98**	**1.77**	**1.87**	**2.23**	**0.4**	**0.4**	**0.3**	**0.5**

3.3. Berry physical properties

3.3.1. Berry weight (g)

The Statistical analysis which carried out on the data of berry weight (g) of the two examined cultivars in relation to inoculating the vines with AMF, Azospirillum, and Azotobacter bacteria separately or in combinations during 2020 and 2021 seasons are displayed in Table (5). It is clear that the weight of berry was significantly affected by the treatments of bio-fertilization treatments. This response was varied from one cultivar to another and from one season to another. During the first season, all treatments were able to cause a significant increase in the weight of berries of Early Sweet cultivars, except for the inoculations with Azospirillum or Azotobacter bacteria each one individually. However, none of the individual inoculations with the three examined micro-organisms were able to cause any significant promotion on the weight of Thompson Seedless *cv.*, during the first season. Contrary, all combined inoculations lead to significant increase in berry weight of both cultivars.

During the second season, none of the individual treatments with the three micro-organisms were able to produce any significant increase in berry weight of Early Sweet cultivar. Contrary, the all combined inoculation with the three micro-organisms was capable to improving the berry weight of Early Sweet, during the second season. Regarding the Thompson Seedless cultivar, it was highly responsive to bio-fertilization treatments with the three micro-organisms, all treatments (either individual or combined) led to a significant promotion in berry weight during the second season. In addition, the data clearly shows that, the vines inoculated with the three micro-organisms in combination (AMF + Azospirillum + Azotobacter) produced the highest berry weight of the two examined *cvs.*, during the two seasons respectively. In the contrary, non-inoculated vines produced the lowest berry weight.

3.3.2. Berry length (cm)

The length of berry significantly affected by the treatments of bio-fertilization using the individually or combined inoculation with AMF, Azospirillum and Azotobacter bacteria during 2020 and 2021 seasons (Table 6). This response was varied between the two cultivars from year to other.

During the first season, the individual inoculations with Azospirillum or Azotobacter was failed to increase the berry equatorial significantly Early Sweet and Thompson Seedless cultivars. While, the individual inoculation with AMF significantly improved the berry equatorial (cm) of the two examined cultivars. Moreover, all co-inoculations treatments led to a significant improvement of berry equatorial during the first experimental season for the two cultivars under study, with the exception of inoculation Thompson Seedless cultivar with Azospirillum + Azotobacter. The obtained data declare that the vines inoculated with AMF + Azospirillum + Azotobacter produced the maximized berry equatorial (cm). While, non-inoculated vines produced the lowest berry equatorial compared to the other treatments (cm), these findings were true during the two experimental seasons respectively.

3.3.3. Berry diameter (cm)

Berry diameter was significantly improved as a result of inoculated the vines with AMF, Azospirillum, and Azotobacter bacteria individually or in combination, during 2020 and 2021 seasons (Table 6). It is clearly from the obtained data that, during the two experimental seasons (2020 and 2021), the obtained results shoes that, both Early Sweet and Thompson Seedless cultivars took the same trend, as the individual inoculation with AMF as well as all combined inoculations was able to produce a significant improvement in the berry diameter (cm), while the individual inoculation with Azospirillum or Azotobacter failed to produce any significant effect on berry diameter.

Table (6). Effect of inoculation with AMF, Azospirillum and Azotobacter on equatorial and diameter of berry weight (g), during 2020 and 2021 seasons

Treatments	Berry equatorial (cm)				Berry diameter (cm)			
	Early Sweet		Thompson Seedless		Early Sweet		Thompson Seedless	
	2020	2021	2020	2021	2020	2021	2020	2021
Control	2.01	2.09	2.29	2.30	1.99	2.01	1.78	1.82
AMF	2.21	2.29	2.49	2.52	2.19	2.23	1.98	1.99
Azospirillum	2.19	2.21	2.39	2.45	2.09	2.12	1.88	1.89
Azotobacter	2.13	2.19	2.30	2.38	2.11	2.10	1.75	1.77
AMF + Azospirillum	2.25	2.28	2.51	2.54	2.21	2.26	2.39	2.42
AMF + Azotobacter	2.29	2.31	2.54	2.55	2.26	2.29	2.29	2.43
Azospirillum + Azotobacter	2.21	2.23	2.37	2.41	2.17	2.20	2.29	2.27
AMF + Azospr. + Azotob.	2.38	2.41	2.52	2.59	2.31	2.39	2.50	2.53
New LSD $_{5\%}$	**0.20**	**0.19**	**0.20**	**0.19**	**0.19**	**0.19**	**0.18**	**0.17**

3.3.4. Berry juice %

The data illustrated in Table (7) shows that, regarding the Thompson Seedless cultivar, all treatments failed to significantly increase the juice percentage, either individually or in combinations, during the two experimental seasons. Concerning the Early Sweet cultivars, during the first season all individual and combined inoculations failed to improve the percentage of juice during the first season. While, during the second season inoculated the Early Sweet with AMF individually or all combined inoculations were led to significant increment in berry juice %. Contrary, inoculated Early Sweet vines with Azospirillum or Azotobacter individually failed to increase the berry juice % during the second season.

The obtained results are in harmony with the findings of some previous authors on different grapevines cultivars and other fruit trees such as those demonstrated by **Carvajal-Munoz & Carmona-**

Garcia (2012); Mosa *et al.* (2014); Bargaz *et al.* (2018); Ahmed & Ahmed (2020) and Hammad *et al.* (2020).

Table (7). Effect of inoculation with AMF, Azospirillum and Azotobacter on Juice % and TSS%, during 2020 and 2021 seasons

Treatments	Juice %				TSS %			
	Early Sweet		Thompson Seedless		Early Sweet		Thompson Seedless	
	2020	2021	2020	2021	2020	2021	2020	2021
Control	72.9	71.3	74.5	74.3	18.1	17.8	18.9	19.1
AMF	73.3	73.7	73.7	75.3	19.1	19.3	19.7	19.9
Azospirillum	73.3	72.1	74.5	74.5	18.6	19.1	19.5	19.8
Azotobacter	72.5	72.5	72.3	75.4	18.1	18.4	19.4	19.5
AMF + Azospirillum	73.7	73.7	75.1	72.2	19.5	19.6	20.9	21.5
AMF + Azotobacter	73.5	73.5	75.3	74.7	19.0	19.4	20.7	21.2
Azospirillum + Azotobacter	72.5	73.3	74.5	74.9	18.8	19.0	19.9	20.4
AMF + Azospr. + Azotob.	74.5	74.2	74.7	75.1	20.3	20.9	21.7	22.2
New LSD $_{5\%}$	**NS**	**1.8**	**NS**	**NS**	**1.1**	**0.8**	**1.0**	**1.1**

3.4. Berry chemical properties

3.4.1. TSS%

Data illustrated in Table (7) shows that shows the response of TSS% of the two examined cultivars to inoculation with AMF, Azospirillum and Azotobacter during 2020 and 2021 seasons. During the first season (2020), all treatments whether individually or in combinations led to a significant increase in the TSS % in berry juice of 'Early Sweet' berries, the combined inoculations with AMF + Azospirillum and AMF + Azospirillum + Azotobacter led to significant increase in TSS%. While, the other treatments, whether individual or in combination failed to produce any significant promotion of TSS%. However, all the individual inoculation failed to improve the TSS% of Thompson Seedless cultivar. Contrary, all combined inoculation led to significant increase in Thompson Seedless TSS % during the first season. During the second season, the data presented in Table (7) shows that all inoculations were capable to significantly improve the TSS% of Early Sweet cultivar, except the individual inoculation with Azospirillum or Azotobacter. However, all individual inoculation failed to significantly increase the TSS% of Thompson Seedless, but all combined inoculations significantly increase the TSS%, during the second season.

3.4.2. Reducing sugars %

The obtained data shows that The response of reducing sugars % of the two examined cultivars (Early Sweet and Thompson Seedless) to the single or combined inoculations with AM fungi, Azospirillum and Azotobacter, during 2020 and 2021 seasons. This table clearly shows that, the treatments were capable to improve the reducing sugars percentage of the two cvs., during the two experimental seasons. with the exception of inoculating Early Sweet *cv.*, with Azospirillum or Azotobacter individually during 2020 and Azospirillum individually, during the second season.

3.4.3. Total acidity %

The data demonstrated that, during the first season all single and combined inoculations hasn't any significant effect on total acidity of Early Sweet cultivars. However, only the combined inoculation with AMF + Azospirillum bacteria and combined inoculation with the three micro-organisms were capable to decrease the total acidity of Thompson Seedless berries (Table 8) of Thompson Seedless cultivar. During the second season (2021), the individual inoculation with AMF and all combined inoculations were able to reducing the total acidity of Early Sweet during the second season. In the other hand, all individual or combined inoculations were capable to significantly decrease the total acidity % during the second season.

Table (8). Effect of inoculation with AMF, Azospirillum and Azotobacter on Reducing sugars % and total acidity %, during 2020 and 2021 seasons

Treatments	Reducing sugars %				Total acidity %			
	Early Sweet		Thompson Seedless		Early Sweet		Thompson Seedless	
	2020	2021	2020	2021	2020	2021	2020	2021
Control	15.3	15.1	16.6	16.7	0.423	0.431	0.364	0.369
AMF	16.4	16.9	18.2	18.4	9.421	0.399	0.351	0.332
Azospirillum	15.8	15.9	17.9	17.8	0.425	0.412	0.342	0.331
Azotobacter	16.2	16.4	17.8	17.8	0.419	0.419	0.345	0.329
AMF + Azospirillum	16.9	17.2	18.3	18.7	0.418	0.377	0.330	0.321
AMF + Azotobacter	17.2	17.3	18.2	18.4	0.421	0.399	0.341	0.319
Azospirillum + Azotobacter	16.4	16.6	17.9	17.9	0.422	0.401	0.347	0.317
AMF + Azospr. + Azotob.	17.2	17.4	19.4	19.6	0.411	0.355	0.312	0.305
New LSD $_{5\%}$	**1.1**	**0.9**	**1.0**	**1.1**	**NS**	**0.028**	**0.032**	**0.029**

The positive role of bio-fertilization with AMF, AZSB, and AZBB inoculations in increasing TSS % and reducing sugars % as well as decreasing total acidity %, which obtained in this investigation was in harmony with the findings of some previous authors on different grapevines cultivars and other fruit trees such as those demonstrated by **Carvajal-Munoz & Carmona-Garcia (2012); Mosa *et al.* (2014); Bargaz *et al.* (2018); Ahmed & Ahmed (2020) and Hammad *et al.* (2020).**

Bashan and Holguin (1997) concluded that inoculated the grapevines with Azospirillum was more effectiveness when combined with the other microorganisms. However, a synergistic relationship was confirmed between AMF and both Azospirillum and Azotobacter bacteria in the current investigation perhaps due to involving the providing nutrients **(Ibrahim *et al.*, 2009)**, remove inhibitory and facilitate of the process in plant development, such as regulation of plant hormones (**Bashan and Holguin, 1997)**. This synergistic effect allows a significant increment can see in facility viability of mineral nutrition of the host (**Bashan *et al.*, 1989**). The enhancement of berry chemical properties as a result of inoculated the vines with AFM inoculation can explained by the role of AMF in contribute to the physiological function of the host plant, and allow assimilate easily of the nutrients from the soil (**Metawie, 2020**). Similar finding was observed on Red globe grapevines by **(Mekawy and Abd El-Hafeez, 2020), Kanitkar *et al.* (2019)** on ‘Thompson Seedless’ grapevines.

4. Conclusion

The obtained data clearly shows that inoculated the Early Sweet and Thompson Seedless were very effective in improving the nutritional status, productivity and berry physical and chemical properties. The combined inoculations with any two micro-organisms were more effective than the individual inoculations. However, the best results were obtained when the vines received the three micro-organisms in combination. Then, it is strongly recommended to inoculating the Early Sweet and Thompson Seedless *cvs.* grown under El-Minia Governorate conditions in sandy soil with AMF, Azospirillum and Azotobacter in combination, to enhancing the productivity and improving berries physical and chemical properties.

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