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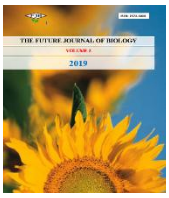

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USING SOME BIOFERTILIZATION TREATMENTS TO PROMOTE YIELD AND BERRIES QUALITY OF FLAME SEEDLESS GRAPEVINES (*Vitis vinifera* L.)

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ABSTRACT: Flame Seedless cultivar is one of the most popular grape cultivar in Egypt. However, in Minia region, it faces some problems such as poor yield and poor coloration of berries, which in turn negatively affect marketing of such grapevine cv. The present study was conducted during two seasons 2017 and 2018 on Flame seedless grapevines in order to study the possibility of using single and combined inoculation with *Arbuscular Mmycorrhizal* fungi (AMF), *Azospirillum brasilense* bacteria (AZSB) and *Azotobacter chrococcum* bacteria (AZBB) on improving productivity and fruit quality of Flame Seedless grapevines grown clay soil under Minia region conditions, Egypt. The obtained results confirmed that inoculation the vines with the three examined microorganisms (AMF, AZSB and AZBB) individually or in combination was remarkably improved the yield and its components, berries physical properties and berries chemical properties comparison to un-inoculated vines. Furthermore, any combined inoculation was more effectiveness on yield and berries physical and chemical than the individual inoculation, during the two experimental seasons. The vines inoculated with the mixture of the three microorganism's AFM+AZSB+AZBB in-combination gave the highest yield and best berries physical and chemical properties.

Key words: Grapevines, *Vitis vinifera*, Flame Seedless, Mmycorrhizal, Azospirillum, Azotobacter.

INTRODUCTION

Grapevine (*Vitis vinifera* L.) is one of the largest and oldest fruit crop on earth, and consider as one of the major horticulture crops throughout the world. It is well known that, vines have great adaptability and thrives in wide range of climatic and soil conditions **(Winkler *et al.*, 1974; Delas 2000; Reynier 2000 and Ibrahim, 2015).** Grapevines are fairly adaptable plants, growing in a wide variety of soil types, from light sand to heavy packed clay, and flourishing around the globe in the temperate bands between 20°C and 50°C Latitude, north or south of the Equator **(Winkler *et al.*, 1974; Reynier 2000 and Srinivasan & Mullins, 2001).** Grapes have been associated with Egyptian culture since ancient times. It was taken care by the ancient Egyptians and they excelled in the ways of raising it. Grapes have multiple uses. In Egypt, grapevine is considered as one of the most important commercial and favorable fruit and occupied the second position of fruit crops, since only citrus crops precede it. Furthermore, grapes are grown successfully in all Egyptian governorates. Flame Seedless cultivar is one of the most popular grape cultivars successfully grown under Egyptian conditions. This cultivar ripens early in the first week of June and sometimes in the last week of May when grown in new Egyptian reclamation sandy soils. It has a great opportunity for export to foreign reign markets due to its early ripening **(Abd- Elwahab, 2015; Saad, 2014 and Ibrahim *et al.*, 2020)**. However, in Minia region it faces some problems such as poor yield and poor coloration of berries, which in turn negatively affect marketing of such grapevine cv. Therefore, many trails and attempts were made for finding out the nontraditional methods for overcoming such problems and at the same time protecting our environment from pollution. Microorganisms can stimulate, inhibit, or be without effect on root growth, depending on the type of microorganism, plant species, and environmental conditions **(Marschner 1995; Ibrahim, 2005; Rao, 2002; Mosa *et al*., 2014 and Ibrahim, 2011).**

The overall aims of this research are to improve understanding the influence of the inoculation with *Arbuscular Mmycorrhizal* fungi (AMF), *Azospirillum brasilense* bacteria (AZSB), and *Azotobacter chrococcum* bacteria on productivity and berries physical and chemical properties of Flame Seedless grapevines frown in clay soil under El-Minia governorate conditions.

MATERIALS AND METHODS

This study was carried out during 2017 and 2018 seasons on 32 uniform in vigour 5 years old Flame Seedless grapevines grown in a private vineyard located at Kom El-Arab village Matay district, Minia Governorate, where the texture of the soil is clay, well drained and water table not less than two meters deep. The selected vines are planted at 2.5 x 3.0 m apart, pruned during the last week of December 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 X ten eyes plus six renewal spurs X two eyes.

Orchard soil analysis

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

Table 1. Physical and chemical analysis of experimental orchard soil

Constituents	Values
Sand %	6.40
Silt %	20.82
Clay %	73.49
Texture	Clay
EC (1 : 2.5 extract) mmhos / cm / 25 C	0.95
Organic matter %	2.62
pH (1 : 2.5 extract)	7.6
Total $CaCO_3$ %	1.79
N %	0.10
Available P (Olsen, ppm)	6.10
Exch. K^+ (mg/100g)	422.10
Exch. Ca^{++} (mg/100g)	20.8

Experimental work

The present trail included the following eight treatments from soil single and combined inoculations of *Arbuscular Mmycorrhizal* fungi (AMF), *Azospirillum brasilense* bacteria (AZSB) and *Azotobacter chrococcum* bacteria (AZBB) were namely: Control (untreated vines); Inoculation with AMF; Inoculation with AZSB; Inoculation with AZBB; Inoculation with AMF combined with AZSB; Inoculation with AMF combined with AZBB; Inoculation with AZSB combined with AZBB; and Inoculation with AMF combined with AZSB + AZBB.

Microorganism strains

The Fungi and bacterial strains used in this experiment were AMF, AZSB, AZBB and their mixtures. Strains of Azospirillum, Azotobacter and Mycorrhiza fungi were kindly isolated and propagated at Laboratory of Microbiology, Minia University, Egypt. Strains of Azospirillum or Azotobacter were grown on Doberiner 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 a rate of 200 ml per vine, however, each ml contain 10^8 cell of Azospirillum or Azotobacter bacteria. However, Arbuscular mycorrhiza fungi were developed on onion plants roots, and so the onion soil added to the vines in order to 200g/vines. However, 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 Mycrrohiza. Before treated the vines with the three tested biofertilizers, each bacterial or fungi treatment as well as each possible combination was mixed with 3 kg of farmyard manure, then add to the vineyard as a soil application. The first dose was added during burst bud stage, the second one during full blooming stage and the third one was applied at one month later.

Experimental design

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

Different measurements: The following measurements were recorded during the two experimental seasons:

Yield and its component: Harvesting took place when TSS/Acid in the berries of the check 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 was recorded.

Cluster properties and Berries quality: four clusters from each vine were taken at random for determination of the following physical and chemical characteristics.

1- Cluster dimensions (length and width, cm.)
2- Average berry weight (g.) and volume (cm^3).
3- Average berry dimensions (longitudinal and equatorial, cm) and berry shape index.

4- Percentage of total soluble solids in the juice by using refractometer.

5- Percentage of titratable acidity (as a tartaric acid/ 100 ml juice) by titration against 0.1N NaOH using phenolphthalein as an indicator **A.O.A.C. (2000)**, as well as TSS/ acid ratio calculated.

6- Percentage of reducing sugars in the juice by **Lane and Eynone (1960)** volumetric method.

7- 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 treatment means were compared using new L.S.D. test at 5% level of probability.

RESULTS AND DISCUSSIONS

1- Effect on yield and its components

Obtained data in Table (2) show that the yield (kg)/vine, cluster numbers/vine "only in the second season", cluster weight, and cluster dimensions of Flame seedless grapevines increased due to inoculated the vines with AMF, AZSB and AZBB each one alone or in mixtures combination, during the two experimental seasons, in comparison with those of un-inoculated ones. Among the individual inoculation of the three examined microorganisms, inoculated the vines with AMF was superior to the two other microorganisms. It is worth noting that, all combined inoculation of the three-microorganism examined was more effectiveness on the yield and its contents comparison to the individual inoculation. The data tack similar trend during the two experimental seasons .

Furthermore, the vines inoculated with the mixture of the three microorganisms (AMF + AZSB + AZBB) in combination present the highest and significant yield/vine (14.4 & 18.1 kg/vine), clusters numbers/vines only in the second season (39 clusters/vine), cluster weight (462 & 465 g), cluster length (25.1 & 26.8 cm) and cluster width (15.8 & 16.9 cm) during the two experimental seasons respectively, with the exception of the case of clusters number/vine in the first season, where non-significant differences between the treatments were observed. On the other hand, the least values of Flame Seedless grapevines yield/vine (10.1 & 10.2 kg/vine), clusters number/vine (30 & 30) cluster weight (335 & 347g), cluster length (16.1 & 16.7cm), and cluster width (12.2 & 12.1 cm) were observed for the un-inoculated vines, during the two experimental seasons respectively .

The positive effect of bio-fertilization on enhancing yield and its component was observed by **Ibrahim *et al.*, (2009); Shaheen *et al.*, (2013); Rozpara *et al.*, (2014) and Reddy *et al.*, (2016)**. Furthermore, the inoculation with nitrogen fixing bacteria (i.e., *Azotobacter cholococcum, Azospirilum brasilense, Azospirilum lipoferum, Sinorhizobium spp., Burkholderia spp.,* and *Pseudomonas spp.*) significantly improved yield of multiple horticulture important crops including fruit trees (**Farag, 2006 a&b; Shata *et al.*, 2007; Carvajal-Munoz & Carmona-Garcia, 2012; Leksono & Yanuwiadi, 2014; Mosa *et al.*, 2015; Shamseldin *et al.*, 2016; Ahmed & Mohamed 2018 and Hammad *et al.*, 2020).**

2- Effect on Berries physical properties

Data presented in Tables (3) shows the effect of AMF, AZSB and AZBB inoculations, each one alone or in combinations, on berries physical properties of Flame seedless grapevines grown under clay soil, during 2017 and 2018 seasons. This Table showed that a significant increase in berry physical properties (berry weight (g), berry volume (cm^3), berry longitudinal (cm), and berry equatorial (cm) compared to the control treatment (un-inoculated vines), during the two experimental seasons. However, the best values in this respect were obtained from the vines received the mixture of the three microorganisms (AMF + AZSB + AZBB) in combination (3.21 & 3.88g for berry weight; 3.08 & 3.27 cm^3 for berry volume; 1.49 & 1.52 cm for berry longitudinal and 1.44 & 1.49 cm for berry equatorial), compared to the other inoculations under study during the two experimental seasons.

It is evident from the same Table that any combined inoculation of AMF, AZSB and AZBB was superior than inoculate the vines with any one alone, in both experimental seasons. On the opposite side, un-inoculated vines produced the minimized values of berries physical properties (2.42 & 2.48 g for berry weight; 1.98 & 2.04 cm3 for berry volume; 1.27 & 1.28 cm for berry longitudinal; 1.24 & 1.26 cm for berry equatorial) during the two experimental seasons respectively.

The role of AMF, AZSB and AZBB as a biofertilizers treatment in improving berry physical properties, which obtained in the present study, was in accordance with the results of some other studies such as those obtained by **Fathi *et al*., (2002); Abdel-Hamid *et al*., (2004); Farag (2006a); Ibrahim *et al*., (2009); Carvajal-Munoz & Carmona-Garcia (2012); Ibrahim and Gad El-Kareem (2014); Mosa *et al*., (2014); Bargaz *et al*., (2018); Ahmed & Ahmed (2020) and Hammad *et al*., (2020).**

Table 2. Effect of AMF, AZSB and AZBB inoculations on cluster number/vine, cluster weight (g), and yield (kg)/vine, as well as cluster dimensions of Flame Seedless grapevines during 2017 and 2018 seasons

Treatments	Clusters number/vine		Cluster weight (g)		Yield (kg/vine)		Cluster length (cm)		Cluster diameter (cm)	
	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
Control	30	30	335	347	10.1	10.2	16.1	16.7	12.2	12.1
Mycrrohiza	31	34	438	442	13.6	15.0	19.9	21.9	14.1	15.2
Azospirilum	30	32	409	439	12.3	14.1	19.5	20.3	13.6	13.9
Azotobacter	31	31	412	423	12.8	13.1	19.2	21.3	14.0	14.4
Mycrrohiza + Azospirilum	30	39	451	457	13.5	17.8	22.3	25.2	14.9	15.1
Mycrrohiza + Azotobactre	30	37	452	455	13.6	16.8	23.1	23.9	15.4	15.9
Azospirilum + Azotobactre	31	37	441	449	13.7	16.6	22.0	22.2	14.1	14.9
Mycrrohiza + Azospirilum + Azotobactre	31	39	462	465	14.4	18.1	25.1	26.8	15.8	16.9
New LSD 5%	NS	3.0	24.2	22.3	0.20	0.15	1.7	1.5	0.88	1.00

Table 3. Effect of AMF, AZSB and AZBB inoculations on berry physical properties of Flame Seedless grapevines grown under clay soil conditions during 2017 and 2018 seasons

Treatments	Berry weight (g)		berry volume (cm^3)		Berry longitudinal (cm)		Berry equatorial (cm)		Shape index	
	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
Control	2.42	2.48	1.98	2.04	1.27	1.28	1.24	1.26	1.02	1.02
Mycrrohiza	2.91	2.98	2.73	2.82	1.38	1.41	1.36	1.40	1.01	1.01
Azospirilum	2.88	2.93	2.73	2.88	1.42	1.44	1.40	1.41	1.01	1.02
Azotobacter	2.97	3.01	2.89	2.97	1.44	1.47	1.41	1.43	1.00	1.03
Mycrrohiza + Azospirilum	3.19	3.28	3.07	3.11	1.41	1.48	1.39	1.43	1.02	1.04
Mycrrohiza + Azotobactre	3.23	3.37	3.21	2.33	1.50	1.50	1.46	1.44	1.03	1.04
Azospirilum + Azotobactre	3.11	3.22	3.13	3.21	1.44	1.46	1.40	1.39	1.03	1.05
Mycrrohiza + Azospirilum + Azotobactre	3.21	3.38	3.38	3.27	1.49	1.52	1.44	1.49	1.03	1.02
New LSD 5%	0.13	0.15	0.11	0.12	0.03	0.02	0.03	0.04	NS	NS

3- Effect on Berries chemical properties

Data concerning the effect of AMF, AZSB, and AZBB inoculations on TSS% and reducing sugars% berries juice of Flame Seedless grapevines, grown under clay and sandy soils conditions, during 2017 and 2018 seasons are shown in Table (4).

Significant increase in juices total soluble solids, reducing sugars and total anthocyanin were obtained from the vines inoculated with AMF, AZSB and AZBB were recorded, during the two experimental seasons. The same Table declared that, during the two experimental seasons, inoculate the vines with AMF, AZSB and AZBB was associated with remarkable and significant decrease in juice total acidity, spatially when the vines inoculated with the mixture of the three examined microorganisms (AMF+AZSB+AZBB).

This decrement was clearer in the second season than those of the first season (Table 4). Furthermore, the same table declared that, increasing the TSS% and decreasing the total acidity lead to remarkable and significant increasing in the TSS/acidity ratio rather than un-inoculated vines, during the two experiment seasons.

The illustrated data declaring that, all combined inoculations were more effectiveness than the individual inoculation of each microorganism alone. The data was true for all chemical parameters, during the two experimental seasons (2017 & 2018).

Furthermore, in both seasons, the vines inoculated with the mixture of the three examined microorganisms (AMF + AZSB + AZBB) in combination gave the berries with highest TSS% (20.7% & 20.9%), reducing sugars% (18.9 & 19.1 %) and total anthocyanins contents (120 & 139 mg/100g F.W.) as well as low total acidity% (0.642 & 0.636%), during the two experimental seasons respectively. On the opposite side, the lowest TSS % (17.9 & 17.8 %), reducing sugars % (15.2 & 15.4%), and total anthocyanins contents (69 & 65 mg/100g F.W.), as well as highest total acidity% (0.780% & 0.784%), during the two experimental seasons respectively.

The role of AMF, AZSB, and AZBB as a bio-fertilizers treatments in improving berries chemical properties, which obtained in the present study was in accordance with the results of some studies carried out on some grapevines cultivars or other fruit spices, such as those obtained by **Abd El-Migeed *et al.*, (2006); Abou El-Yazied & Sellim (2007); Shata *et al.*, (2007); Ibrahim *et al.*, (2009); Carvajal-Munoz & Carmona-Garcia (2012); Mosa *et al.*, (2014); Reddy *et al.*, (2016); Wang *et al.*, (2017); Bargaz *et al.*, (2018); Ahmed & Ahmed (2020) and Hammad *et al.*, (2020).**

Table 4. Effect of AMF, AZSB and AZBB inoculations on berries chemical properties of Flame Seedless grapevines grown under clay soil conditions, during 2017 and 2018 seasons

Treatments	TSS %		Reducing sugars %		Total acidity %		TSS/acid Ratio		Total Anthocyanin (mg/100g F.W.)	
	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
Control	17.9	17.8	15.2	15.4	0.780	0.784	22.9	22.7	69	65
Mycrrohiza	19.1	19.4	16.7	16.9	0.745	0.738	25.6	26.3	83	92
Azospirilum	18.2	18.3	16.1	16.4	0.766	0.764	23.8	23.9	78	77
Azotobacter	18.0	18.4	16.0	16.5	0.768	0.767	23.4	24.0	73	79
Mycrrohiza + Azospirilum	20.2	20.4	18.1	18.8	0.659	0.641	30.7	31.8	99	104
Mycrrohiza + Azotobactre	19.2	19.5	17.2	17.6	0.619	0.606	30.5	32.2	101	109
Azospirilum + Azotobactre	19.2	19.9	17.7	17.9	0.722	0.651	26.6	30.6	97	106
Mycrrohiza + Azospirilum + Azotobactre	20.7	20.9	18.9	19.1	0.642	0.636	32.2	32.9	120	139
New LSD 5%	0.7	0.7	0.5	0.7	0.034	0.038	1.3	1.3	9.1	10.5

Conclusion

Based on Obtained results, it is may be strongly recommended to inoculate Flame Seedless grape vines grown under clay soil conditions in El-Minia Governorate and resembling conditions, with the mixture of AFM+AZSB+AZBB three times during vegetative growth cycle, in order to improve the yield and berries physical and chemical properties.

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