Minufiya J. Agric. Res. Vol.36 No. 4:1077 -1098 (2011) "http://www.mujar.net"

EFFICIENCY OF *AZOTOBACTER*, DILUTED SULFURIC ACID AND VINASSE ON SOME CALCAREOUS SOIL PROPERTIES AND PRODUCTIVITY OF ONION

Faten M. Mohamed, S.F. Mansour and M.R.M. Ahmed
Soils, Water and Environment Research Institute, ARC, Giza, Egypt
(Received: Mar. 9, 2011)

ABSTRACT: *In an endeavor to alleviate the problems of calcareous soil, some possible manipulation of soil was evaluated .Two separate experiments were carried out to achieve the main objective of this research. The first was laboratory experiment, using qualitative and quantitative analyses to estimate the efficiency of Azotobacter spp isolate on dissolving $CaCO_3$, decreased pH and solubility of nutrients in both specific solid and liquid media, which were being inoculated with Azotobacter spp and incubated at 30 °C for 0, 3, 5, 7, 10 and 14 days, results obtained increased with prolonging the incubation periods up to 10 days.*

The second was a field experiment, being carried out on a calcareous soil located at Cairo El-Ismailiya Desert Road (Km 38), during two successive seasons (2009-2010), to study the effect of certain treatments on amelioration of calcareous soil properties and its productivity. The soil was amended with diluted sulphuric acid (5%) at rates of (5,10,15 $m^3 fed^{-1}$), vinasse at rats of (1, 2 and 3 ton fed^{-1}) and/or Azotobacter isolate carried on vinasse as a carrier (with a final density of 10^8 cells ml^{-1} liquid culture), applied at rates of (15, 30 and 45 L fed^{-1}). Seedlings of Onion (Allium Cepa) cv. Giza 20 were transplanted and drip irrigated with saline well water (EC_w 4.2 dSm^{-1}).

The results obtained showed that the values of soil pH, , total $CaCO_3$, active $CaCO_3$, bulk density and fine capillary pores trended to decrease with increasing the applied rates of each of Azotobacter spp., diluted sulphuric acid and vinasse. On the other hand, EC, , available Ca^{2+}, Aggregation %, percentage of stable aggregate (PSA), hydraulic conductivity and total porosity exhibited pronounced increases, and these were reflected on onion yield and quality, as well as increase enzyme activities (Nitrogenase and dehydrogenase) and CO_2 evolution especially with adding Azotobacter spp. at any rate and vinasse 3 ton fed^{-1}.

Economically, cost of the treatment of 15 m^3 fed^{-1} diluted sulfuric acid was 1200 L.E. for decreasing about 40% of total $CaCO_3$ and 60% of active $CaCO_3$, while vinasse 750 L.E. for 3 ton fed^{-1} decrease $CaCO_3$ % about 30% and about 43% active $CaCO_3$. Values concerning the Azotobacter treatment were 270 L.E. for 45 Lfed^{-1} to achieve about 27 and 37 % decreased in both total and active $CaCO_3$, respectively. Such treatments provided favorable conditions for nutrients availability for the growing plants and microbial population in soil.

Key words: *calcareous soils, Physiochemical properties, diazotrophy, total $CaCO_3$, Active $CaCO_3$, percent stable aggregates, sulphoric acid, Vinasse, N_2-ase, CO_2 evolution , Allium Cepa.*

INTRODUCTION

The soils of Egypt are located in the arid region, 30% or more of the desert area of Egypt is calcareous in nature. However, relatively high amounts of CaCO3 are originated and distributed in different soil fractions. However the occurrence of total $CaCO_3$ content at 14% or more as well as 4% or more of active carbonate in the soil, should be defined as calcareous soil Shawke *et al.* (2004).

Nowadays the extension of desert reclamation and cultivation in Egypt has become urgent and essential demand, due to the tremendous increase in population. Most of the soils are calcareous in nature such soils are suffering from some problems, i.e. Surface liem crust due to high percentage of $CaCO_3$ which cause the high EC and/or pH values, micronutrients fixation and/or precipitation. Also, the organic matter content of these soils is generally low due to the high temperature and arid climate. In addition, the intensive use of inorganic fertilizers caused the pollution of such soil, water and plant. Thus, utilization of Bio-organic fertilizers in the calcareous soil may decrease soil pollution and improves its physical, chemical and biological properties. The uses of bio-organic conditioners are more economic and much better than that of in organic chemicals. Fenn *et al.* (1990) and Marriano *et.al.* (2009) stated that the addition of acid or acidic forming materials such as sulfur and Vinasse oftenly reduced soil reaction (pH) and enhanc microbial densities and activities. Mansour (2007) found that the addition of diluted sulfuric acid reduced soil reaction, soil bulk density penetration resistance, total $CaCO_3$ and active $CaCO_3$. While, total porosity, quickly drainable pores, available water and hydraulic conductivity were increased.

Vinasse is a byproduct of sugar cane complementary industries, produced from the fermentation of molasses and it can be used as a source of potassium. Gomez and Rodriguez (2000) found that the application of vinasse would substitute for 55% of N, 72% of P_2O_5 and 100% of the K_2O required for sugar cane crop in Venezuela. Arafat and Yassen (2002) concluded that application of vinasse increased crop because it is a good source of many of nutrients which plants needed to grow.

The stimulatory effect of diazotrophs on increasing crop yield attributed to not only to N_2-fixation activity or production of some compounds like polysaccharides, peptides, lipids, growth promoters, which induced the proliferation of lateral roots and root hairs that increase nutrient absorbing surface, as well as produced organic acids which solubulize both organic and inorganic forms of elements that are unavailable to plants (Aly, 2003 and Massoud *et al.*, 2009).

Therefore, this work was carried out to evaluate the beneficial effects of *Azotobacter* spp., diluted sulfuric acid and vinasse, as acidic forming on $CaCO_3$ dissolution and improving some properties of a calcareous soil and its productivity of onion crop.

MATERIALS AND METHODS

To achieve the main objective of this research, experiments were carried as follows:

Experiment (1):

The purpose of this experiment was to estimate the efficiency of *Azotobacter* isolate on dissolving $CaCO_3$ and/or decreasing pH (producing acids) through qualitative and quantitative analyses in both solid and liquid specific media.

Preparation of *Azotobacter* isolate:

Three surface soil samples (saline sodic soils) were collected from El - Tina plain, Gelbana Village No.7, Port Said Governorate (pH 8.25, EC_e 47.6 dSm^{-1}, ESP 16.6 % and $CaCO_3$ 7.7 %) for isolation, purification and counting of *Azotobacter* spp., which ,were then grown on modified Ashby ' s media (Abd El-Malak,1968) to determine some characteristics. (Table,1).

Table (1): Some characteristics of *Azotobacter* isolate.

Shape	Gram reaction	Count (c.f.u ml^{-1} liquid culture)	N_2-ase activity (m mole C_2H_4 ml^{-1} hr^{-1})	Production of phytohormones			Exopoly-saccharides
				Auxins	Gibberellins	Cytokinins	
Spherical	Negative	1.7×10^8	58.8	+	+++	++	+++

Morphological tests:

Cell morphology and gram-stain reaction were investigated for cells grown on the specific media of *Azotobacter* spp., 30°C after 16 hours of inoculation. Gram staining was carried out according to the method described by Cerny (1979). Cell shape was studied by the electron microscopy. Cells were prepared for scanning electron microscopy by fixation in 2% glutaldehyde solution in potassium phosphate buffer (pH 7.0) for 2-4 hours. Fixed cells were washed several times in the buffer and post fixed in 2% O_sO_4 for 2 hours. Following fixation, the samples were rinsed twice in phosphate buffer, dehydrated in an ethanol series, freeze-dried coated with gold and finally examined by scanning electron microscope. (Plate, 1).

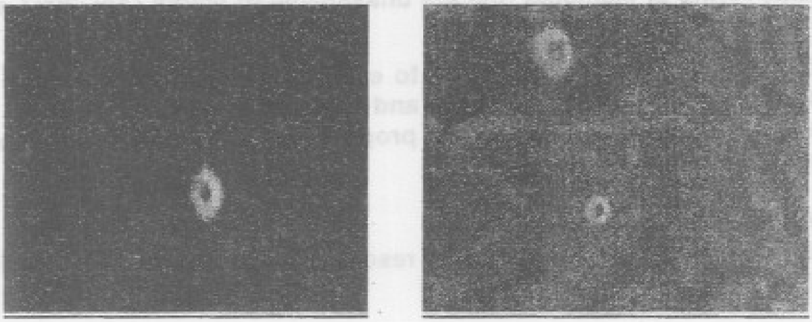


Plate (1): Morphology of *Azotobacter* isolate

Qualitative analyses:

The surface inoculated plates with *Azotobacter* isolate were incubated at 30 °C for 0, 3, 5,7,10 and 14 days. After incubation period culture was examined for the area of the clear zones formed around the bacterial colonies, compared to the dark color appeared for the rest of media. Clear zone declare the efficiency of microbes to dissolve $CaCO_3$ in specific solid media (0.5% $CaCO_3$).

Quantitative analyses:

Azotobacter isolate was purified and examined for its ability to produce acids (pH decrease) , dissolve $CaCO_3$, EC and soluble nutrients in inoculated specific liquid media (100 ml), were determined after 0,3,5,7,10 and 14 days from incubation periods at 30°C.

Experiment (2):

The investigation was extended to study the possibility to ameliorate some biological, chemical and physical properties of a calcareous soils and its crop productivity, under salinity stress, by using some of inorganic and bio-organic acidic materials i.e., sulfuric acid, *Azotobacter* and vinasse.

A Field experiment was carried out during two successive seasons (2008 - 2009 and 2009-2010) on a calcareous soil in a private farm located at Cairo - El-Ismailiya Desert Road (km38), Helwan governorate. Data in Table (2) represent some initial soil physical and chemical properties of the experimental soil. Also, analysis of applied vinasse (sugar industrial wastes) was presented in Table (3).

Table (2): Some physical and chemical properties of the experimental soil.

Soil characteristics	Value
<u>Particle size distribution</u>	
Coarse sand %	10.80
Fine sand %	30.10
Silt %	16.10
Clay %	43.00
Soil texture	Clay
Bulk density (Mg m^{-3})	1.57
Hydraulic conductivity (cm h^{-1})	0.78
$CaCO_3$ %	32.10
Organic matter %	0.23
pH (1:2.5 soil-water suspension)	8.39
EC (dSm^{-1})	1.90
Available Ca (meq100 g^{-1} soil)	33.30

Table (3): Chemical characteristics of vinasse used.

Characteristics	Value
pH	4.0
Organic matter %	46.5
Total Nitrogen (mgL^{-1})	1072.0
Total P (mgL^{-1})	210.0
Total K (mgL^{-1})	4360.0
Total Ca (mgL^{-1})	401.2
Total Mg (mgL^{-1})	264.1
Total Fe (mgL^{-1})	47.6
Total Mn (mgL^{-1})	2.0
Total Cu (mgL^{-1})	25.0
Total Zn (mgL^{-1})	7.0
Polysaccharides %	46.3

The experimental design was randomized complete block design with three replicates. The plot consisted of 7 rows, each 3 m long with 0.5 m apart giving a plot area of about 10.5 m^2. The experimental soil ploughed twice and recommended dose of inorganic fertilizer (100 kg ammonium nitrate, 33.5 % N; 150 kg super phosphate, 15 % P_2O_5 and 50 kg potassium sulphate, 48 % K_2O) were applied. Nitrogen applied at five equal split doses (at transplantation and then every 20 days). The experimental treatments consisted of control, *Azotobacter* isolate carried on diluted liquid vinasse as a carrier (with a final density of 10^8 cells ml $^{-1}$ liquid culture) at rates of 15, 30 and 45 L fed^{-1}, applied by using a procedure of spraying soil surface 3 times for each rate, after transplantation and then every 25 days. Diluted sulfuric acid solution (5%) was applied at rates of 5, 10 and 15 m^3 fed^{-1} and vinasse at rates of 1, 2 and 3 ton fed^{-1}. Sulfuric acid and vinasse were uniformly sprayed on soil surface and then followed by irrigation and kept to dry, welting and drying recycles were repeated 2 times before transplantation. Seedlings of onion (*Allium Cepa*) Giza 20 were transplanted and drip irrigated with a saline well water (Ec_w 4.2 dS m $^{-1}$) using.

At harvest (after 150 days from transplanting), the total yield of each plot was recorded (ton fed^{-1}). In addition, a random sample of 10 bulbs was collected from each plot to determine the average dry and fresh weights as well as bulb diameter. Disturbed and undisturbed soil samples were taken from 0 –30 cm depth, to determine physical and chemical properties, as well as rhizospheric soil samples were taken to determine biological properties according to the standard methods (Table, 4).

Table (4): Soil properties as determined by the standard methods described by the different publishers.

Property	Publishers
• Particle size distribution (%).	Gee and Bauder (1986)
• Bulk density (Mgm^{-3}).	Vomocil (1965)
• Pore size distribution • Stability index = 1/instability index • Instability index= (mean weight diameter of air dry soil before wet sieving) – (mean weight diameter of the same soil after wet sieving).	De Leenheer and De Boodt (1965)
• Saturated hydraulic conductivity.	Klute and Dirksen (1986)
• Total calcium carbonate (%), soil reaction (pH), EC (dS m^{-1}) and available Ca^{2+} (meq 100 g^{-1} soil).	Page *et al.* (1982)
• Active $CaCO_3$ content.	Yaalon (1957)
• Dry sieving and Aggregation (%). Aggregation (%) = (weight of aggregates>2 m.m)/ (total weight of soil) ×100.	Sharma D.K. and De Boodt (1982)
• Wet sieving.	Kemper and Rosenau (1986)
Percentage of stable aggregates (P.S.A) = aggregates (%) × stability index. • Classification is used for the P.S.A: P.S.A soil structure 40 acceptable 60 good 80 excellent	Sharma D.K. and De Boodt (1982)
• Nitrogenase activity.	Hegazi *et al.* (1979)
• Gram staining.	Cerny (1976)
• Dehydrogenas activity and CO_2 evolution.	Casida *et al.* (1964)

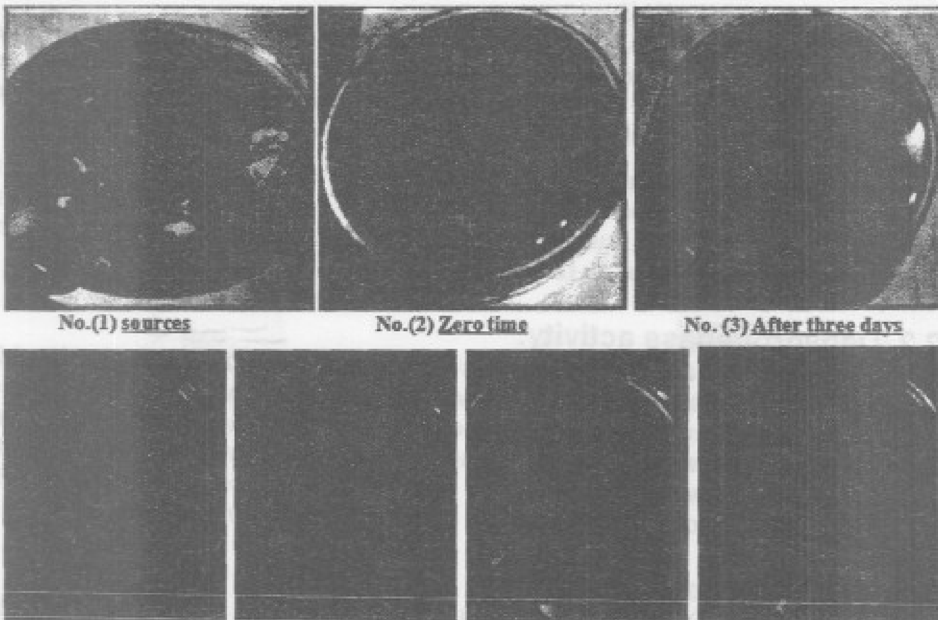
RESULTS AND DISCUSSION

1. Efficiency of *Azotobacter* to producing acids:

The following trails were measured in *Azotobacter* which grown on both solid and liquid media, as indicator for its ability to produce acidic substances. The obtained results were thought to be presented under two subheadings.

1.1. $CaCO_3$ dissolution in *Azotobacter* solid culture medium:

The ability of *Azotobacter* isolate to dissolve $CaCO_3$ in a specific solid media through different incubation periods showed in plate (2).



No.(1) sources; No.(2) Zero time; No. (3) After three days; No.(4) After five days; No.(5) After seven days; No.(6) After ten days; No.(7) After fourteen days

Plate (2): Efficiency of *Azotobacter* on dissolving $CaCO_3$ in specific solid media.

Generally, the obtained data showed that the clear zone were increased with increasing the incubation periods up to 10 days conversely to the dark zone. This may be attributed to increasing the population of *Azotobacter* cells and its ability to dissolve $CaCO_3$ in the medium through producing acidic substances.

1.2. $CaCO_3$, pH, EC and soluble elements in *Azotobacter* liquid culture medium:

Efficiency of *Azotobacter* isolate to produce acids in a liquid culture media was determined by qualitatively i.e., $CaCO_3$ percentage, pH, EC and soluble nutrients (Table, 5).

Generally, data showed that pH values and $CaCO_3$ percentage decreased with increasing the incubation periods up to 10 days, whereas opposite trend was obtained in both EC and soluble nutrients.

2. Microbiological properties in the rhizospheric soil of onion plant:

2.1. Nitrogenase activity:

Table (6) revealed the changes in nitrogenase activity in the calcareous subjected to the experimental treatments. Actually, N_2-ase was more favoured by the addition of vinasse, *Azotobacter* and diluted sulphuric acid, as its activity increased at any rate of addition compared with control.

A comparison between the effect of the different` amendment treatments on N_2-ase activities, indicated the following descending order: vinasse >*Azotobacter* > diluted sulphuric acid. These results supported by the conclusion of Massoud *et al.* (2009).

2.2. Dehydrogenase activity:

Dehydrogenase activity represents the overall measurement of microbial biomass in soil. Data in Table (6) revealed that application of inorganic or bio-organic amendments under consideration to the studied calcareous soil under salinity stress of irrigation water, improved dehydrogenase activity, since it increased with increasing the rate of each addition,with a superiority of *Azotobacter* ,followed desendengly sulphuric acid then vinasse.

2.3. CO_2 evolution:

Data in Table (6) showed that evolution rates of CO_2 were highly affected by the different soil amendments under study, as they increased with increasing the rates of *Azotobacter*, sulphuric acid and vinasse. These results are harmony with those reported by Mohamed *et al.* (2010). Vinasse exhibited the relatively highest value as compared with the other amendments. Crivelaro (2005) showed an increase in the microbial population with vinasse addition.

Table (5): Effect of *Azotobacter* on $CaCO_3$, pH, EC and soluble elemnts in the liquid culture medium under different incubation periods.

Incubation periods (days)	$CaCO_3$ (%)	pH in liquid culture medium	EC (dS m^{-1})	Soluble elements (ppm)							
				N	P	K	Ca	Fe	Zn	Mn	Cu
Zero time (control)	0.46	8.2	1.14	450	750	1400	8215	14.6	1.00	6.00	15.30
Three	0.40	8.1	2.06	460	762	1631	8173	16.0	1.71	6.00	16.20
Five	0.33	7.7	2.55	489	821	1900	12311	17.3	5.01	6.18	20.30
Seven	0.20	6.6	4.51	506	883	2173	33170	20.3	12.30	6.23	26.10
Ten	0.13	5.0	5.03	560	985	2380	38540	22.9	14.60	6.55	33.00
Fourteen	0.13	5.0	5.11	561	990	2389	38614	23.1	14.80	6.61	33.80

Table (6): Effect of applied *Azotobacter* ,diluted sulphuric acid and vinasse on nitrogenenaes and dehydrogenase activity as well as CO_2 evaluation in rhizospheric soil ,on the 80th day of transplanting onion seedling during two seasons (combined analysis).

Treatments	Rate (per fed)	N_2-ase (μ mole C_2H_4 dry^{-1} soil hr^{-1})	Dehydrogenase activity (μTPF g^{-1} dry soil day^{-1})	CO_2 evolution (mg 100 g^{-1} soil)
Control	--	26.9	18.6	60.0
Azotobacter Spp.				
B1	15 L	56.3	39.6	118.3
B2	30 L	71.7	53.5	128.1
B3	45 L	82.3	68.7	134.6
mean		70.1	53.9	127.0
Diluted sulphuric acid				
H1	5 m^3	43.8	26.7	115.2
H2	10 m^3	69.9	38.1	122.3
H3	15 m^3	72.1	45.2	135.3
mean		61.9	36.7	124.2
Vinasse				
V1	1 ton	74.6	38.9	127.1
V2	2 ton	96.2	80.1	144.8
V3	3 ton	81.7	107.5	152.3
mean		84.2	75.5	141.4

3. Chemical properties of the calcareous soil treated with the various amendments.

3.1. Soil reaction (pH) and soil salinity (EC).

Data in Table (7) showed that conditioning the calcareous soil with inorganic and bio-organic acidic materials i.e., diluted sulphuric acid, *Azotobacter* and vinasse respectively, improved its soil pH, positively proportional with the rate of addition. These results are mainly attributed to the highly acidic effect of this material. Similar findings were obtained by Awaad *et al.* (2010).

Table (7): Effect of applied *Azotobacter*, diluted sulphuric acid and vinasse on soil pH, EC, and available calcium in the studied calcareous soil after harvesting during two seasons (combined analysis).

Treatments	Rate (per fed)	pH (1:2.5)	EC (dSm^{-1})	Ca^{2+} (meq100 g^{-1} soil)
Control	-	8.30	1.90	33.30
Azotobacter SPP.				
B1	15 L	8.01	1.60	47.10
B2	30 L	7.83	1.87	50.3
B3	45 L	7.78	1.95	52.4
mean		7.87	1.81	49.9
Diluted sulphuric acid				
H1	5 m^3	7.96	2.14	57.3
H2	10 m^3	7.62	2.82	60.2
H3	15 m^3	7.46	3.16	66.6
mean		7.67	2.71	61.4
Vinasse		7.52	3.06	52.7
V1	1 ton			
V2	2 ton	7.40	3.25	58.2
V3	3 ton	7.32	3.37	66.8
mean		7.41	3.23	59.2

An Opposite trend was noticed for EC. This could be attributed to the released organic acids via bio-activity of *Azotobacter* and/or decomposition processes of vinasse, thus encouraging the solubility and availability of Ca^{2+}. These results were in full agreement with these reported by Mariano *et al.*

(2009) who observed that vinasse can modify temporarily some soil chemical and biological characteristics, such as concentrations of calcium, magnesium, organic matter and mainly potassium, causing an elevation of the soil electrical conductivity.

3.2. Solubility of soil calcium:

Data in Table (7) depicted the effect of applied treatments to the calcareous soil on its availability of Ca^{2+} after onion harvesting. It was clear that all of the applied treatments used increased available Ca^{2+} compared to the untreated soil (control). Efficiency of the different treatments on Ca^{2+} values could be arranged in an ascending order, i.e. $V_3 > H_3 > H_2 > V_2 > H_1 > V_1 > B_3 > B_2 > B_1 >$ control .This may be due to the influence of such treatments on reducing soil pH values and its releasing Ca^{2+} due to carbonation process. On the other hand, *Azotobacter* activity and vinasse decomposition enhancing released inorganic and organic acids which encourage the liberation of Ca^{2+}.

4. Physical properties of the calcareous soil related with the various amendments:

4.1. Total and activity of calcium carbonate:

Data presented in Table (8) showed that the effect of different applied treatments on the properties of the calcareous soil. The data revealed that increasing the applied rate led to decrease total calcium carbonate values and consequently decrease of active carbonate, comparing with untreated soil (control), revealing the ascending order $H_3 > H_2 > H_1 > V_3 > B_3 > B_2 > B_1 > V_2 > V_1 >$ Control, after onion harvesting. These results may be attributed to the acidic action of the amendments tested. These results are in agreement with Mace *et al.* (1999) and Mansour (2007).

It was apparent that the active calcium carbonate content in the different fractions followed the order clay > silt for all treatments (Table, 8), as the majority of $CaCO_3$ within the clay fraction. These indicate that the clay fraction is the main factor controlling the active carbonate content. On the other hand, presence of $CaCO_3$ in clay or silt fraction will be more effective on lime crust formation in the calcareous soil. These results are in agreement by Wahba (2003) who found that the total carbonate content is not the only effective parameter in the soil but the proportion of the active $CaCO_3$ which influence the pedometers and consequently the physio-chemical and hydrological reactions.

Table (8): Effect of applied *Azotobacter*, diluted sulphuric acid and vinasse on particle size distribution and CaCO3 in the studied calcareous soil after harvesting during two seasons (combined analysis).

Treatments	Rate per fed.	Status of Carbonates	Particle size distribution (%)			$CaCO_3$ (%)		Relative decrease of $CaCO_3$	
			Clay	Silt	Sand	Total	Active	Total	Active
Control		Before removing	43.0	16.1	40.9				
	----	After removing	28.0	7.2	32.7				
		Distribution	15.0	8.9	8.2	32.1	23.9	—	—
Azotobacter SPP.		Before removing	44.4	14.2	41.4				
B1	15 L	After removing	30.5	9.6	32.7				
		Distribution	13.9	4.6	8.7	27.2	18.5	15.3	22.6
		Before removing	41.9	15.3	42.8				
B2	30 L	After removing	30.2	10.9	32.6				
		Distribution	11.7	4.4	10.2	26.3	16.1	18.1	32.6
		Before removing	42.3	16.8	40.9				
B3	45 L	After removing	31.1	12.2	33.2				
		Distribution	11.2	4.6	7.7	23.5	15.1	26.8	36.8
Diluted sulphuric acid		Before removing	42.1	15.7	42.2				
H1	5 m^3	After removing	32.6	13.2	31.8				
		Distribution	9.5	2.5	10.4	22.4	12	30.2	49.8
		Before removing	43.5	15.8	40.7				
H2	10 m^3	After removing	35.6	13.6	29.9				
		Distribution	7.9	2.2	10.8	20.9	10.1	34.9	57.7
		Before removing	42.9	17.2	39.9				
H3	15 m^3	After removing	36.2	14.4	30.2				
		Distribution	6.7	2.8	9.7	19.2	9.5	40.2	60.3
Vinasse		Before removing	42.6	17.5	39.9				
V1	1 ton	After removing	32.4	10.1	27.2				
		Distribution	10.2	7.4	12.7	30.3	17.6	5.61	26.4
		Before removing	42.8	17.1	40.1				
V2	2 ton	After removing	32.9	11.6	28.1				
		Distribution	9.9	5.5	12.6	27.4	15.4	14.6	35.6
		Before removing	42.9	16.9	40.2				
V3	3 ton	After removing	34.2	11.9	31.4				
		Distribution	8.7	5.0	8.8	22.5	13.7	29.9	42.8

4.2. Stability Index (SI), Aggregation (%) and the percentage of stable aggregates (P.S.A):

From the data presented in Table (9) it could concluded that adding diluted sulfuric acid to calcareous soil at any rate resulted in an increase of stability index (SI) higher than vinasse treatment followed by *Azotobacter*. While the influence of the diluted sulphuric acid on the aggregation % behaved an appositively. On the other hand, the percentage of the stable aggregates was not high enough to be appreciated to have an acceptable good soil structure, except the diluted sulphuric acid then the vinasse treatment at the rate of 3 ton fed^{-1}. Whereas the percentage of stable aggregates was good and acceptable at the applied rates of 5, 10 and 15 $m^3 fed^{-1}$ of the diluted sulphuric acids and. 3 ton fed^{-1} of the vinasse.

As a conclusion it can be said that the application of any rate of diluted sulphuric acid increased the percentage of stable aggregates, while the applied any rate of *Azotobacter*, and low rates of vinasse (1& 2 ton fed^{-1}) exhibited no clear effects. So it is available to treat the soil with *Azotobacter* at a rate higher than 15 $Lfed^{-1}$., while vinasse should be applied rate of 3 ton fed^{-1}..

Table (9): Effect of, applied *Azotorbacter*, diluted sulphuric acid and vinasse on the percentage of Stable aggregates (PSA) in the studied calcareous soil after harvesting during two seasons (combined analysis).

Treatments	Rate per fed.	M.W.D*		Stability index (%)	Aggregation (%)	(PSA) %	Soil structure classification
		Dry sieving	Wet sieving				
Control	—	3.62	0.84	0.36	40.9	14.7	—
Azotobacter SPP.							
B1	15L	3.82	0.84	0.33	48.9	16.1	—
B2	30L	4.62	0.97	0.27	66.3	17.9	—
B3	45L	7.72	1.46	0.31	73.7	22.9	—
Diluted sulphuric acid							
H1	$5m^3$	2.86	2.15	1.40	45.8	64.1	Good
H2	$10m^3$	2.92	2.20	1.39	46.6	64.8	Good
H3	$15m^3$	3.28	2.37	1.10	61.7	67.9	Good
Vinasse							
V1	1 ton	3.16	1.12	0.49	50.3	24.6	—
V2	2 ton	3.34	1.20	0.47	64.6	30.4	—
V3	3 ton	3.81	2.28	0.65	77.9	50.6	acceptable

* MWD= Mean weight diameter

4.3. Bulk density (B.D) and total porosity (T.P):

It is well known that bulk density and total porosity are mostly affected by soil structure. Total porosity provides also valuable information about soil structure and is aversely correlated with bulk density. Mean values of bulk density (Mgm^{-3}) and total porosity (%) after onion harvesting are presented in Table (10). Data revealed that increasing the applied rate of any treatment (*Azotobacter,* vinasse and diluted sulphuric acid) to the calcareous soil led to reduce the values of soil bulk density, and consequently caused an increase in total porosity relative to the control (untreated soil). The best treatments occurred with the diluted sulphuric acid, followed by (V3) and then by (B3), since these treatments caused the lowest values of bulk density ($0.99Mgm^{-3}$) and the highest values of total porosity (52.9%). These results may be attributed to *Azotobacter*, vinasse and diluted sulphuric acid which enhanced the formation of large soil aggregates. This could be due to the dominance of soluble Ca^{+2} on the exchange complex. Such results are in agreement with Mansour (2007) who reported that the addition of diluted sulfuric acid led to reduce of soil bulk density and increase of total porosity. Also, El-Sersawy *et al.* (1997) and Reda *et al.* (2006), reported that improvement for both soil bulk density and total prosity were ameliorated as a result of biofertilization with *Azotobacter chroococcum* refering to control.

4.4. Hydraulic conductivity (HC):

Data in Table (10) revealed that the values of soil hydraulic conductivity increased with increasing the rates of applied each of *Azotobacter* , vinasse and diluted sulphuric acid as compared with the control. The hydraulic conductivity were increased from 1.78 cm h^{-1} in the untreated soil to 2.94, 2.87 and 2.64 cm h^{-1} then 2.01 cmh^{-1} under diluted sulphuric acid (15, 10 m^3fed^{-1}) and vinasse (3 ton fed^{-1}) then *Azotobacter,* (45 L fed^{-1}) respectively, this could be attributed to the effect of such treatments increasing the macro pores and decreasing the micro pores. Similar results were obtained by Mace *et al.* (1999) and Mansour (2007) using sulphuric acid, Reda *et al.* (2005) using biofertilization with diazotrophs and Mariano (2009) using vinasse. At the same time, a similar trend was observed with total porosity.

4.5. Pore size distribution:

Mean values of pore size distribution of soil after onion harvesting, as affected by applied different treatments *Azotobacter,* diluted sulphuric acid and vinasse was shown in Table (10). Data indicated that the fine capillary pores in the control represented the greatest percent of total pores by volume in the soil. On the other hand, the fine capillary pores are progressively decreased with increasing the applied rates of previous treatments. Generally, the data showed that increasing the applied rate of *Azotobacter,* diluted sulphuric acid or vinasse separately led to increasing the total porosity and decreasing of fine capillary pores. This result may be attributed to increase the solubility of Ca^{2+} that enhanced soil aggregation and the percentage of stable aggregates (P.S.A). These results are in agreement with obtained by Miyamoto and Stroehlein (1986) and Mansour (2007).

Table (10): Effect of applied Azotobacter, diluted sulphuric acid and vinasse on bulk density, hydraulic conductivity, total porosity and Pore size distribution in the studied calcareous soil, after harvesting during two seasons (combined analysis).

Treatments	Rate per fed.	B.D ($Mg\ m^{-3}$)	H.C (cmh^{-1})	T.P (%)	Pore size distribution (%)			
					Q.D.P >28.8μ	S.D.P 28.8 -8.6μ	W.H.P 8.6-0.19μ	F.C.P <0.19μ
Control	--	1.37	1.78	43.8	15.3	8.7	18.1	57.9
Azotobacter SPP. B1	15 L	1.29	1.89	45.4	20.9	12.1	21.4	45.6
B2	30 L	1.27	1.92	48.3	23.0	14.3	26.5	36.2
B3	45 L	1.21	2.01	49.8	23.1	14.7	31.9	30.3
Diluted sulphuric acid H1	5 m^3	1.12	2.66	50.2	25.5	14.7	29.0	33.9
H2	10 m^3	1.08	2.97	51.3	24.8	11.6	30.2	30.0
H3	15 m^3	0.99	2.94	52.9	28.0	15.5	30.8	25.7
Vinasse V1	1 ton	1.31	1.84	44.9	22.1	12.5	22.9	42.5
V2	2 ton	1.28	1.88	45.6	21.5	13.0	20.8	44.7
V3	3 ton	1.13	2.64	50.5	24.8	17.4	25.5	32.3

Q.D.P=quickly drainable pores
S.D.P=slowly drainable pores
W.H.P=water holding pores
F.C.P=fine capillary pores

5. Onion yield:

Data presented in Table (11) showed the effect of inoculation with *Azotobacter* and addition of the vinasse (as a byproduct of the sugar industrial wastes), as well as diluted sulphuric acid on onion yield and bulb quality (dry weight and diameter), under salinity stress in the calcareous soil. The applied treatments led to increase onion yield and bulb quality (dry weight and diameter) in comparison to the control. Furthermore, the data showed that the effect of vinasse (V_3) was more pronounced, followed by *Azotobacter* (B_3) then the diluted sulfuric acid (H_3). This increase may be due to that vinasse contains pronounced content of micronutrients (Zn, Fe, Cu and Mn) and macronutrients (N, P and K), besides its role on reducing soil pH and improving soil physical and biological properties. Similar results were obtained by Awaad et al. (2010) and Arafat and Yassen (2002).While, Aly (2003) and Massoud (2009) stated that some bacteria such as *Azotobacter chroococcum* are capable to produce some hormones namely indol 3-butyric acid, indol lactic acid, absesic acid, gibberellins and cytokinins, which induced the proliferation of lateral roots and root hair that increase nutrient absorbing surfaces, as well as produce organic acids which solubulizing inorganic and organic forms of mineral elements that are unavailable to plants.

Table (11): Onion yield, bulb quality parameters at harvesting as affected by applied different treatments during two growth seasons (combined analysis).

Treatments	Rate per fed.	Yield (ton fed^{-1})		Onion bulb quality	
		Fresh	Dry	Dry weight one bulb (g)	Bulb diameter (cm)
Control	-----	3.15	0.28	60	2.45
Azotobacter SPP.					
B1	15 L	8.75	0.82	96	2.50
B2	30 L	9.56	0.88	109	2.65
B3	45 m^3	10.90	1.01	121	2.80
Diluted sulphuric acid,					
H1	5m^3	6.20	0.58	106	2.55
H2	10 m^3	8.56	0.79	113	2.70
H3	15 m^3	10.80	1.00	129	2.90
Vinasse					
V1	1 ton	7.60	0.71	112	3.00
V2	2 ton	9.70	0.91	122	3.20
V3	3 ton	12.70	1.19	131	3.50

6. Economics of using treatments:

The rate of increase the availability of Ca^{+2} and onion yield with increasing the applied rate of the used amendments, is more attributed to the pronounced reduction of soil pH, total $CaCO_3$ and active $CaCO_3$, consequently helping to solve soil lime crust formation problems and plant nutritional in the calcareous soil.

A. *Azotobacter.*

1- Economic cost for (15 L^3 fed^{-1}) *Azotobacter* = 5×15=75 L.E.
2- Economic cost for (30 L^3 fed^{-1}) *Azotobacter* = 5×30=150 L.E.
3- Economic cost for (45 L^3 fed^{-1}) *Azotobacter* =5×45=225 L.E.

B. Diluted sulfuric acid 5% (D.S.A).

1- Economic cost for (5 m^3 fed^{-1}) D.S.A = 250×1.6 = 400 L.E.
2- Economic cost for (10 m^3 fed^{-1}) D.S.A= 500 ×1.6= 800 L.E.
3- Economic cost for (15 m^3 fed^{-1}) D.S.A = 750×1.6 = 1200 L.E.

C. Vinasse.

1-Economic cost for (1 ton fed^{-1}) vinasse concent.100% = 1×250 = 250 L.E.
2-Economic cost for (2 ton fed^{-1}) vinasse concent.100% = 2×250 = 500 L.E.
3-Economic cost for (3 ton fed^{-1}) vinasse concent.100% = 3×250 = 750L.E.

Conclusion

Based on the aforementioned discussion, it could be concluded that the usage of any treatment (*Azotobacter,* diluted sulphuric acid and vinasse) could positively affect the soil physio-chemical and biological properties i.e., pH, total $CaCO_3$, active $CaCO_3$, available Ca^{2+}, EC_e, bulk density and fine capillary pores. On the contrary, hydraulic conductivity, total porosity, aggregation %, percentage of stable aggregates (PSA), total yield and bulb diameter increased. Furthermore, enzymes activities (nitrogenase and dehydrogenase) and CO_2 evolution was increased especially by the application of *Azotobacter* at any rate and vinasse at a rate of 3 ton fed^{-1}.

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كفاءة بكتريا الأزوتوباكتر وحمض الكبرتيك المخفف والفيناس على بعض خواص الأراضى الجيرية وإنتاجيتها للبصل

فاتن محى الدين محمد - صبحى فهمى منصور – محمد رضا محمود أحمد
معهد بحوث الاراضى والمياة والبيئة – مركز البحوث الزراعية الجيزة – مصر

الملخص العربي

فى مسعى جاد لتخفيف مشاكل الأراضى الجيرية أجريت بعض المعالجات المتاحة على التربة من خلال تجربتين منفصلتين :-

التجربة الأولى:-

أجريت تجربة معملية باستخدام كل من المزرعة الصلبة والسائلة لعزلة الأزوتوباكتر المعزوله من الأراضى الملحية بسهل الطينة بعد تنقيتها وتحضينها على فترات صفر، ٣، ٥، ٧، ١٠، ١٤ يوم على درجة حرارة ٣٠ درجة مئوية بغرض تقييم كفاءة العزلة الميكروبية على إذابة كربونات الكالسيوم وصفياً وكمياً وكذلك على إنخفاض درجة الحموضة.
وتبين من النتائج أن عزلة الازوتوباكتر لها القدرة على إذابة كربونات الكالسيوم بالمزرعة وكذا على إنخفاض درجة الحموضة وزيادة محتوى العناصر الذائبة.

التجربة الثانية :-

إمتد البحث بإجراء تجربة حقلية بمزرعة خاصة بطريق الإسماعيلية الصحراوى (الكيلو ٣٨) محافظة حلوان خلال الموسمين (٢٠٠٩ – ٢٠١٠) بغرض تحديد أفضل معالجة ممكنة لتحسين خواص الارض الجيرية (الحيوية والكميائية والطبيعة) وزيادة إنتاجيتها من محصول البصل صنف جيزة ٢٠ تحت نظام الرى بالتنقيط بمياه آبار ملحية، بإستخدام بعض المواد ذات الصفة الحامضية، معدنية مثل (حامض كبريتك ٥%) بمعدلات ٥ ، ١٠، ١٥ متر مكعب للفدان، وعضوية مثل الفيناس (أحد مخلفات صناعة السكر) بمعدلات ١، ٢، ٣ طن للفدان والحيوية مثل (الازوتوباكتر المحملة على الفيناس المخفف) بمعدلات ١٥، ٣٠ ، ٤٥ لتر للفدان.

أظهرت النتائج أن زيادة معدلات الإضافة لأى من الازوتوباكتر والفيناس والحامض أدت إلى زيادة فى نشاط إنزيم النيتروجينيز والديهيدروجينيز ومعدل إنطلاق ثانى أكسيد الكربون وتيسر الكالسيوم وملوحة التربة والتجمعات الارضية ودرجة ثابتها والمسامية الكلية وإنتاجية محصول البصل وجودته، وعلى العكس من ذلك أدت زيادة معدلات الإضافة إلى إنخفاض حموضة التربة والنسبة المئوية لكربونات الكالسيوم الكلية والنشطة والكثافة الظاهرية ونسبة مسام الصرف الدقيقة مقارنة بالكنترول.

وقد تبين من التكلفة الاقتصادية للمعاملات الثلاثة أن إضافة حامض الكبريتيك المخفف (٥%) بمعدل ١٥ متر مكعب/فدان أدت إلى إنخفاض نسبة كل من الكربونات الكلية والنشطة بمعدل ٤٠، ٦٠% على الترتيب مقابل مبلغ ١٢٠٠ جنية مصرى. بينما كانت تكلفة إضافة ٤٥ لتر/فدان من الأزوتوباكتر المحملة على الفيناس المخفف أدت الى إنخفاض نسبة كل من الكربونات الكلية والنشطة بمعدل ٢٧ ، ٣٧ % على الترتيب بمبلغ ٢٧٠ جنيهاً مصرى، وكذلك كانت التكلفة الاقتصادية باستخدام ٣ طن/فدان من الفيناس هى مبلغ ٧٥٠ جنيها مصرى مقابل إنخفاض نسبة كل من كربونات الكالسيوم الكلية والنشطة بمعدل ٣٠ ، ٤٣ % على الترتيب.

علاوة على ما سبق، فإن كل من المعاملتين الحيوية والفيناس تتفوق على حامض الكبريتيك فى قدرتها على تهيئة الظروف المناسبة من زيادة الأعداد الميكروبية للتربة ونمو النبات.