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ATTEMPT TO IMPROVE PRODUCTIVITY AND BERRIES QUALITY OF FLAME SEEDLESS GRAPEVINES GROWN IN SANDY SOIL, THROUGH SOME BIOFERTILIZERS TREATMENTS

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ABSTRACT: This investigation aimed to study the effect of some biofertilizers strains namely: Arbuscular mycorrhizal fungi (AMF), *Azospirillum brasilense* bacteria (AZSB) and *Azotobacter chroococcum* bacteria (AZBB) as well as their combinations on productivity and berries quality of Flame Seedless grapevines. The study was carried out during two seasons 2018 and 2019 on grapevines grown under new reclamation sandy soil conditions at Matay distract, El Minia Governorate – Egypt. Results showed that the three examined microorganisms strains were capable to significantly increase yield/vine and improve berries physical &chemical properties in both seasons. Inoculation with AMF showed superiority than AZSB or AZBB. Also, combined inoculations were more effective than single inoculations. However, the vines treated with the triple mixture (AMF , AZSB and AZBB) gained the highest yield (kg/vine), berry weight(g), berry dimensions (cm), juice TSS (%) , juice sugar content (%) and juice total anthocyanins (mg/100g F.W.) during the two experimental seasons.

Key words: Grapevines, Flame Seedless, Arbuscular Mmycorrhizal fungi, Azospirillum brasilense and Azotobacter chrococcum bacteria, sandy soil.

INTRODUCTION

Grapevine (*Vitis vinifera* L.) is considered as one of the major fruit crops throughout the world, based on cultivated area and economic value. Grapes are one of the most desirable fruit in Egypt, it ranked the second positions in fruit crops after the citrus. In 2017, fruiting area and total production of grapevines in Egypt reached 189762 feddans and 1758979-ton fruits, respectively (2017 statistics in ARE, annual reports, Ministry of Agriculture and Land Reclamation).

Minia governorate, where the present study was carried out, occupied the second position in grapevines cultivation and production. Flame Seedless cultivar is a newly introduced in Egypt that has a high economic importance among the grapevines cultivars due to its early ripening and successfully adapt in Egyptian new reclamation desert land conditions **Ibrahim (2011)**. Furthermore, actually it considered as a prime and popular cultivar in Egypt.

Continuous use of mineral fertilizers maybe leads to deterioration of soil texture and fertility and accumulation of heavy metals in plant tissues, effecting fruit nutritional value and edibility **(Ibrahim *et al.*, 2010; Druva-Lusite and Levinsh 2010; Ibrahim 2011; Shaheen *et al.*, 2013; Ibrahim and Gad El-Kareem 2014; Mosa *et al.*, 2014; Ibrahim, 2015 and Bargaz *et al.*, 2018)**. In arid and semi-arid regions such as Egyptian desert reclamation lands, the soil is nutritionally deficient and moisture limitation.

Furthermore, high temperature during the most months of year, for these reasons chemical fertilizers cannot be applied in adequate quantities to fruit trees. Grapevines grown in such areas; therefore, it is preferred that partial replacement nitrogen fertilizers by biological nitrogen fixation microorganisms such as a non-symbiotic microaerophilic nitrogen fixer Azospirillum and Azotobacter strains. Thus, possibly promoting yield and fruit quality, as well as nutritional status of grapevines. However, the arbuscular mycorrhizal fungi (AMF) form is a symbiotic association with more than 80% of land plant families **(Shata *et al.*, 2007; Ibrahim *et al.*, 2010; Ibrahim 2011; Wang *et al.*, 2011; Wang *et al.*, 2017 and Bargaz *et al.*, 2018).** AMF consists of an internal phase inside the root and an external phase, or extra radical mycelium phase, which can form an extensive network within the soil **(Druva-Lusite & Levinsh 2010; Neagoe *et al.*, 2013 and Ibrahim 2015).** AMF benefit their host principally by enhancing uptake of relatively immobile phosphate ions, due to the ability of the fungal to gridge beyond the phosphate depletion zone that quickly develops around the roots **(Wang *et al.*, 2011; Neagoe, 2013; Grzyb *et al.*, 2015 and Wang *et al.*, 2017).**

The main objective of this work, investigate the response of flame seedless grapevines grown under sandy soil to some biofertilization treatments on yield and some cluster and berry characteristics.

MATERIALS AND METHODS

This study was carried out during 2018 and 2019 seasons on 32 uniform in vigor 11 years old flame seedless grapevines grown in a private vineyard located at East desert road, facing to Matay district, Minia Governorate, where the texture of the soil is sandy, well drained and water table not less than two meters deep. The selected vines are planted at 2.5 × 3.0 m apart, pruned during the first week of January in the two seasons using cane pruning method with the assistance of gable supporting system. Vine load was 72 eyes for all the selected vines on the basis of six fruiting canes × ten eyes plus six renewal spurs × two eyes.

Mechanical, physical and chemical analysis of the orchard soil as well as irrigation water were carried out at the start of the experiment according to the procedures of **Wilde *et al.* (1985) and Walsh & Beaton (1986)** so, the data of soil and water sample analyses are shown in Table 1.

Table 1. Physical and chemical analysis of orchard soil and irrigation water

Soil analysis		Water analysis	
Constituents	**Values**	**Constituents**	**Values**
Sand %	79..9	E.C (mmhos/cm/25C)	1. 4
Silt %	12.3	Hardness	17.9
Clay %	9.8	pH	7.42
Texture	Sandy	Ca (mg/L)	49.1
EC (1:2.5 extract) mmhos/cm/ 25 C	3.2	Mg (mg/L)	26.3
Organic matter %	0.31	K (mg/L)	4.17
pH (1 : 2.5 extract)	8.32	Na (mg/L)	75.6
Active lime ($CaCO_3$ %)	8.43%	Sum of Cations (mg/L)	8.26
Total N %	0.08	Alkalinity (mg/L)	179
Available Phosphorus (ppm)	2.80	Chlorides (mg/L)	113
Available Ca (meq/100g)	19.9	Nitrate (mg/L)	12.0
Available Mg (meq/100g)	2.33	Sulphates (mg/L)	44.9
Available K (meq/100g)	0.56	Sum of anions (mg/L)	8.19
C/N Ratio	15.2	SAR	2.94

This study included seven treatments of soil application with three microorganisms; arbuscular mycorrhizal fungi (AMF), *Azospirillum brasilense* bacteria (AZSB) and *Azotobacter chroococcum* bacteria (AZBB) each alone or combined, in addition to the control as following:

1- Control (untreated vines).
2- Inoculation with arbuscular mycorrhizal fungi (AMF).
3- Inoculation with *Azospirillum brasilense* bacteria (AZSB).
4- Inoculation with *Azotobacter chroococcum* bacteria (AZBB).
5- Inoculation with arbuscular mycorrhizal fungi combined with *Azospirillum brasilense* bacteria.
6- Inoculation with arbuscular mycorrhizal fungi combined with *Azotobacter chroococcum* bacteria.
7- Inoculation with Azospirillum *brasilense* bacteria combined with Azotobacter *chroococcum* bacteria.
8- Inoculation with arbuscular mycorrhizal fungi combined with *Azospirillum brasilense* bacteria and Azotobacter *chroococcum* bacteria.

Strains of arbuscular mycorrhizal fungi (AMF), *Azospirillum brasilense* bacteria (AZSB) and *Azotobacter chroococcum* bacteria (AZBB) were kindly isolated and propagated at laboratory of Microbiology, Minia University, Egypt. Strains of Azospirillum or Azotobacter were grown on Dobereiner medium. Strains were grown in liquid medium on a rotary shaker at 30 °C and 120 rpm, then the culture were added to the vines, three times/year at rate of 200 ml per vine, each ml contain 10^8 cell of Azospirillum or Azotobacter bacteria. Moreover, arbuscular mycorrhiza fungi were developed on onion plants roots, and so the onion soil added to the vines in order to 200g/vines, each 1 gram contained 10^8 spores. The bio-fertilizers were applied either separately or in a mixture three times to the soil around each vine at 100 ml/vine for Azospirillum or Azotobacter and 100g/vine for Mycorrhiza. Before treated the vines with the three tested biofertilizers, each bacterial or fungi treatment as well as each possible combination was mixed with 5 kg of farmyard manure, then add to the vineyard as a soil application. The first dose was added during bud burst stage, the second one during full blooming stage and the third was applied at one month later.

Experimental design: Randomized complete block design was followed where the experiment consisted of eight treatments, each treatment was replicated four times, one vine per each **(Rangaswamy, 1995)**.

Different measurements: The responses of grapevine to biofertilization treatments were evaluated through the following parameters:

Yield/vine and some cluster characteristics: Harvesting took place when TSS/acid in the berries of the checked treatment reached 22: 1, at the last week of June in the two seasons, according to (**Winkler *et al.,* 1974 and Weaver, 1976)**. The yield per vine expressed in weight (kg.) and number of clusters per vine. Four clusters from each vine were taken at random to determine the cluster length and width (cm.).

Berry physical and chemical properties: Samples of 50 berries were randomly taken from cluster samples of each replicate to calculated the following parameters:

1- Average berry weight (g).

2- Berry dimensions (length and diameter, cm) and shape index.

3- Total soluble solids (TSS%) percentage in the juice by using handy refractometer.

4- Percentage of reducing sugars in the juice by **Lane and Eynon (1960)** volumetric method as described in **A.O.A.C. (1995).**

5- Titratable acidity percentage (as a tartaric acid/ 100 ml juice) by titration against 0.1N NaOH using phenolphthalein as an indicator **A.O.A.C. (1995).**

6- Total anthocyanin content (Mg/100g F.W.) was extracted from one-gram berry skin (fresh weight) with 100 ml of Acidified methanol (0.1% HCL). The solution was filtered through a centered glass funnel G-3 and absorbance was measured at wavelength 520 nm by Spekol 11 spectrophotometer **(Geza *et al*., 1983).**

Statistical analysis: The obtained data were tabulated and significantly analyzed according to **Snedecor and Cochran (1980).** Differences between treatments means were compared using new L.S.D. test at 5%.

RESULTS AND DISCUSSION

Yield/vine and some cluster characteristics

Data in Table (2) showed that all biofertilization treatments failed to affect significantly the number of clusters/ vine in the first season, while in the second season the differences between all treatments were significant compared with control.

The double and triple combination biofertilizers improved total yield /vine and cluster characteristics than single application in both seasons.

The double treatments arbuscular mycorrhizal fungi + *Azospirillum brasilense* bacteria (AMF+ AZSB) and triple arbuscular mycorrhizal fungi + *Azospirillum brasilense* bacteria + *Azotobacter chroococcum* bacteria (AMF + AZSB + AZBB) recorded highest yield (kg), cluster weight (g), cluster length and width in the two seasons without differences between them (double & triple). The least values of all properties were from the control in both seasons.

The positive effect of bio-fertilization on enhancing yield and its component was observed by **El-Sayed (2001); Abdel-Hamid *et al.* (2004); Ibrahim (2005); Shaheen *et al.* (2013) and Rozpara *et al.* (2014).**

The aforementioned findings are in harmony with **Farag, 2006; Shata *et al.*, 2007; Carvajal-Munoz and Carmona-Garcia, 2012 and Leksono and Yanuwiadi, 2014** that inoculation with nitrogen fixing bacteria (i.e., *Azotobacter cholococcum, Azospirillum brasilense, Azospirillum lipoferum, Sinorhizobium* spp., *Burkholderia* spp., and *Pseudomonas* spp.) significantly improved yield of multiple important horticultural crops including fruit trees.

Ibrahim *et al*. (2010) studied the response of Balady guava trees to bio-fertilization with arbuscular mycorrhizal fungi (AMF) and/or Phosphate dissolving bacteria under sandy calcareous soil conditions. The authors confirmed that guava trees inoculated with AMF produced higher and significantly yield/tree (kg) rather than un- inoculated trees. **Shamseldin *et al*., (2016)** stated that the yield and fruit dimensions of Washington Navel orange were significantly developed with inoculated the trees with bio-fertilizers containing nitrogen fixers (namely: *Azospirillum brasilense*, *Azotobacter chroococcum* and *Pseudomonas fluorescence*) as compared to the un-inoculated trees. Furthermore, **Rozpara *et al*. (2014)** and **Mosa *et al.* (2015)** reported that apple trees treated with arbuscular mycorrhizal fungi, *Pseudomonas fluorescence* and *Rhizobium* Sp. bacteria, significantly increased number of fruits/ tree, fruit weight (g) and fruit dimensions (cm).

Berry physical properties

Data presented in Table (3) demonstrated that, grapevines amended with arbuscular mycorrhizal fungi (AMF), *Azospirillum brasilense* bacteria (AZSB) and *Azotobacter chroococcum* bacteria (AZBB) each alone or combined significantly increased berry physical properties as: berry weight (g), berry volume (cm^3), berry longitudinal (cm) and berry equatorial (cm) compared to the control (un inoculated vines). The best values came from the triple combined treatment (AMF, AZSB and AZBB) during the two experimental seasons. The control treatments gave least values of all berry physical properties in both seasons. On the other side, shape index showed no significant effect between all the treatments in both seasons.

The obtained result is in agreement with **Fathi *et al.* (2002); Abdel-Hamid *et al.* (2004); Farag (2006); Ibrahim *et al.* (2010); Carvajal-Munoz and Carmona-Garcia (2012); Ibrahim and Gad El-Kareem (2014); Mosa *et al.* (2014) and Bargaz *et al.* (2018).**

Berry chemical constituents

Concerning berries chemical properties, data in Table (4) demonstrated that grapevines inoculated with double treatments (AMF+ AZSB) and triple treatment (AMF + AZSB + AZBB) gave the highest significant values of TSS, reducing sugars and TSS/acid ratio in both seasons without differences between them (double & triple). Otherwise, increasing the TSS and decreasing the total acidity led to significant increasing in the TSS/acidity ratio compared to untreated vines. Triple treatment (AMF + AZSB + AZBB) recorded uppermost values of anthocyanin (152 and 167 Mg/100g F.W.) in the two seasons, respectively.

The aforementioned findings are in harmony with **Abou El-Yazied and Sellim (2007); Shata *et al.* (2007); Ibrahim *et al.* (2010); Carvajal-Munoz and Carmona-Garcia (2012); Mosa *et al.* (2014); Reddy *et al.* (2016); Wang *et al.* (2017) and Bargaz *et al.* (2018)** on some grapevines cultivars and other fruit species inoculated with some bio-fertilizers**.**

Table 2. Effect of some biofertilization treatments on yield /vine (kg) and some cluster characteristics of Flame Seedless during 2018 and 2019 seasons

Treatments	Clusters number/vine		Cluster weight (g)		Yield (kg/vine)		Cluster length (cm)		Cluster diameter (cm)	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
Control	28.00	29.00	275.00	277.00	7.70	7.76	14.90	15.20	11.10	11.60
Mycorrhiza	29.00	32.00	298.00	312.0	8.64	9.98	18.60	18.80	12.70	13.10
Azospirillum	28.00	31.00	290.00	292.00	8.12	9.06	16.60	17.20	11.60	12.10
Azotobacter	28.00	30.00	285.00	299.00	7.98	8.97	16.20	16.40	11.60	12.00
Mycorrhiza + Azospirillum	29.00	37.00	310.00	347.00	8.99	12.84	19.90	21.20	13.30	13.70
Mycorrhiza + Azotobacter	28.00	35.00	305.00	331.00	8.54	11.59	18.10	18.60	12.90	13.60
Azospirillum + Azotobacter	29.00	35.00	296.00	325.00	8.58	11.38	17.30	17.80	12.30	12.90
Mycorrhiza + Azospirillum + Azotobacter	29.00	37.00	324.00	344.00	9.39	12.73	19.90	21.20	14.30	14.90
New LSD at 5%	NS	2.00	18.90	17.10	0.82	0.79	1.80	1.60	0.99	1.10

Table 3. Effect of some biofertilization treatments on berries physical properties of Flame Seedless during 2018 and 2019 seasons

Treatments	Berry weight (g)		Berry volume (cm^3)		Berry length (cm)		Berry diameter (cm)		Berry shape index	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
Control	2.22	2.28	1.91	1.94	1.17	1.18	1.12	1.16	1.05	1.02
Mycorrhiza	2.59	2.68	2.33	2.45	1.28	1.31	1.23	1.28	1.05	1.03
Azospirillum	2.39	2.47	2.21	2.29	1.21	1.22	1.21	1.21	1.00	1.01
Azotobacter	2.36	2.48	2.15	2.22	1.21	1.22	1.19	1.22	1.02	1.00
Mycorrhiza + Azospirillum	3.01	3.18	2.87	2.93	1.32	1.34	1.29	1.31	1.03	1.03
Mycorrhiza + Azotobacter	3.03	3.07	2.78	2.83	1.31	1.33	1.29	1.31	1.00	1.02
Azospirillum + Azotobacter	2.87	3.00	2.49	2.77	1.30	1.33	1.25	1.30	1.05	1.03
Mycorrhiza + Azospirillum + Azotobacter	3.21	3.32	3.08	3.17	1.39	1.38	1.33	1.36	1.05	1.02
New LSD at 5%	0.12	0.13	0.11	0.12	0.03	0.03	0.04	0.03	NS	NS

Table 4. Effect of some biofertilizers treatments on berries chemical properties of Flame Seedless grown under sandy soil conditions, during 2018 and 2019 seasons

Treatments	TSS (%)		Reducing sugars (%)		Total acidity (%)		TSS/acid ratio		Anthocyanin (Mg/100g F.W.)	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
Control	18.20	18.30	16.20	16.40	0.779	0.781	23.30	23.40	82.00	79.00
Mycorrhiza	20.10	20.40	18.10	18.70	0.772	0.778	26.10	26.20	119.00	121.00
Azospirillum	19.10	19.80	17.70	17.90	0.776	0.781	24.60	25.40	89.00	92.00
Azotobacter	19.20	19.60	17.10	17.50	0.778	0.779	25.00	25.10	90.00	96.00
Mycorrhiza + Azospirillum	20.80	21.30	19.30	19.90	0.669	0.647	31.00	32.90	111.00	116.00
Mycorrhiza + Azotobacter	20.10	20.90	19.10	19.60	0.666	0.656	30.10	31.80	108.00	115.00
Azospirillum + Azotobacter	20.20	20.40	18.10	18.70	0.711	0.659	28.40	30.90	101.00	106.00
Mycorrhiza + Azospirillum + Azotobacter	20.90	21.10	19.90	20.20	0.622	0.606	33.60	34.80	152.00	167.00
New LSD at 5%	0.80	0.70	0.60	0.70	0.010	0.025	1.20	1.00	12.10	10.30

Conclusion: The present study confirmed that, inoculated Flame Seedless grapevines grown under new reclamation sandy soil with arbuscular mycorrhizal fungi (AMF), *Azospirillum brasilense* bacteria (AZSB), *Azotobacter chroococcum* bacteria (AZBB) three times yearly obviously enhanced yield (kg)/ vine, berry physical properties and berry chemical properties of Flame seedless grapevines.

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