Bio-fertilizers as a partial substitute for mineral nitrogen and its effect on vegetative growth, yield and fruit quality of Thompson Seedless Grapevine

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ABSTRACT

This investigation was carried out during two successive seasons of 2008 and 2009 on 15- year old Thompson Seedless grapevines grown in clay soil at 1.8 x 2 m to study the effect of biofertilizers as alternative nitrogen source to mineral nitrogen fertilization in vineyards. Mineral nitrogen fertilization (MN) was added at 80, 60, 40, 20 and 0 units per feddan with adding different biofertilizers (BF) i.e., *Azotobacter chroococum, Azospirillum brasilens,* Biogen, Microben and Nitrobene at 0, 25, 50 and 100 g per vine, respectively. Biofertilizers application significantly improved the vegetative growth indices i.e., leaf area, shoot length, wood ripening and cane's carbohydrates content. Cluster weight and yield/ vine were improved with application of 60 unites MN per feddan + 25 g Microbien per vine. Fruit quality was improved in term of soluble solid content: titratable acidity ratio, whereas nitrate and nitrite contents in berries juice were significantly reduced through using biofertilizers.

Key words: Grapevine, biofertilizers, yield, fruit quality

1. INTRODUCTION:

Grape (*Vitis vinifera* L.) ranks first among fruit crops grown all over the world. It is the second fruit crop in Egypt after citrus and mainly consumed as fresh table grapes. One of the most important problems facing grape growers concerning the use of mineral nitrogen fertilizers are the high cost of the manufactured fertilizers needed and shortage in these fertilizers. The mineral nitrogen fertilizers cause a major pollution of ground water with nitrate and accumulate harmful residual substances, such as nitrate and nitrite in berries and leaves of grapevines [Ibraheem, 1994; Montaser et al, 2003]. Using the bio-fertilizers is considered a promising alternative for chemical fertilizers. It is very safe for human, animals and environment [Suba-Rao, 1984; Abdel-Hamid, 2002; El-Akkad, 2004]. In addition, the bio-fertilizers

play a major role in facilitating the fixation of atmospheric N as well as activating the availability uptake and translocation of most nutrients, that accelerate carbohydrate and protein synthesis and movement leading to encouraging cell division and the development of meristematic tissues. Moreover, it enhances the resistance of plants to root diseases and controlling vegetative growth of trees, leading tale improve its productivity [Suba-Rao, 1984; Gaur et al, 1980]. Bio-fertilizers application improves plant growth, fruit yield and chemical composition through the exertion of plant promoting substances mainly IAA, gibberellic acid and cytokinin like substances, vitamins and amino acid content [Abd El-Mouty et al, 2001]. Supplying various grapevine cultivars with bio-fertilizers only or beside mineral-N source caused a pronounced increase in vegetative growth and nutritional status of vines as well as yield components, cluster traits and berry quality [Abdel-Hady, 2003; Abbas et al, 2006; Mostafa, 2008].
This study was carried out to evaluate the partial substitute of biofrtilizers Viz, *Azotobacter chroococum*, *Azospirillum brasilens*, Biogen, Microben and Nitrobene as alternative fertilizer source for mineral nitrogen fertilizer (Ammonium nitrate 33.5%) at different levels on growth productivity fruit quality of Thompsons Seedless grapevine cultivar.

2. MATERIALS AND METHODS:

This study was carried out during two successive seasons of 2008 and 2009 on 15 years old Thompson Seedless grapevines, planted in a clay soil at 1.8 x 2 meters. The vines were trained on four wires system and cane pruned. The experiment consists of 21 treatments arranged as a factorial experiment with two factors (source of biofertilizers and its application levels) in a complete randomize block design, each treatment include three replicates of two uniform vines. The vines were pruned to 6 canes with 12 eyes at each cane along with 6 renewal spurs, in all 48 eyes were left per vine. Mineral nitrogen was added at rate of 0, 20, 40, 60 and 80 units per feddan and the selected vines were (in both seasons), inoculated with microbial inoculums at rate of 100, 75, 50 and 0 g /vine, respectively. The biofertilizers (BF) were thoroughly mixed with sand then applied for vines and then covered with soil then supplied with water. Equal doses for each rate of ammonium nitrate (33.5% N) (MN) and biofertilizer source (BF) was added at growth onset (15 and 20 April at 2008 and 2009 seasons, respectively), and added again at full bloom stage (15 and 25 May at 2008 and 2009 seasons). The control vines received only the recommended mineral nitrogen dose as 80 units N per feddan. Other horticultural practices needed for grapevines (P and K fertilization, irrigation, weed and pest control as well as winter pruning) were applied for all vines as practically followed in the experimental vineyard). Regarding the effect of the tested treatments on vegetative growth, 12 shoots / vine was tagged during the growing seasons. Fifteen leaves per vine were randomly collected from the first fully mature leaves from the top of the previously tagged shoots and the leaf area was measured using a planimeter at the end of both growing seasons. The tagged shoots were used to follow up their length and measured at the end of each season (September) and the rate of wood ripening was calculated by dividing length of the ripened part (brown color) by the total length of the shoot [Rizk and Rizk, 1994]. Samples of 0.2 g from fine powder of dried canes were taken from the middle part of the canes at winter pruning. Total carbohydrates content was determined [Schaffer and Hartman, 1921] and calculated as g/100g dry weight. At harvest time, (15 and 25 July in the two seasons, respectively) total yield (kg per vine) and yield per feddan (ton/Fed.) were determined. A sample of five clusters was randomly taken from the harvested yield of each replicate for quality determination. In each sample, cluster weight was determined and soluble solids content (SSC) was measured as a percentage in juice of mature fresh berries, by Carlsize hand refractometer. Titratable acidity (TA) was determined by titrating 10 ml of clear juice against 0.1 N NaOH using phenolphethalein as an indicator and expressed as gram of tartaric acid in 100 ml juice. SSC: TA ratio was calculated. Nitrate and nitrite contents in fresh berry juice were determined [Singh, 1988]. The randomized complete blocks design arranged as a factorial experiment was applied to analyze the obtained data [Sndedecor and Cochran, 1972]. Means for treatments were compared by Duncan's Multiple Range Tests at 5% level of probability.

3. RESULTS AND DISCUSSION:

Data in Table (1) reveal that Azospirillum application induced short shoot length, higher wood ripening, large leaf area and high cane carbohydrates content compared with the other BF sources. Biofertilizers application significantly increased the vegetative growth indices i.e., leaf area and shoot length of inoculated vines compared with the control vines. The data also revealed that 75 BF + 20 MN treatments had higher vegetative growth indices as compared with the other treatments in the two seasons. Similar results had been reported previously [Mahmoud and Mahmoud, 1999; AbdEl-Naby and Gomaa, 2000; Sudhakar et al 2000; Khalil et al 2011]. They all concluded that, using biofertilizers may increase growth indices of the inoculated trees. The beneficial effect of biofertilizer in this respect may be attributed to its effect on increasing nitrogen fixation, production of growth promoting substances or organic acids, enhancing nutrient uptake or protecting vines against certain pathogens [Samah, 2002]. Moreover, it is reported that the increment of plant growth due to inoculation with N fixed bacteria could be attributed to the capability of these organisms to produce growth regulators such as auxins, cytokinins and gibberellins which affect production of root biomass and nutrients uptake [Abou El-Khashab, 2002].

It is clear from Table (2), that Microben biofertilizers significantly increased clusters weight, yield / vine and yield/ feddan compared with the other biofertilizers. In both seasons, the higher values of clusters number, cluster weight, yield / vine and yield / feddan were recorded by vines treated with microbial inoculated compared with the recommended dose of mineral fertilizers. The highest values of clusters number and yield per vine were recorded for 75% BF + 20 MN treatments in the two seasons. The positive effect of biofertilizers may due to the role of free nitrogen fixing bacteria in producing adequate amounts of growth regulators, improving availability of nutrients which promoted cell division and cell enlargement [Gaur et al, 1980] and the vegetative growth to go forward and then affect yield as well as clusters weight. These findings go in line with those previously obtained [Abdel-Hady, 2003; El-Sabagh et al, 2011; Abd El-Monem et al, 2008; Ibrahim, 2009]. The improvement occurred in vines growth and nutritional status certainly reflected their effect on improving yield as well as clusters weight.

Data in Table (3) reveal that the SSC: TA ratio was significantly increased by application of Azotobacter compared to the other BF sources, as a result of increasing SSC and decreasing TA. The highest SSC: TA ratio was showed in 25 BF + 60 MN treatments. The positive action of biofertilizers on the quality of the berries could be attributed to their effect on increasing carbohydrates and accelerating cluster ripening. These results were supported by the previous results [Shaheen et al, 2012; Mostafa, 2008; 27; 28]. The obtained data revealed also that application of Microbien significantly reduced nitrite and nitrate percentages in the berry juice compared with the other BF sources.

Application of 25 BF + 60 MN markedly reduced nitrite and nitrate berry contents. These results were emphasized by previous results [**Montaser et al, 2003**, **Abd El-Monem et al, 2008;** 28]

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الأسمدة الحيوية كبديل جزئي للنيتروجين المعدني وأثرها على النمو الخضري والمحصول وجودة ثمار صنف العنب طومسون سيدلس

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أجريت هذه التجربة لدراسة تقليل التسميد النتروجينى المعدني من ٨٠ الى ٦٠، ٤٠، ٢٠، ٠ وحدة نتيروجين معدنى للفدان بتكملة إضافات مختلفة من التسميد الحيوى مثل بكتيريا الازوتوباكتر والازوسبيريللم والبيوجين والميكروبين والنتروبين بمعدلات: صفر، ٢٥، ٥٠، ٧٥، ١٠٠ جرام لكل شجيرة على التوالي وتأثير ذلك على النمو الخضري ومحصول وجودة ثمار صنف العنب طومسون سيدلس. نفذت هذة التجربة على شجيرات عمره ١٥عاما خلال موسمى ٢٠٠٨، ٢٠٠٩ منزرعة فى أرض طميية على مسافة ١.٨* ٢ م ومرباة تربية قصبية على أربعة أسلاك. أظهرت النتائج أن إضافة الأسمدة الحيوية أدت إلى تحسن معنوي في مؤشرات النمو الخضري المتمثلة في مساحة الورق وطول الأفرع ونسبة الخشب الناضج ومحتوي القصبات من الكربوهيدرات. كما أدت إضافة ٢٥ جم ميكروبين للشجيرة + ٦٠ وحدة نيتروجين معدني للفدان إلى زيادة وزن العنقود ومحصول الشجيرة وأن إستخدام الأسمدة الحيوية أدى الى تحسن جودة الثمار من خلال زيادة نسبة المواد الصلبة الذائبة: الحموضة الكلية وإنخفاض محتوي العصير من كل من النترات والنيتريت.

الكلمات المفتاحية: الكرمة، الأسمدة الحيوية، والغلة، جودة الثمار

Table 1: Effect of biofertilizers sources and partial substitute for mineral nitrogen on some vegetative growth incidence of Thompson Seedless grapevines (2008 and 2009 seasons).

Treatments	Leaf area (cm^2)		Shoot length (cm)		Wood ripening (%)		Carbohydrates cane content (%)	
	2008	2009	2008	2009	2008	2009	2008	**2009**
A: Bio-fertilization								
Microbien	152.3 c	158.9 b	156.3 b	155.7 b	81.91 b	83.08 b	18.04 d	**18.97 c**
Azotobacter	140.7 e	139.5 d	152.5 d	151.7 d	81.98 b	80.96 d	19.02 a	**19.22 ab**
Azospirillum	156.1 b	162.7 a	151.8 e	151.0 e	82.74 a	83.86 a	18.48 b	**19.30 a**
Biogen	163.3 a	162.1 a	155.6 c	154.8 c	79.83 d	80.40 e	18.34 c	**19.09 bc**
Nitrobien	144.1 d	149.4 c	159.5 a	156.9 a	81.46 c	82.02 c	18.48 b	**19.29 a**
B: Doses of bio and mineral nitrogen								
0 BF + 80 MN	143.8 d	143.0 c	149.2 e	149.3 d	80.53 d	80.23 d	18.28 c	**18.57 c**
25 BF +60 MN	154.7 c	160.1 b	156.1 c	154.9 c	78.03 e	79.29 e	16.97 e	**18.19 d**
50 BF +40 MN	136.8 e	141.7 c	161.1 a	158.7 a	82.03 c	82.86 c	18.79 b	**19.31 b**
75 BF +20 MN	162.1 a	167.6 a	158.8 b	157.5 b	84.37 a	84.38 a	20.21 a	**21.28 a**
100 BF + 0MN	**159.2 b**	**160.2 b**	**150.5 d**	**149.7 d**	**82.96 b**	**83.57 b**	**18.10 d**	**18.51 c**

- Means followed by the same letter (s) in the same column are not significantly different by DMRT at 0.05. *BF :biofertelizer source (g / vine), MN: mineral nitrogen (unit / feddan).

Table 2: Effect of biofertilizers sources and partial substitute for mineral nitrogen on number of clusters per vine, cluster weight (g), yield per vine (kg) and yield per feddan (ton) of Thompson Seedless grapevines (2008 and 2009 seasons).

Treatments	Number of cluster per vine		Cluster weight (g)		Yield per vine (kg)		Yield per feddan (ton)	
	2008	2009	2008	2009	2008	2009	2008	2009
Bio-fertilization (A)								
Microbien	14.6 c	15.7 b	546.8 a	522.4 a	7.9 a	8.1 a	8.6 a	8.9 a
Azotobacter	17.0 a	17.6 a	456.7 c	431.9 c	7.5 b	7.4 b	8.2 b	8.1 b
Azospirillum	14.4 c	14.4 d	394.9 e	398.0 e	5.5 e	5.7 d	6.1 e	6.2 d
Biogen	15.0 b	14.9 c	480.1 b	456.3 b	6.9 c	6.7 c	7.5 c	7.4 c
Nitrobien	13.8 d	14.7 c	443.3 d	423.3 d	6.2 d	5.8 d	6.8 d	6.3 d
Doses of bio and mineral nitrogen (B)								
0 BF+ 80 MN*	11.3 d	13.9 d	442.5 c	406.7 e	5.3 c	5.5 e	5.8 d	6.0 e
25 BF + 60 MN	14.5 c	14.7 c	462.5 b	459.2 b	6.6 b	6.7 d	7.3 c	7.4 d
50 BF + 40 MN	16.8 a	16.6 b	456.1 b	440.1 d	7.4 a	7.2 b	8.1 a	7.9 b
75 BF +20 MN	16.6 a	17.5 a	460.7 b	451.6 c	7.3 a	7.4 a	7.9 b	8.1 a
100 BF + 0 MN	15.6 b	14.6 c	500.0 a	474.3 a	7.4 a	6.9 c	8.1 a	7.5 c

- Means followed by the same letter (s) in the same column are not significantly different by DMRT at 0.05. *BF :biofertelizer source (g / vine), MN: mineral nitrogen (unit / feddan).

Table 3: Effect of biofertilizers source and partial substitute for mineral nitrogen on some characters of fruit quality of Thompson Seedless grapes (2008 and 2009 seasons).

Treatments	Soluble solid content (%)		Titriable Acidity (%)		SSC: TA ratio		NO_3 content (ppm)		NO_2 content (ppm)	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	**2009**
Bio-fertilization (A)										
Microbien	19.7 c	19.1 c	0.524 a	0.497 b	37.75 d	38.62 d	13.17 c	13.13 c	0.29 c	**0.27 c**
Azotobacter	20.7 a	20.6 a	0.479 d	0.477 d	43.34 a	43.38 a	9.39 e	9.82 e	0.23 d	**0.20 d**
Azospirillum	19.3 d	19.1 c	0.509 b	0.514 a	37.97 d	37.22 e	10.06 d	10.32 d	0.23 d	**0.20 d**
Biogen	20.4 b	20.2 b	0.499 c	0.512 a	41.10 c	39.59 c	15.44 b	15.48 b	0.39 b	**0.33 b**
Nitrobien	20.8 a	20.5 a	0.498 c	0.484 c	41.94 b	42.54 b	18.69 a	16.85 a	0.45 a	**0.39 a**
Doses of bio and mineral nitrogen (B)										
0 BF+ 80 MN	18.9 d	19.7 d	0.503 b	0.504 b	37.53 c	39.04 d	26.42 a	30.36 a	0.74 a	**0.66 a**
25 BF + 60 MN	21.3 a	20.6 a	0.489 d	0.487 d	43.86 a	42.31 a	8.65 e	7.20 d	0.23 b	**0.18 c**
50 BF + 40 MN	21.3 a	20.3 b	0.491 c	0.490 c	43.51 a	41.62 c	10.52 c	9.17 c	0.22 bc	**0.18 c**
75 BF + 20 MN	20.0 b	20.0 c	0.503 b	0.478 e	39.96 b	42.04 b	9.77 d	9.55 b	0.19 d	**0.16 d**
100 BF + 0 MN	**19.4 c**	**18.9 e**	**0.523 a**	**0.524 a**	**37.23 c**	**36.33 e**	**11.39 b**	**9.32 c**	**0.21 c**	**0.21 b**

- Means followed by the same letter (s) in the same column are not significantly different by DMRT at 0.05. *BF :biofertelizer source (g / vine), MN: mineral nitrogen (unit / feddan).