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EFFECT OF SOME AZOSPIRILLUM STRAINS AS A PARTIAL REPLACEMENT OF INORGANIC N FERTILIZERS ON YIELD AND QUALITY OF SUPERIOR GRAPEVINES

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ABSTRACT: *About 91 mutants were obtained from exposing Azospirillum brasilense to different concentrations of Acriflavine mutagen and different time exposure. Out of those three new N microbial strains namely Methionineless, Threonineless and Tyrosineless were tested on Superior grapevines after their showed highest growth on artificial media. Superior grapevines were supplied with these microbial strains at 5 to 20 ml/vine and three slow release fertilizers (Methylene urea, Sulphur coated urea and urea formaldehyde) at 25 to 75% as partial replacement of the fast release N fertilizer namely ammonium nitrate during 2015 and 2016 seasons.*

Supplying Superior grapevines with N through 50 to 75 % inorganic N + 25 to 50% slow release N fertilizers (Methylene urea, Sulphur coated urea and urea formaldehyde) + bacteria strains (Methionineless, Threonineless and Tyrosineless) at 5 to 10 ml/vine proved to be very effective in stimulating all growth traits, leaf pigments and nutrients , berry setting %, yield and quality of the berries relative to using N as 100 % mineral N or when mineral N was added at 25%. An obvious reduction was observed on both nitrate and nitrite in the juice, with reducing percentages of mineral N from 100 to 25% as well as increasing percentages of the three release N fertilizers from 25 to 75% and the three bacterial strains from 5 to 20 ml/vine. The best bacterial strains in this respect were Methionineless, Theronineless and Tyrosineless, in ascending order. The best slow release fertilizer was methylene urea followed by sulphur coated urea.

Amending Superior grapevines with N as 50% inorganic N (ammonium nitrate) + 50% slow release N fertilizer (methylene urea) + Tyrosineless at 10 ml/vine was responsible for promoting yield and fruit quality.

Key words: *Fast and slow release N fertilizers, bacterial strains, growth, yield, berries quality, Superior grapevines.*

INTRODUCTION

Superior grapevines cv. is considered a prime and favourite grape cv successfully grown under Egypt conditions. Due to its early in ripening season cv. it has a greater potentially for export to foreign markets. For enhancing export efficiency and protecting our environment from pollution, it is reliable to adjust the amount of mineral N.

Nitrogen fertilization is considered an important and limiting factor for fruiting of different grapevine cvs. Using N in the form of mineral at higher amounts causes severe damage to environment and could result in enhancing growth of the vines at the expense of fruiting status.

Nowadays, slow release N fertilizers, plant wastes and biofertilizers were used as partial replacement of mineral N fertilizers in most fruit orchards. In organic farming system microorganisms were used as biological for sustainable agriculture (Wani and Lee, 1995). Application of chemical fertilizers in the fruit orchards causes some problems especially for exportation. It is well known that nitrogenous fertilizers are lost via nitrate reduction, denitrification and ammonia volatilization. Moreover, some nitrogenous fertilizers can be leached to the surface and underground water causing environmental pollution (Attia, 1990). Mahendran and Chandramani, (1998) reported that dual inoculation of plants with the above mentioned microorganisms increased soil availability of N, P and K.

The economical point account, and the high prices of chemical fertilizers may increase the production costs. Therefore, the use of biofertilizers and organic manures of particular interest to avoid the previously mentioned problems. Biofertilizers application with a half dose of chemical nitrogen fertilizer provided to be an efficient tool in increasing the availability of nutrients in soil as well as growth performance and yield of cultivated crops is improved. Several investigators indicated that inoculation with *Azotobacter* and *Azospirillum* improved growth and yield of horticultural crop (Kannaiyan, 2002).

At one time, it was thought that the counterpart of *Azospirillum*, which is found in legumes had been found for cereals, and by exploiting the capabilities of *Azospirillum Sp*. it would be possible to supply nitrogen to crops of economic importance (Klingmuller, 1982).

The search for a mechanism by which *Azospirillum* promotes plant growth is somewhat more complicated (Vande Broek and Vanderleyden, 1995). *Azospirillum* mutants have been induced by chemical radiation, Tn_5 mutagenesis and gene replacement. Using N- methyl-N-Nitro-N nitroguanidine mutagenesis, NIF-mutants, defective in nitrogen fixation (Pedrosa and Yates, 1984). Tn_5 nutagenesis has been used to obtain auxotrophic and IAA-over producing mutants of *Azospirillum* (Klingmuller, 1982).

During the last few years, several controlled –release N fertilizers were developed mainly to improve the efficiency of N used by plants, minimize the loss of nutrients via leaching and to reduce to the lower extent the great pollution occurred in our environment (Wang and Alva, 1996). They considered new approaches for amending the plants with their requirements from nutrients at the optimum rate.

Inoculation with Argininelss 1, methionineless, Threonineless, Alanineless, Adenineless, Prolineless, Thymineless, Lysineless, Agrginineless 2 and Histidineless 2, in ascending order was favourable for improving growth, yield and quality parameters of Banaty grapevines. Putting in our consideration the yield and fruit quality, fertilizing mature Banaty grapevines with N at 100 g/vine through the *Azotobacter vinelandii* mutant Histidineless 2 at 100 ml/vine plus 150 g ammonium nitrate/vine proved to be very effective for obtaining high and better fruit quality. (Uwakiem, 2006 and Ahmed *et al.,* 2007).

Organic and biofertilzation when used as a partial replacement of inorganic N fertilizers were responsible for enhancing N fixation, organic matter, water retention, availability of nutrients, root development, the biosynthesis of hormones and antibiotics and reducing soil pH (El-Sisy, 2000; Kannaiyan, 2002 and Cabrera *et al*., 2003).

Shaaban, (2014) pointed out that supplying Superior grapevines with mineral N, P and K fertilizers at 50% plus 50% plant compost, rock phosphate, feldspar and biofertilzation namely Biogen, phosphorin and potassiumage was very effective in improving growth and fruiting over the application of mineral N alone. Previous studies showed that using organic and biofertilization as partial replacement of inorganic N fertilizers was very beneficial in improving growth, vine nutritional status, yield and berries quality (Dakhly *et al.,* 2007; Uwakiem, 2011; Abou El-Lail, 2012; Allam-Aida *et al*., 2012; Shaheen *et al*., 2013; Ahmed *et al*., 2014; Abd El-Kareem, 2014; El-Wany, 2015; Abd El-Reheem, 2015; Aly-

Samar, 2015; Tony, 2016; Ahmed *et al*., 2017 and El-Kady-Hanaa, 2017).

Slow release N fertilizers were responsible for improving yield and berries quality in different fruit crops (Ibrahim-Asmaa, 2001; Gobara *et al*., 2003 and Ahmed and Abada, 2012).

The target of this study was examining the effect of some newly microbial strains produced from *Azospirillum brasilense* and some slow release N fertilizers as partial replacement of mineral N fertilizers on fruiting of Superior grapevines.

MATERIALS AND METHODS

This study was carried out during the two consecutive seasons of 2015 and 2016 on sixty uniform in vigour 8-years old Superior grapevines grown in a private vineyard located at El-Hawarta Village, Minia district, Minia Governorate where the soil texture is clay (Table 1) and well drained water since water table depth is not less than two meters. The chosen vines are planted at 2 x 3 meters apart. Cane pruning system was followed at the first week of January leaving 84 eyes per vine (on the basis of six fruiting canes x 12 eyes plus six renewal spurs x two eyes) with the assistance of Gable shape supporting system. The vines were irrigated through surface irrigation system using Nile water.

Except those dealing with the present treatments (all sources of N and biofertilization) , all the selected vines (60 vines) received the usual horticultural practices which are commonly used in the vineyard.

1- Production of different N microbial strains:

Lab. Experiment was carried out during the 2014 at the biofertilizer center of Fac. of Agric., Minia Univ.

1-1 The used microorganisms:

Azospirillum brasilense and their mutants were provided from the biofertilizers center of Fac. Agric. Minia Univ.

2- Inocula preparation:

For preparation of *Azospirillum brasilense* and their mutants inocula in malate medium (in grams per litter: KH_2Po_4 0.4; K_2 $HP0_4$ 0.1; Mg $So_4.7H_2O$ 0.2 ; NaCl 0.1; CaCl 0.02; $FeCl_3$ 0.01; $Na_2MO_4.2$ H_2O 0.002; sodium malate 5.0; yeast extract 0.05 and pH 7. 2- 7.4) was used.

Minimal medium (MM) of Mckenney and Melton, (1986) was used to isolate the auxotrophic mutants of *Azospirillum*, composed of Mannitol 10.0 gm, K_2HP0_4 0.5gm, Mg 50_4 $7H_2O$ 0.20 gm, NaCl 0.20 gm, Mn $S0_4.4$ H_2O trace, $FeCl_3$ trace, N_2 molybdate trace, $CaCO_3$ 5.0 gm, Agar 20.0 gm and distilled water up 1000 ml.

3- Inoculation process:

Each vine inoculated with 5,10 and 20 ml from cell suspension diluted in 2 liter water and then the vines were irrigated.

This study included the following ten treatments:

1. Using N as 100% Mineral Nitrogen (MN) (179.1g ammonium nitrate).
2. Using N as 75% MN (134.4 g ammonium nitrate) + 25% $slow_1$ Ureaformaldehyde (41% N) (36.6 g) + $strain_1$ (Methionineless) at 5 ml/vine
3. Using N as 50% MN (89.6 g ammonium nitrate) +50% $slow_1$ Ureaformaldehyde (41% N) (73.2 g) + $strain_1$ (Methionineless) at 10 ml/vine
4. Using N as 25% MN (44.8 g ammonium nitrate) +75 %$slow_1$ Ureaformaldehyde (41% N) (109.8 g) + $strain_1$ (Methionineless) at 20 ml/vine
5. Using N as 75% MN (134.4 g ammonium nitrate) + 25% $slow_2$ (Sulphur – coated urea (41% N) (36.6 g) + $strain_2$ (Threonineless) at 5 ml/vine
6. Using N as 50% MN (89.6 g ammonium nitrate) + 50% $slow_2$ (Sulphur – coated urea (41% N) (73.2 g) + $strain_2$ (Threonineless) at 10 ml/vine
7. Using N as 25% MN (44.8 g ammonium nitrate) + 75% $slow_2$ (Sulphur – coated urea (41% N) (109.8 g) + $strain_2$ (Threonineless) at 20 ml/vine

8. Using N as 75% MN (134.4 g ammonium nitrate) + 25% $slow_3$ (Methylene urea (22% N) (68.2 g)+ $strain_3$ (Tyrosineless) at 5 ml/vine
9. Using N as 50% MN (89.6 g ammonium nitrate) + 50% $slow_3$ (Methylene urea (22% N) (136.4 g)+ $strain_3$ (Tyrosineless) at 10 ml/vine
10. Using N as 25% MN (44.8 g ammonium nitrate) + 75% $slow_3$ (Methylene urea (22% N) (204.6 g)+ $strain_3$ (Tyrosineless) at 20 ml/vine

Each treatment was replicated three times, two vines per each. Ammonium nitrate (33.5 % N) as a source of inorganic N was divided into three unequal batches as 40% at growth start (1st week of March), 40% just after berry setting (3rd week of April) and 20 % 30 day after harvesting. All bacterial strains were added once in shallow holes 20 cm apart from the trunk and covered with moist soil. The three slow release fertilizers $Slow_1$= Ureaformaldehyde (41% N) , $Slow_2$= Sulphur – coated urea (41% N) and $Slow_3$= Methylene urea (22% N) were added once before growth start (1st week of March) 50 cm far from the vine trunk in drenches (50×50×50 cm dimensions). The organic fertilizers namely plant compost (2% N) was added at fixed rate namely 0.25 kg / vine. It was added once just after winter pruning (3^{rd} of January) in shallow holes 20 cm a part from the trunk and covered with soil. Analyses of plant compost are given in Table (2).

Randomized complete block design (RCBD) was followed (Rangaswamy, 1995), where the experiment consisted of ten treatments, each treatment was replicated three times, two vines per each.

During both seasons, the following measurements were recorded:

1. Growth aspects namely main shoot length (cm), leaf area $(cm)^2$ (Ahmed and Morsy, 1999), pruning wood weight/vine, wood ripening coefficient (Bouard, 1966) and cane thickness (cm)..
2. Leaf chemical components namely chlorophylls a, b, total chlorophylls (mg/1g F.W) (Von- Wettstein, 1975), N, P and K (Wilde *et al.* 1985 and Balo *et al,* 1988).
3. Percentage of berry setting.
4. Percentage of total carbohydrates in canes (Smith *et al.*,1956).

Table (1): Analysis of the tested soil:

Constituents	Values
Particle size distribution:	
Sand %	7.0
Silt %	21.5
Clay %	71.5
Texture	Clay
pH(1:2.5 extract)	7.95
EC (1 :2.5 extract) (dsm^{-1}) 1 cm / 25ºC.	0.97
O.M. %	2.01
$CaCO_3$ %	2.41
Total N %	0.11
Available P (Olsen, ppm)	3.11
Available K (ammonium acetate, ppm)	405.9

Table (2): Analysis of plant compost.

Parameters	Values
Cubic meter weight (kg.)	600.0
Moisture %	29.0
Organic matter %	30.7
Organic carbon %	28.56
pH (1: 2.5 extract)	27.25
EC (dsm^{-1}) (1: 2.5 extract)	10.25
C/N ratio	14.28
Total N %	2.0
Total P %	1.02
Total K %	1.21
Total Ca %	1.25
Total Mg %	1.30
Total Fe (ppm)	18.5
Total Mn (ppm)	37.55
Total Zn (ppm)	43.22
Total Cu (ppm)	17.40

5. Yield expressed in weight (kg.) and number of clusters/vine as well as cluster weight and dimensions (length and shoulder).
6. Percentages of shot berries.
7. Physical and chemical characteristics of the berries namely weight, longitudinal and equatorial, T.S.S. %, total acidity%, reducing sugars% (Lane and Eynon,1965 and A.O.A.C, 2000) and nitrite in the juice (ppm) (Ridnour-Lisa *et al*., 2000), total count of bacteria in the soil was counted (cfug)/1.0g soil (Cochran,1950).

Statistical analysis was done and treatment means were compared using new L.S.D. at 5% (according to Rao, 2007).

RESULTS AND DISCUSSION

1-Number and percentages of *Azospirillum brasilense* survivals after treatment with different concentrations of Acriflavine mutagen:

It is clear from the obtained data in Tables (3 and 4) and Fig. (1) that exposing *Azospirillum* wild type to Acriflavine for three concentrations was accompanied with decreasing numbers and percentages of *Azospirillum brasilense* survivals compared to control (non treatment).

Treating *Azospirillum brasilense* with Acriflavine produced 2500 number and 100 percentage of survival. However, numbers of survivals were 500, 150, 50 after treatment with Acriflavine for 0.01, 0.05 and 0.10 mg/ml cell suspension however,percentage of survivals recorded 20.0, 6.0 and 2.0 percentage of survivals, respectively. Many nif^- mutants have been characterized biochemically (Roberts and Brill, 1981). Santero *et al.,* (1986) reported the isolation of mutants, selected on the basis of their chlorate resistance that are affected in a general control of nitrogen metabolism.

Fong and Bockrath, (1979) suggested that, at a concentration insufficient to alter survival of unirradiated cells of E.coli, Acriflavine (2 Mg/ml) inhibited both simglestrand deoxyribonucleic acid breakage and post replication repair after ultraviolet irritation.

Table (3): Number and percentages of *Azospirillum brasilense* survivals after treatment with different concentrations of Acriflavine for 1 and 2 hours.

Organism	AC. conc.	Time (hrs)	No. and percentage of survivals	
			No	%
Azospirillum brasilense	Control	1 (hr)	12500	100
	0.01		9725	77.80
	0.05		6250	50.00
	0.10		2200	17.60
	Control	2 (hrs)	12500	100
	0.01		5125	41.00
	0.05		1975	15.80
	0.10		0.000	00.00

Table (4): Percentages and Numbers of characterization single mutants resulted by concentrations of Acriflavine in *Azospirillum brasilense*

Time	AC. Conc	No.of colonies tested	No.of mutants	Mutants freq. %	No. and percentage of characterization					
					Meth-	His-	Arg-	Thr-	Tyro-	Ala-
1 (hr)	0.01	278	8	2.87	1	1	3	0	1	2
	0.05	350	10	2.85	2	2	1	1	2	2
	0.10	379	15	3.95	2	3	3	4	1	2
2 (hrs)	0.01	300	30	10	10	2	7	8	2	1
	0.05	250	28	11.2	3	5	6	3	3	8
	0.10	0	0	0.0	0	0	0	0	0	0

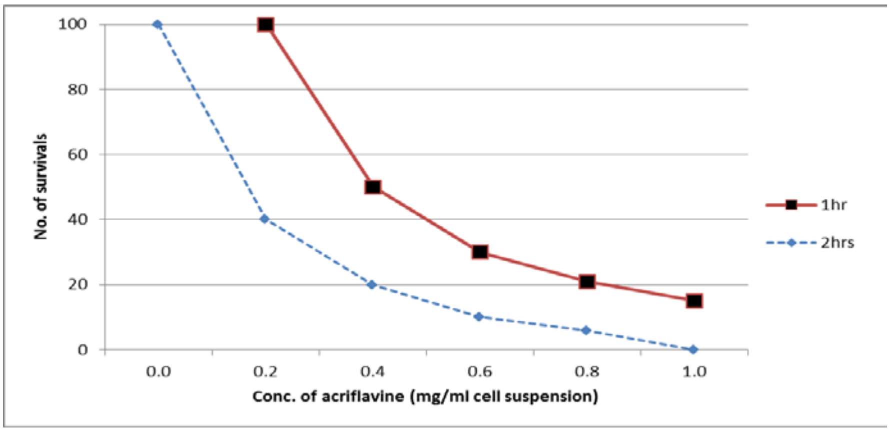

Figure (1): *Azosperillum brasilense* survived different acriflavine concentrations and incubations for 1 and 2 hours at 30ºc.

2- Some vegetative growth characteristics:

Data in Table (5) clearly show that the six growth aspects namely main shoot length, number of leaves/ shoot, leaf area, wood ripening coefficient, pruning wood weight and cane thickness were significantly affected by varying N management. Using mineral N (ammonium nitrate) at 50 to 75%, the three slow release N fertilizers (Methylene urea, sulphur coated urea and urea formaldehyde) at 25 to 50% + bacteria strains (Methionineless, Threonineless and Tyrosineless) each at 5 to 20 ml/ vine significantly were followed by enhancing the six growth characteristics namely main shoot length, number of leaves/ shoot, leaf area, wood ripening coefficient, pruning wood weight and cane thickness over the application of N as 100 % inorganic N alone or when mineral N was applied at 25% of the suitable N even with the application of slow release N fertilizers and the three namely N strains. Increasing percentages of inorganic N from 50 to 75 % and at the same time increasing percentages of the three slow release fertilizers from 25 to 50% and bacterial strains from 5 to 10 ml/vines significantly was followed by stimulating the previously mentioned six growth aspects. The best percentage of ammonium nitrate was 50%. The beneficial effects of these slow release N fertilizers on enhancing growth aspects can be arranged as follows in ascending order ureaformaldehyde, sulphur coated and methylene urea.

The best bacterial strains was Tyrosineless followed by Threonineless and Methionineless. The maximum values of shoot length (116.7 & 117.5 cm), number of leaves/shoot (27.0 & 27.8), leaf area (112.0 & 112.6 cm^2), wood ripening coefficient (0.81 & 0.83), pruning wood weight (2.41 & 2.50 kg) and cane thickness (1.40 & 1.42 cm) were recorded on the vines that received N as 50 % mineral N + 50 % methylene urea and Tryosineless N strains at 10 ml/vine. The lowest values of these growth traits were recorded on the vines that received N as 25% mineral N + 75 % the slow release N fertilizer namely ureaformaldehyde and the N strain namely methionineless at 20 ml/vine. These results were true during the two seasons.

3- Leaf and cane chemical components:

It is clear from the obtained data in Tables (6 & 7) that the supplying the vines with N as 50 to 75 % inorganic N + any one of the three slow release N fertilizer (Methylene urea, Sulphur coated urea and urea formaldehyde) + bacteria strains (Methionineless, Threonineless and Tyrosineless) each at 5 to 10 ml/vine significantly was followed by enhancing the seven chemicals namely cane total carbohydrates % as well as percentages of N, P, K, Mg, Ca and S in the leaves relative to using N via 100% MN or when N was added as 25 % MN.

There was a gradual promotion on these nutrients and total carbohydrates with reducing the percentages of inorganic N from 100 to 50 % and at the same time increasing the percentages of the three slow release N fertilizers from 25 to 50 % and bacterial strains from 5 to 10 ml/vine.

The best slow release fertilizer and N bacterial strains applied with mineral N was methylene urea and N strain namely Tyrosineless, respectively.

The maximum values of cane total carbohydrates (17.3 & 17.1 %), N (2.11 & 2.13), P (0.161 & 0.156 %), K (1.84 & 1.75 %), Mg (0.89 & 0.93), Ca (2.61 & 2.70%) and S (0.99 & 0.98 %) were recorded on the vines that received N as 50 % MN + 50 % methylene urea and Tyrosineless bacterial at 10 ml/vine during both seasons, respectively. The vines fertilized with N as 25 % MN + 75 % the slow release N fertilizers urea formaldyhde + bacterial strain namely methioneless at 20 ml/vine gave the lowest values . These results were true during both seasons.

Table 5

Table 6

Table 7

4- Leaf pigments:

As shown in Table (7) plant pigments namely chlorophyll a & b, total chlorophylls and total carotenoids were significantly varied among the ten N management treatments. These pigments were significantly enhanced in response to supplying the vines with N via 50 to 75 % MN + any one of the slow release N fertilizer Methylene urea, Sulphur coated urea and urea formaldehyde) + bacteria strains (Methionineless, Threonineless and Tyrosineless) each at 5 to 10 ml/vine relative to the application of N as 100 % inorganic N or when inorganic N was applied at 25 % even with the application of slow release N fertilizer each at 75 % and bacterial strains at 20 ml/vine. The three bacterial strains could be arranged as follows regarding their positive action on leaf pigments Methionineless, Threonineless and Tyrosineless in ascending order. The best results were observed due to using urea formaldehyde, sulphur coated urtea and methylene urea, in ascending order. The promotion was associated with reducing mineral N percentage from 100 to 50% and increasing percentages of slow release N fertilizers from 25 to 75 % and N bacterial from 5 to 10 ml/vine. Supplying Superior grapevines with N via 50 % mineral N + methylone urea at 50% + N bacterial strain namely Tyrosineless at 10 ml/vine gave the maximum values. The lowest values were recorded on the vines that supplied with N as 25 % MN + 75 % the slow release N fertilizers urea formaldyhde + bacterial strain namely methioneless at 20 ml/vine. Similar results were announced during the two seasons.

5- Berry setting %, yield and cluster aspects:

It is worth to mention from the data in Table (8) that berry setting %, yield expressed in weight and number of clusters /vine, weight, length and shoulder of cluster were significantly varied among the ten N management.

Amending the vines with MN at 50 to 75% + 25 to 50 % any slow release N fertilizer (Methylene urea, Sulphur coated urea and urea formaldehyde) + bacteria strains (Methionineless, Threonineless and Tyrosineless) each at 5 to 10 ml/vine had significant promotion on berry setting %, yield and cluster aspects relative to the application of N via MN alone or when MN was added via 25% even with the application of slow release N fertilizers and bacterial strains. The promotion was significantly associated with reducing inorganic N from 100 to 50% and at the same time increasing percentages of the slow release N fertilizers from 25 to 50 % and levels of bacterial strain from 5 to 10 ml/vine. A significant reduction on these parameters was detected with reducing the percentages of inorganic N from 50 to 25% regardless the application of slow release N fertilizer and bacterial strains. Using N as 100% inorganic N was significantly preferable than using N as 25 %inorganic N + 75% any slow release N fertilizers (Methylene urea, Sulphur coated urea and urea formaldehyde) + bacteria strains (Methionineless, Threonineless and Tyrosineless) at 20 ml/vine. The maximum yield/vine (8.87 & 11.9 kg/vine) during both seasons,respectively were recorded on the vines that received N as 50 % MN + 50% methylene urea + 10 ml tyrosineless bacterial strain. The vines treated with N completely via inorganic N form gave 7.5 & 9.0 kg during both seasons, respectively. The percentage of increment on the yield above the check treatment (using N as 100% N reached 13.5 & 31.5%) during both seasons, respectively. Number of clusters /vine in the first season was unaffected by the present treatments in the first season of study. The previous beneficial effect of slow release N fertilizers as well as organic and biofertilizers on growth and vine nutritional status surely reflected on improving berry setting and cluster weight consequently the yield.

Table 8

6- Percentage of shot berries:

Table (9) shows that shot berries % was significantly regulated with supplying the vines with MN as 25 to 75 % inorganic N + 25 to 75 % any one of the three slow release N fertilizers or bacterial strains relative to application of N as 100% MN. The reduction was significantly in proportional to the reduction on the percentage of MN and at the same time the increase in concentration of slow release N fertilizer and the three bacterial strain and the best slow release fertilizers in controlling shot berries % were ureaformaldehyde, sulphur coated urea and methylene urea, in ascending order. The lowest values (5.5 & 5.2 %) were recorded on the vines that received N as 50 % MN + 50% methylene urea + 10 ml Tyrosineless bacterial strain. The highest values (10.6 & 11.0 %) were recorded on the vines received N as 100 % inorganic N. These results were true during both seasons. The reducing effect of the slow release N fertilizers and bioforms of N on shot berries might be attributed to their important roles in obtaining a good balance between various nutrients in plant tissues and enhancing nutritional status of the vines.

7- Physical and chemical characteristics of the berries:

It can be stated from the data in Tables (9 & 10) that treating the vines with N as 25 to 75 % MN + 25 to 75 % slow release N fertilizers (Methylene urea, Sulphur coated urea and urea formaldehyde) + bacteria strains (Methionineless, Threonineless and Tyrosineless) each at 5 to 20 ml/vine significantly was favourable in enhancing quality of the berries in terms of increasing berry weight and dimensions (longitudinal and equatorial), T.S.S.% and reducing sugars and decreasing total acidity% and both nitrate and nitrite in the juice relative to the application of N as 100 % inorganic N. There was a gradual promotion on quality of the berries with reducing percentages of inorganic N and the same time increasing the percentages of slow release N fertilizers and the levels of N bacterial strains. The important roles of the three slow release N fertilizers on improving quality of the berries could be arranged as follows in descending order ureaformaldehyde, sulphur coated urea and methylene urea. The best results with regard to fruit quality were observed due to treating the vines with bacterial strains namely Methionineless, Threonineless and Tyrosineless, in descending order. The best results were obtained due to treating the vines with 25% MN + 75% the slow release N fertilizer ureaformaldehyde at 75 % and bacterial strains namely Methionineless at 20 ml/vine. Unfavourable effects on quality of the berries were attributed to using N as 100 % inorganic. The lowest values of nitrate (1.50 & 1.41 ppm) and nitrite (0.50 & 0.47 ppm) were recorded on the vines that received N as 25 % inorganic N + 75% ureaformaldehyde + Methionineless bacterial strain at 20 ml/vine. Similar trend was noticed during both seasons. The benefits of slow release N fertilizers and bioforms of N on adjusting N uptake as well as enhancing the uptake of Mg surely reflected on enhancing the biosynthesis of sugars and leaf pigments and advancing maturity stage and berries quality.

8- Total counts of bacterial in the soil:

Table (10) shows that all treatments involved the application of inorganic, slow release N fertilizers and bacterial strains significantly enhanced total counts of bacterial in the soil relative to using N as 100 % inorganic N. The promotion on total counts of bacterial in the soil was significantly related to increasing percentage of slow release N fertilizer as well as levels of bacterial strains from 5 to 20 ml / vine. The best slow release fertilizer in this respect were urea formaldehyde, sulphur coated urea and methylene urea, in ascending order. Using bacterial strains namely Methionineless, Threonineless and Tyrosineless, in ascending order was very

Table 9

Table 10

effective in enhancing total counts of becteria in the soil. Subjecting the vines to N as 25% MN + 75% methylene urea + 20 ml Tyrosineless bacterial strain gave the maximum values (142.2 & 144.0 CFU /g soil) during both seasons, respectively. Similar trend was obvious during both seasons.

Economical study for the recommended treatment it applied in one feddan:

As shown in Table (11) net profit gained by the application of the recommended treatment (50% Mineral N+ 50% methylene urea + 10 ml strain Tyrosineless /vine) it applied in one feddan contains 700 vines reached 7098 (L.E) and 14578 (L.E) while in the control vines (100% M.N) reached 4720 (L.E) and 7200 (L.E) during both seasons, respectively. The increase on net profit due to application of the recommended treatment over the control reached 2378 (L.E) and 7378 (L.E) during both seasons, respectively

Discussion:

Azospirillum produces several phytohormones including IAA, gibberellins, cytokinins and ethylene (Bashan and Holguin, 1997). Plant growth hormones produced by bacteria can increase growth rates and improve yields of host plants (Glick, 1995). IAA production has beneficial effects on the plant – *Azospirillum* association.

The production of phytohormones by plant growth promoting rhizobacteria is considered to be an important mechanism by which these bacteria promote plant growth.

Slow release fertilizers release their own from nutrients at longer times. The continuous providing of the vines with their requirements from different nutrients can be realized by using these fertilizers. They protect nutrients from fixation , leaching and volatilization (Wang and Alva, 1996).

Organic and biofertilzation when used as a partial replacement of inorganic N fertilizers were responsible for enhancing N fixation, organic matter, water retention, availability of nutrients, root development, the biosynthesis of hormones and antibiotics and reducing soil pH (El-Sisy, 2000; Kannaiyan, 2000 and Cabrera *et al*., 2003).

Table (11): Economical study for the recommended treatment if it applied in one feddan.

Recommended treatment	2015	2016
Costs Hort. Practices (L.E)	17000	18000
Costs of mineral N (L.E)	188	188
Costs of Methylene urea (L.E)	400	400
Costs of Tyrosineless bio (L.E)	154	154
Total costs (L.E)	17742	18742
Yield/fed (tons)	6.21	8.33
Price of yield/fed	24840	33320
Net profit (L.E)	7098	14578
Control		
Costs Hort. Practices (L.E)	17000	18000
Yield/fed (tons)	5.43	6.30
Price of yield/fed (L.E)	21720	25200
Net profit (L.E)	4720	7200
Increase over control	2378	7378

*Price of ton grapes in the first (2015) and second season (2016) were 4000 (L.E).

The beneficial effect of organic manures in enhancing growth, vine nutritional status of Superior grapevines might be attributed to their important roles on enhancing soil fertility, organic matter, availability of all nutrints as well as the biothensises nutral hormones B vitamins and antibiotics as well as lowering soil pH and salinity (Mengel *et al*., 2001). These results are in agreement with those obtained by (Dakhly *et al.,* 2007; Uwakiem, 2011; Abou El-Lail, 2012; Allam-Aida *et al*., 2012; Shaheen *et al*., 2013; Ahmed *et al*., 2014; Abd El-Kareem, 2014; El-wany, 2015; Abd El-Reheem, 2015; Aly-Samar, 2015; Tony, 2016; Ahmed *et al*., 2017 and El-Kady-Hanaa, 2017).

Conclusion:

Amending Superior grapevines with N as 50% inorganic N (ammonium nitrate) + 50% slow release N fertilizer (methylene urea) + Tyrosineless at 10 ml/vine was responsible for promoting the yield. Treating the vines with (25% MN + 75% the slow release N fertilizer ureaformaldehyde + bacterial strains namely Methionineless at 20 ml/vine) gave the best results with regard to berries quality.

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تأثير بعض سلالات الأزوسبيريللام كبديل جزئى للسماد النيتروجينى الغير عضوى علي المحصول وجودة حبات العنب السوبيريور

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الملخص العربي

تم الحصول على91 طفرة ناتجة من تعريض بكتريا الأزوسبيريللام براسيلنز لتركيزات ومواعيد مختلفة من مطفر الاكريفلافين. تم اختبار ثلاث سلالات بكتيرية منها وهي Methionineless ، Threonineless و Tyrosineless بناء علي قوة النمو علي البيئة الغذائية.

وتم تلقيح كرمات العنب السوبيريور بهذه السلالات البكتيرية بمعدل 5 إلى 20 مل / كرمة وثلاثة أسمدة نيتروجينية بطيئة التحلل وهي اليوريا الفورمالديهايد ، واليوريا المغلفة بالكبريت والميثيلين يوريا بنسبة 25 إلى 75٪ كبديل جزئي للسماد االنيتروجينى السريع التحلل وهو نترات الأمونيوم خلال موسمي 2015 و 2016.

كان تسميد كرمات العنب السوبيرويور بالنيتروجين من 50 إلى 75٪ تسميد غير عضوى+ اسمدة بطيئة التحلل بنسبة 25 إلي 50% + سلالات البكتريا الثلاثة بمعدل 5 إلي 10 مل/ الكرمة فعالا جدا فى تحسين جميع صفات النمو الخضرى والصبغات والعناصر الغذائية فى الأوراق والنسبة المئوية لعقد الحبات وكمية المحصول وخصائص الجودة للحبات وذلك بالمقارنة بالتسميد باستخدام النيتروجين الغير عضوى بنسبة 100 % أو بالمقارنة بالنيتروجين المعدنى عندما يستخدم بنسبة 25 % وكان هناك انخفاض واضح فى محتوى العصير من النترات والنيتريت عند نقص النسبة المئوية المستخدمة من النيتروجين الغير عضوى من 100 إلي 25 % كذلك مع زيادة النسبة المئوية المستخدمة من الأسمدة بطيئة التحلل من 25 إلي 75% وسلالات البكتريا من 5 إلي 20 مل للكرمة ويمكن ترتيب الأسمدة بطيئة التحلل الثلاثة حسب تأثيرها الايجابي علي النحو التالي الميثيلين يوريا ، واليوريا المغلفة بالكبريت واليوريا الفورمالديهايد مرتبة ترتيبا تنازليا ويمكن ترتيب السلالات البكترية الثلاثة حسب أهميتها علي النحو التالي Methionineless , Threonineless , Tyrosineless مرتبة ترتيبا تصاعديا.

توصى الدراسة بتسميد كرمات العنب السوبيريور بالنيتروجين بنسبة 50 % تسميد نيتروجينى غير عضوى (فى صورة نترات الأمونيوم) بالاضافة الي السماد البطئ التحلل الميثيلين يوريا بنسبة 50% + السلالة البكترية ذات العوز الغذائي لل ` Tyrosineless بمعدل 10 مل للكرمة وذلك لأجل تحسين كمية المحصول. بينما تم الحصول علي افضل النتائج بخصوص جودة الحبات عند تسميد الكرمات بنسبة 25% تسميد نيتروجيني معدني (في صورة نترات الامونيوم) + 75% سماد بطئ التحلل يوريا فورمالدهيد + السلالة البكتيرية ذات العوز الغذائي لل Methionineless بمعدل 20 مل للكرمة.

الكلمات الدالة: الأسمدة النيتروجينية السريعة والبطيئة التحلل – السلالات البكترية – النمو – كمية المحصول – خصائص الجودة – العنب السوبيريور.

Table (5): Effect of replacing mineral N fertilizers partially by using some slow release N fertilizers and different microbial strains on some vegetative growth characteristics of Superior grapevines during 2015 and 2016 seasons.

Treatments	Shoot length(cm)		No. of leaves/shoot		Leaf area (cm)^2		Wood ripening coefficient		Pruning wood weight (kg.)		Cane thickness (cm.)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
100 % Mineral Nitrogen (MN)	96.3	97.0	18.1	19.0	99.0	99.5	0.53	0.51	1.91	2.00	0.84	0.81
75% MN+25% slow$_1$+strain$_1$ at 5 ml/vine	99.9	100.6	19.3	20.2	101.9	102.4	0.64	0.65	1.99	2.08	0.95	0.95
50% MN +50% slow$_1$ + strain$_1$ at 10 ml/vine	105.9	106.6	20.8	21.7	103.0	103.6	0.68	0.69	2.06	2.15	1.01	1.02
25% MN + 75 %slow$_1$ + strain$_1$at 20 ml/vine	90.0	90.7	16.0	16.8	94.0	94.5	0.46	0.47	1.80	1.89	0.71	0.72
75% MN + 25 %slow$_2$ + strain$_2$ at 5 ml/vine	108.0	108.6	21.9	22.8	105.6	106.1	0.71	0.72	2.15	2.24	1.12	1.14
50% MN+ 50 %slow$_2$ + strain$_2$ at 10 ml/vine	111.0	111.7	23.0	23.8	108.6	109.1	0.75	0.77	2.25	2.34	1.20	1.22
25% MN + 75%slow$_2$ + strain$_2$ at 20 ml/vine	91.5	92.2	17.1	18.0	95.5	96.0	0.48	0.49	1.84	1.93	0.75	0.76
75% MN + 25%slow$_3$ + strain$_3$ at 5 ml/vine	112.9	113.5	25.0	25.8	110.0	110.4	0.78	0.80	2.31	2.40	1.31	1.33
50% MN + 50%slow$_3$ + strain$_3$ at 10 ml/vine	116.7	117.5	27.0	27.8	112.0	112.6	0.81	0.83	2.41	2.50	1.40	1.42
25% MN + 75%slow$_3$ + strain$_3$ at 20 ml/vine	92.9	93.6	17.8	18.4	97.0	97.6	0.50	0.51	1.87	1.96	0.78	0.79
New L.S. D at 5%	1.1	1.0	1.0	1.0	1.1	1.3	0.03	0.02	0.06	0.05	0.05	0.06

Slow$_1$= Ureaformaldehyde (41% N) Strain$_1$= Methionineless
Slow$_2$= Sulphur – coated urea (41% N) Strain$_2$= Threonineless
Slow$_3$= Methylene urea (22% N) Strain$_3$= Tyrosineless

Table (6): Effect of replacing mineral N fertilizers partially by using some slow release N fertilizers and different microbial strains on percentages of cane total carbohydrates and N, P, K, Mg and Ca in the leaves of Superior grapevines during 2015 and 2016 seasons.

Treatments	Cane total carbohydrates %		Leaf N %		Leaf P %		Leaf K %		Leaf Mg %		Leaf Ca %	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
100 % Mineral Nitrogen (MN)	14.1	14.0	1.60	1.62	0.116	0.110	1.41	1.32	0.64	0.66	2.01	2.10
75% MN + 25 % $slow_1$ + $strain_1$ at 5 ml/vine	14.9	14.7	1.69	1.71	0.119	0.113	1.49	1.40	0.69	0.71	2.09	2.18
50% MN + 50 % $slow_1$ + $strain_1$ at 10 ml/vine	15.5	15.3	1.78	1.80	0.125	0.119	1.59	1.50	0.73	0.75	2.20	2.28
25% MN + 75 %$slow_1$ + $strain_1$at 20 ml/vine	13.2	13.1	1.50	1.52	0.101	0.095	1.20	1.11	0.51	0.53	1.91	2.00
75% MN + 25 %$slow_2$ + $strain_2$ at 5 ml/vine	15.9	15.7	1.85	1.87	0.131	0.126	1.66	1.57	0.76	0.78	2.31	2.40
50% MN + 50 %$slow_2$ + $strain_2$ at 10 ml/vine	16.4	16.2	1.94	1.96	0.141	0.135	1.72	1.62	0.80	0.82	2.41	2.50
25% MN + 75 %$slow_2$ + $strain_2$ at 20 ml/vine	13.5	13.4	1.53	1.55	0.105	0.100	1.25	1.16	0.55	0.57	1.95	2.04
75% MN + 25 %$slow_3$ + $strain_3$ at 5 ml/vine	16.9	16.7	2.01	2.03	0.151	0.146	1.79	1.69	0.85	0.87	2.51	2.60
50% MN + 50 %$slow_3$ + $strain_3$ at 10 ml/vine	17.3	17.1	2.11	2.13	0.161	0.156	1.84	1.75	0.89	0.93	2.61	2.70
25% MN + 75 %$slow_3$ + $strain_3$ at 20 ml/vine	13.9	13.7	1.56	1.59	0.110	0.105	1.30	1.21	0.58	0.60	1.96	2.06
New L.S. D at 5%	0.6	0.4	0.05	0.04	0.02	0.02	0.04	0.03	0.03	0.03	0.05	0.04

$Slow_1$= Ureaformaldehyde (41% N)
$Slow_2$= Sulphur – coated urea (41% N)
$Slow_3$= Methylene urea (22% N)

$Strain_1$= Methionineless
$Strain_2$= Threonineless
$Strain_3$= Tyrosineless

Table (7): Effect of replacing mineral N fertilizers partially by using some slow release N fertilizers and different microbial strains on percentage of S and leaf pigments (mg/1 g F.W) of Superior grapevines during 2015 and 2016 seasons.

Treatments	Leaf S %		Chlorophyll a (mg/1g F.W)		Chlorophyll b (mg/1g F.W)		Total chlorophylls (mg/1g F.W)		Total carotenoids (mg/1g F.W)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
100 % Mineral Nitrogen (MN)	0.70	0.69	2.11	2.20	1.71	1.75	3.82	3.95	1.60	1.63
75% MN + 25 % slow$_1$ + strain$_1$ at 5 ml/vine	0.74	0.73	2.21	2.30	1.74	1.78	3.95	4.08	1.65	1.68
50% MN + 50 % slow$_1$ + strain$_1$ at 10 ml/vine	0.79	0.78	2.31	2.40	1.80	1.83	4.11	4.23	1.71	1.74
25% MN + 75 %slow$_1$ + strain$_1$at 20 ml/vine	0.60	0.59	2.00	2.08	1.59	1.64	3.59	3.72	1.47	1.50
75% MN + 25 %slow$_2$ + strain$_2$ at 5 ml/vine	0.84	0.83	2.41	2.50	1.88	1.92	4.29	4.42	1.80	1.83
50% MN + 50 %slow$_2$ + strain$_2$ at 10 ml/vine	0.90	0.89	2.51	2.61	1.98	2.02	4.49	4.62	1.86	1.89
25% MN + 75 %slow$_2$ + strain$_2$ at 20 ml/vine	0.66	0.65	2.05	2.14	1.64	1.68	3.69	3.82	1.52	1.55
75% MN + 25 %slow$_3$ + strain$_3$ at 5 ml/vine	0.95	0.94	2.61	2.70	2.11	2.15	4.72	4.85	1.90	1.93
50% MN + 50 %slow$_3$ + strain$_3$ at 10 ml/vine	0.99	0.98	2.71	2.80	2.21	2.25	4.92	5.05	1.95	1.98
25% MN + 75 %slow$_3$ + strain$_3$ at 20 ml/vine	0.69	0.70	2.10	2.19	1.69	1.74	3.79	3.93	1.56	1.60
New L.S. D at 5%	0.03	0.03	0.06	0.05	0.03	0.03	0.04	0.04	0.04	0.04

Slow$_1$= Ureaformaldehyde (41% N)
Slow$_2$= Sulphur – coated urea (41% N)
Slow$_3$= Methylene urea (22% N)

Strain$_1$= Methionineless
Strain$_2$= Threonineless
Strain$_3$= Tyrosineless

Table (8): Effect of replacing mineral N fertilizers partially by using some slow release N fertilizers and different microbial strains on percentage of berry setting, yield as well as weight, length and shoulder of cluster of Superior grapevines during 2015 and 2016 seasons.

Treatments	Berry setting (%)		No. of clusters/vine		Yield/vine (kg.)		Cluster weight(g.)		Cluster length(cm.)		Cluster shoulder(cm.)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
100 % Mineral Nitrogen (MN)	10.1	10.5	21.0	24.0	7.75	9.00	369.2	375.0	19.3	19.0	8.8	9.0
75% MN + 25 % $slow_1$ + $strain_1$ at 5 ml/vine	11.0	11.3	21.0	23.0	7.88	8.74	375.0	380.1	21.0	20.7	9.1	9.3
50% MN + 50 % $slow_1$ + $strain_1$ at 10 ml/vine	11.9	12.9	21.0	25.0	8.09	9.78	385.0	391.0	21.8	21.5	9.4	9.7
25% MN + 75 %$slow_1$ + $strain_1$at 20 ml/vine	8.3	8.7	21.0	22.0	7.50	7.92	357.1	360.0	17.1	16.6	7.9	8.2
75% MN + 25 %$slow_2$ + $strain_2$ at 5 ml/vine	12.5	12.9	21.0	25.0	8.09	9.78	385.2	391.0	22.9	22.4	9.7	10.0
50% MN + 50 %$slow_2$ + $strain_2$ at 10 ml/vine	13.0	13.4	22.0	27.0	8.71	10.83	396.0	401.1	24.0	23.7	10.1	10.4
25% MN + 75 %$slow_2$ + $strain_2$ at 20 ml/vine	8.8	9.2	21.0	23.0	7.58	8.37	360.9	364.0	17.8	17.5	8.2	8.5
75% MN + 25 %$slow_3$ + $strain_3$ at 5 ml/vine	13.9	14.4	22.0	27.0	8.58	10.67	390.0	395.0	25.0	24.7	10.5	10.8
50% MN + 50 %$slow_3$ + $strain_3$ at 10 ml/vine	14.5	14.9	22.0	29.0	8.87	11.90	403.1	410.0	25.9	25.6	10.8	11.1
25% MN + 75 %$slow_3$ + $strain_3$ at 20 ml/vine	9.2	9.6	22.0	25.0	8.01	9.24	364.0	369.7	18.1	17.7	8.8	8.8
New L.S. D at 5%	0.6	0.6	NS	2.0	0.13	0.16	5.1	4.9	0.6	0.5	0.4	0.4

$Slow_1$= Ureaformaldehyde (41% N)
$Slow_2$= Sulphur – coated urea (41% N)
$Slow_3$= Methylene urea (22% N)

$Strain_1$= Methionineless
$Strain_2$= Threonineless
$Strain_3$= Tyrosineless

Table (9): Effect of replacing mineral N fertilizers partially by using some slow release N fertilizers and different microbial strains on percentage of shot berries as well as physical and chemical characteristics of the berries of Superior grapevines during 2015 and 2016 seasons.

Treatments	Shot berries%		Berry weight (g.)		Berry longitudinal (cm.)		Berry equatorial (cm.)		T.S.S%	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
100 % Mineral Nitrogen (MN)	10.6	11.0	3.5	3.5	2.00	2.01	1.91	1.94	17.1	17.0
75% MN + 25 % $slow_1$ + $strain_1$ at 5 ml/vine	9.6	9.4	5.0	5.1	2.27	2.29	2.14	2.14	19.0	19.1
50% MN + 50 % $slow_1$ + $strain_1$ at 10 ml/vine	9.2	9.0	5.3	5.4	2.33	2.35	2.18	2.19	19.4	19.5
25% MN + 75 %$slow_1$ + $strain_1$at 20 ml/vine	10.5	10.3	5.6	5.7	2.37	2.38	2.23	2.24	19.8	20.0
75% MN + 25 %$slow_2$ + $strain_2$ at 5 ml/vine	8.0	7.8	4.4	4.5	2.16	2.17	2.04	2.05	18.2	18.3
50% MN + 50 %$slow_2$ + $strain_2$ at 10 ml/vine	7.4	7.2	4.8	4.9	2.22	2.23	2.10	2.11	18.6	18.7
25% MN + 75 %$slow_2$ + $strain_2$ at 20 ml/vine	10.0	9.8	5.3	5.2	2.27	2.30	2.14	2.15	19.0	19.1
75% MN + 25 %$slow_3$ + $strain_3$ at 5 ml/vine	6.0	5.8	3.7	3.8	2.06	2.08	1.95	1.96	17.4	17.5
50% MN + 50 %$slow_3$ + $strain_3$ at 10 ml/vine	5.5	5.2	4.0	4.0	2.11	2.12	2.00	2.01	17.8	17.9
25% MN + 75 %$slow_3$ + $strain_3$ at 20 ml/vine	9.6	9.4	4.3	4.4	2.16	2.17	2.04	2.05	18.2	18.3
New L.S. D at 5%	0.8	0.8	0.2	0.3	0.04	0.04	0.04	0.03	0.4	0.3

$Slow_1$= Ureaformaldehyde (41% N)
$Slow_2$= Sulphur – coated urea (41% N)
$Slow_3$= Methylene urea (22% N)

$Strain_1$= Methionineless
$Strain_2$= Threonineless
$Strain_3$= Tyrosineless

Table (10): Effect of replacing mineral N fertilizers partially by using some slow release N fertilizers and different microbial strains on some chemical characteristics of the berries and total counts of bacteria of Superior grapevines during 2015 and 2016 seasons.

Treatments	Total acidity %		Reducing sugars %		Nitrite (ppm)		Nitrate (ppm)		Total counts of bacteria (cfug)/1g soil	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
100 % Mineral Nitrogen (MN)	0.683	0.680	15.6	15.5	2.59	2.61	3.60	3.61	121.0	119.0
75% MN + 25 % $slow_1$ + $strain_1$ at 5 ml/vine	0.600	0.599	16.7	16.9	0.90	0.85	1.90	1.81	124.0	125.0
50% MN + 50 % $slow_1$ + $strain_1$ at 10 ml/vine	0.590	0.589	17.0	17.2	0.70	0.66	1.69	1.62	125.5	127.8
25% MN + 75 %$slow_1$ + $strain_1$at 20 ml/vine	0.571	0.560	17.4	17.6	0.50	0.47	1.50	1.41	127.0	129.3
75% MN + 25 %$slow_2$ + $strain_2$ at 5 ml/vine	0.630	0.629	16.5	16.7	1.50	1.47	2.49	2.45	129.5	130.0
50% MN + 50 %$slow_2$ + $strain_2$ at 10 ml/vine	0.610	0.609	16.7	16.9	1.30	1.26	2.28	2.25	131.9	131.5
25% MN + 75 %$slow_2$ + $strain_2$ at 20 ml/vine	0.580	0.584	17.1	17.3	1.10	1.04	2.11	2.04	134.0	133.0
75% MN + 25 %$slow_3$ + $strain_3$ at 5 ml/vine	0.670	0.671	15.9	16.1	2.00	1.99	3.00	2.97	138.0	136.3
50% MN + 50 %$slow_3$ + $strain_3$ at 10 ml/vine	0.650	0.649	16.2	16.4	1.90	1.89	2.89	2.88	140.0	141.0
25% MN + 75 %$slow_3$ + $strain_3$ at 20 ml/vine	0.637	0.629	16.6	16.9	1.80	1.77	2.80	2.70	142.2	144.0
New L.S. D at 5%	0.009	0.008	0.4	0.3	0.09	0.11	0.11	0.08	1.3	1.9

$Slow_1$= Ureaformaldehyde (41% N)
$Slow_2$= Sulphur – coated urea (41% N)
$Slow_3$= Methylene urea (22% N)

$Strain_1$= Methionineless
$Strain_2$= Threonineless
$Strain_3$= Tyrosineless