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MICROBIAL SURVIVAL OF TOSHKY VIRIGIN SOIL AND THE INFLUENCE OF BACTERIAL INOCULATION ON YIELD PRODUCTIVITY OF WHEAT AND CLOVER

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ABSTRACT

In a trail to discover the suitability of the new reclaimed soil in Toshky (high temperature area in South Egypt) for cultivation. The chemical and physical properties of this soil were tested. The main groups of microbial community, *i.e.* total bacteria, actinomycetes and fungi were counted as well as thermophilic bacteria. Results indicated that the growth of all soils microflora was inhibited by increasing soil salinity. Clover inoculated with three strains of *Rhizobium trifolii* cultivated in this soil was failed because of its high salinity and nematode infection. Wheat inoculated with *Azotobacter chroococcum, Azospirillum brasilence and Azorhizobia caulinodans* had successfully grown in this soil after being reclaimed. Wheat inoculated with *Azorhizobium* gave higher biomass and nitrogen content than that inoculated with the other diazotrophs. Bacterial inoculation approximately can save more than 50% of inorganic nitrogen fertilizer in wheat.

INTRODUCTION

Occurrence of microbial population in soil is very useful because microbes excrete growth regulators, help in organic matter decomposition, mineral dissolving and may maintain the nitrogen status in soils through nitrogen fixation, as well as microbial occurrence is considered as an index to soil fertility (Abd-el-Malek, 1971). *Azospirillum*, *Azotobacter* and *Azorhizobia* are the most important nitrogen-fixing bacteria that saving more than half recommended dose of mineral nitrogen fertilizer (Rashid *et al.*, 1998 and Sabry *et al.*,2000). The soil samples that collected from Toshky area are presentable for all soil saline categories (1) salt free EC < 4 dSm^{-1} (2) slightly saline EC: 4- 8 dSm^{-1} (3) moderately saline EC: 8-16 dSm^{-1} (4) strongly saline EC :> 16 dSm^{-1} as mentioned by the United States Salinity Laboratory (USSL, 1954).

MATERIALS AND METHODS

Twenty soil observation sites were chosen to represent the main soil types of an area in Toshky project. The selected area lies between longitudes 31' 19" and 31' 30" E, and latitudes 22' 50" and 23' N. The collected soil surface samples were air dried, crushed and sieved through a 2 mm sieve, and then subjected to determine particle size distribution, $CaCO_3$ (Table 1) and pH in soil saturation paste, EC and soluble ions in soil saturation extract as well as soil texture (Table 2) as described by USDA (1984). Microbial community, total count (Bridson, 1978), total fungi (Martin, 1950) and thermal bacteria (Bridson, 1978) were also determined in all soil samples.

Table (1): Physical analysis of Toshky soil samples

No.	Coarce sand (%	Fine sand %	Silt %	Clay %	Texture class	$CaCO_3$ %
1	24.27	45.15	4.03	26.55	Sandy clay loar	10.30
2	35.40	42.25	1.93	20.42	Sandy clay loar	10.30
3	45.74	29.74	2.48	22.23	Sandy clay loar	7.92
4	47.35	21.95	3.45	27.25	Sandy clay loar	7.13
5	68.02	20.50	1.25	10.23	Loamy sand	11.09
6	26.17	34.75	11.60	27.48	Sandy clay loar	6.34
7	33.35	33.05	2.83	30.77	Sandy clay loar	4.75
8	41.95	37.40	0.95	19.70	Sandy loam	7.92
9	54.70	30.35	1.53	13.42	Loamy sand	8.71
10	66.18	21.45	1.00	11.37	Loamy sand	9.11
11	25.07	37.90	3.43	33.60	Sandy clay loar	7.13
12	30.55	35.70	1.10	32.65	Sandy clay loar	7.52
13	32.89	32.89	5.53	31.33	Sandy clay loar	8.71
14	22.55	53.15	3.55	20.75	Sandy clay loar	7.92
15	24.67	43.15	3.53	28.65	Sandy clay loar	11.09
16	14.60	55.25	4.90	25.25	Sandy clay loar	8.32
17	38.26	33.30	2.20	26.24	Sandy clay loar	7.92
18	59.40	25.75	1.20	13.65	Loamy sand	12.67
19	57.31	24.95	1.95	15.74	Sandy loam	1.58
20	65.15	13.65	2.30	18.87	Sandy loam	2.22

Table (2): Chemical analysis of Toshky soil samples

No.	SP*	pH**	EC***	Anions meq/L			Cations meq/L			
				HCO_3^-	Cl^-	SO_4^{2-}	Ca^{2+}	Mg^{2+}	Na^+	K^+
1	33	7.80	7.21	4.5	60.0	36.19	49.51	14.43	34.30	2.75
2	30	7.81	7.81	4.0	50.0	31.01	27.51	13.40	40.55	3.55
3	31	7.81	13.53	2.5	70.0	83.14	49.51	14.13	88.20	3.80
4	42	7.92	14.85	5.0	110.0	84.02	49.51	27.76	120.50	1.25
5	22	7.73	5.68	2.5	17.0	42.44	27.72	4.09	28.75	1.38
6	50	7.61	18.87	3.0	135.0	87.41	59.41	31.50	150.0	2.50
7	50	7.71	9.35	2.5	55.0	71.73	44.55	28.18	55.0	1.50
8	32	7.81	14.77	2.5	75.0	114.78	54.46	18.27	115.75	3.80
9	25	7.76	6.53	3.0	64.0	2.44	24.55	5.09	39.75	2.05
10	22	8.03	1.88	3.0	6.5	10.57	4.46	2.81	12.60	0.20
11	40	7.89	11.22	2.5	65.0	83.79	34.65	28.99	84.90	2.75
12	46	7.91	8.42	3.5	28.0	60.59	31.68	10.14	48.22	2.05
13	40	7.83	8.29	3.0	70.0	9.10	32.67	10.06	36.67	2.70
14	38	7.75	3.66	2.0	9.0	32.90	30.69	6.58	6.55	1.08
15	42	7.87	18.91	2.0	141.0	156.14	44.55	19.09	231.50	4.00
16	50	8.01	11.1	2.5	25.0	110.29	44.55	9.99	79.45	3.80
17	32	7.95	10.00	2.0	105.0	110.50	49.51	5.04	90.25	3.50
18	25	7.97	7.32	2.5	52.0	28.98	24.75	5.25	52.48	1.00
19	23	7.99	7.44	2.5	52.5	29.92	23.76	5.33	55.00	0.83
20	21	7.95	8.17	2.5	61.0	55.01	28.94	8.57	80.00	1.00

*Saturation percent ** Soil reaction *** Electric conductivity

seeds:

Clover seeds (*Trifolium alexandrinum*) cvs. Gemeza 1, Giza 6 and sror 1 and wheat seeds of (*Triticum acsativum* L.) cvs. Seds 1 and Sakha93 were kindly provided by Field Crops Institute, Agric. Res. Center (ARC), Giza.

Bacterial strains:

Three rhizobial strains from *Rhizobium trifolii* viz. ARC; 102, 103 and 111 were supplied by The Unit of Biofertilizers, Department of Microbiology, ARC, Giza. The strains were grown and maintained on yeast extract manitol agar medium (Vincent, 1970). *Azotobacter chrooccoum* was isolated from soil by selective medium (Hegazi and Neimela, 1976), while *Azospirillum brasilence* was isolated by the use of nutrient agar (Tarrand *et al.*, 1978). *Azorhizobium caulinodans* strain ORS 571 (IRBG314) was supplied by J. K. Ladha, Soil Microbiology Department, International Rice Research Institute (IRRI), Los Banos, Philippines, the strain was grown and maintained on TGYE medium (Ladha *et al.*, 1989).

Bacterial inoculum preparation:

A broth culture contains approximately 10^8 cell ml^{-1} from each single bacterial strain was used individually as inoculum or mixed in equal portions to obtain the mixed bacterial inoculum.

Method of inoculation:

Broth culture was added to each pot at planting over the seeds and the other after week.

The bacterial broth culture was inoculated to seeds twice as initially at planting time and one week later.

Experimental design:

Two experiments were carried out using soil sample No. 20 chosen because it contains high number of the thermophilic (800 cfu g dry $soil^{-1}$) and characterized by 8 dSm^{-1}. In both experiments, pots were 15 cm in diameter and filled with 2 kg soil.

Clover experiment

Pots were seeded with 15 seeds and after germination the plants were thinned to 5 healthy seedlings. The hills containing seeds were inoculated with rhizobia strains. The recommended nitrogen rate of 20 kg N fed^{-1} the nitrogen were applied in three split doses at 10, 20 and 30 days from cultivation. Phosphorus (super phosphate 15.5% P_2O_5) and potassium sulfate (48%K) were added in uniform doses as 200 and 100 kg fed^{-1}, respectively. The experiment comprises the following treatments:

1- 100% N dose.
2- Strain ARC 102 plus 15% N dose
3- Strain ARC 103 plus 15% N dose
4- Strain ARC 111 plus 15% N dose
5- Mixture of strains 102+103
6- Mixture of strains 102+111
7- Mixture of strains 103+111
8- Mixture of strains 102+103+111

Wheat experiment

The remained soil in pots after clover plants deteriorated due to high salinity and nematode infection was treated with both nemaliss (an anti nematode biological agent) and leaching to reduce the EC from 8.17 to 5.5 dSm-1. Soil was then used for wheat cultivation.

Each pot was seeded with 5 wheat seeds and after germination the plants were thinned to 3 healthy seedlings. The hills containing seeds were inoculated with *Azotobacter, Azospirillum and Azorhizobia*. Nitrogen were added at the rate of 0, 25, 50 and 100 kg N fed^{-1} as ammonium sulfate (20.5 % N) in three split doses at 10, 20 and 30 days from cultivation. The experiment includes treatments as the following:

1- *Azotobacter* + 25% N dose.
2- *Azospirillum* +25% N dose.
3- *Azorhizobia* + 25% N dose.
4- *Azotobacter*, *Azospirillum* and *Azorhizobia* +25% of N dose.
5- *Azotobacter* +50 % N dose.
6- *Azospirillum* + 50 % N dose
7- *Azorhizobia* + 50 % N dose.
8- *Azotobacter*, *Azospirillum* and *Azorhizobia* + 50 % N dose.
9- 25% of N dose.
10- 50 % of N dose.
11- 100 % of N dose.

RESULTS

Microbial counts in Toshky soils

Microbial count of the twenty soil samples presented in Table (3) revealed that the lowest bacterial count of 0.14×10^4 cfu. g.dw. $soil^{-1}$ was obtained by the strongly saline soil (EC >18.9 dSm^{-1}). It is also clearly shown that bacterial counts were much higher in saline free soil samples (EC. 3.66 dSm^{-1} soil No. 14) and (EC. 1.88 dSm^{-1} soil No. 10) than the saline soil samples and ranged between 89×10^6 and 292×10^6 cfu g dry weight $soil^{-1}$, respectively, while the numbers of bacteria in the slightly saline soil (EC 5.68 dSm^{-1} soil No.5) and (EC 7.81 dSm^{-1} soil No.2) ranged between 87×10^6 and 36×10^4 cfu g dw $soil^{-1}$. In moderately saline soils the numbers of bacteria ranged between 35×10^4 and 1.4×10^4 cfu g dw $soil^{-1}$ against 0.24×10^4 cfu g dw $soil^{-1}$ in highly saline soil (EC 14.85 dSm^{-1} and soil No. 4). The recorded fungi counts were ranged between 85×10^4 in saline soil and 9.1×10^2 cfu g dw $soil^{-1}$ in strongly saline soil. Actinomycetes counts ranged between 26×10^4 cfu g dw $soil^{-1}$ in non saline soil and 0.4×10^2 cfu g dw $soil^{-1}$ in strongly saline soil.

Effect of inoculation with diazotrophs on growth of clover in Toshky soil

Clover exhibited good resistance to saline during the first period of cultivation. This behavior was clear in all clover varieties when seeds were inoculated with a mixture containing ARC 102 plus ARC 111 *Rhizobia* strains. However, the same trend was observed with mixtures of ARC 103 plus ARC 111 *Rhizobia* strains in varieties Gemeza 1 and serror 1, while

Gemeza 1 was positively responded either to the mixture of ARC 102 plus ARC 103 or the mixture of ARC 103 plus ARC 111 strains. Giza 6 also had positively responded either to the mixture of st. ARC 102 plus st. ARC 103 or the mixture of st. ARC 102 plus st. ARC 111. All bacterial mixture strains positively affected the variety Serror 1. Seedlings did not respond positively to any individual inoculation. After 30 days clover seedlings being deteriorated and could not tolerate the level of salinity (EC 8.17 dSm^{-1} soil No. 20) and thereafter all plants failed to grow in parallel to the appearance of root-knot nematodes.

Table (3): Population densities of bacteria, fungi, actinomycetes and thermal bacteria in the collected soil samples

Soil samples		Bacteria *	Fungi**	Actinomycetes**	Thermal bacteria
samples	EC (dSm^{-1})				
1	17.21	1900	5300	140	-
2	7.81	36	230	70	-
3	13.53	2.3	20	1.6	10
4	14.85	0.24	12	1.4	-
5	5.68	8700	7200	800	-
6	18.87	0.19	10	0.5	-
7	9.35	1.6	31	11	-
8	14.77	1.4	21	2.2	-
9	6.53	5400	6400	150	-
10	1.88	29200	8500	2600	-
11	11.22	4.9	23	6	-
12	8.42	28	50	20	-
13	8.29	30	60	27	10
14	3.66	8900	4200	100	-
15	18.91	0.14	9.1	0.4	-
16	11.1	15	26	7	-
17	10.0	17	30	9	150
18	7.32	96	360	70	-
19	7.44	98	3800	80	10
20	8.17	35	100	37	800

*: x 10^4 c.f.u g^{-1} oven dry soil * *: x 10^2 c.f.u g^{-1} oven dry soil

Effect of inoculation with diazotrophs on growth of wheat

Data presented in Table (4) showed the effect of inoculation with *Azotobacter*, *Azospirillum* and *Azorhizobium*. Plants inoculated and uninoculated that received quarter and half recommended dose of nitrogen fertilizer resulted in increases in shoots and roots dry weight of inoculated plants compared to those uninoculated plants receiving the same level of nitrogen dose. Inoculation with *Azorhizobia* combined with quarter and half nitrogen doses recorded the highest dry weight of roots and shoots. These increases compared to uninoculated in cv. Seds1 were 212 and 105.5% for *Azorhizobia* fertilized with quarter and half of nitrogen, respectively. Plants inoculated with *Azorhizobia* and received 25% nitrogen dose showed significant increases in shoots and roots dry weight compared to plants inoculated with *Azorhizobia* combined with 50% nitrogen dose and those uninoculated combined with 100% of N dose. *Azospirillum* was in the second level of increasing when compared to uninoculated plants. Inoculation with *Azospirillum* combined with 25 and 50% N doses increased dry weight by 112 and 61%, respectively. In the third level where plants inoculated with the

mixture, significant increases in dry weight of shoots and roots of 75% and 100% were achieved by applying 25% and 50% N doses, even when plants were inoculated with *Azotobacter*. A significant increase of 24% was noticed with the use of 25% N dose.

Concerning wheat cv. Sakha 93, the increases in shoots and roots dry weight in plants inoculated with *Azorhizobia* and *Azospirillum* were in parallel with those inoculated with *Azorhizobia* and fertilized by 25 and 50% N doses. The corresponding increases in shoots and roots dry weight where 80% for both over those of plants received 25%N dose only and 21% over those of plants received 50% N dose only, respectively. Plants inoculated with *Azospirillum* combined with 25% N dose had recorded significant increases in shoots and roots dry weight over those fertilized with 25% N dose only. Plants fertilized with nitrogen showed increases over the inoculated plants.

Table (4): Biomass yield (mg plant^{-1}) of two varieties of wheat inoculated with diazotrophs and supplemented with various N fertilization doses

Treatments	Nitrogen fertilizer Kg fed^{-1}	Shoot weight mg plant^{-1}		Root weight mg plant^{-1}	
		Se ds1	S akha93	Se ds1	S akha93
1-*Azotobacter*	25	410	350	390	140
2-*Azospirillum*	25	700	570	250	290
3-*Azorhizobia*	25	1030	720	500	470
1+2+3	25	660	510	380	320
1-*Azotobacter*	50	340	290	200	120
2--*Azospirillum*	50	580	390	290	210
3-*Azorhizobia*	50	740	510	440	240
1+2+3	50	630	410	330	220
Uninoculated	25	330	400	200	290
Uninoculated	50	360	420	230	180
Uninoculated	100	480	640	260	320
Control	0	300	100	180	75
L.S.D. (0.05)					
Variety		14.512		16.623	
Treatment		35.55		40.718	
Interaction		50.062		57.241	

In Table (5) the plant height showed that *Azorhizobia* inoculation beside 25% N dose was preceding significantly all treatments, while those inoculated with a mixture of the diazotrophs combined with 50% N dose were in the second order and then those inoculated with *Azospirillum* besides 25%N dose and *Azorhizobia* combined with 50% N dose in descending order, which indicate that in cv. Seds 1 was preceded cv. Sakha93.

In Table (5) nitrogen content of plants inoculated with any of the diazotrophs combined with 25% N dose had recorded significant increases compared to uninoculated plants received 25% of N dose. The plants received 50% of N dose beside inoculation (except *Azotobacter*) gave significant increases in nitrogen content as in wheat cv. Seds1. *Azarhizobium* was superior to *Azospirillum* which, was in the second order even when compared to uninoculated plants received 100% of N dose.

Table (5): Height (cm plant^{-1}) and nitrogen content (mg plant^{-1}) of two variety of wheat inoculated with diazotrophs and supplemented with various N fertilization.

Treatments	Nitrogen fertilizer Kg fed^{-1}	Height cm plant^{-1}		N-content mg plant^{-1}	
		Seds1	Sakha 93	Seds1	Sakha 93
1-*Azotobacter*	25	47	41	32.0	17.1
2-*Azospirillum*	25	57	48	69.3	32.4
3-*Azorhizobia*	25	63	54	101.9	47.9
1+2+3	25	51	41	32.0	29.0
1-*Azotobacter*	50	42	36	15.3	18.56
2-*Azospirillum*	50	49	45	54.8	23.0
3-*Azorhizobia*	50	55	45	89.3	30.1
1+2+3	50	62	46	40.3	28.3
Uninoculated	25	35	37	12.9	26.9
Uninoculated	50	37	39	19.8	29.6
Uninoculated	100	45	42	38.7	53.4
Control	0	23	21	10.0	9.9
L.S.D. (0.05)					
Variety		1.828		0.643	
Treatment		4.46		1.575	
Interaction		6.230		2.218	

In wheat cv. Sakha 93 *Azospirillum*, *Azorhizobium* and/or their mixture increased the nitrogen contents of the plant over the uninoculated plants, although they all received 25% of N fertilizer. In plants received 50% N dose, only plants inoculated with *Azorhizobia* showed no significant increases nitrogen content over the uninoculated plants. Plants received 100% N dose gave higher nitrogen content over both inoculated and uninoculated plants.

DISCUSSION

Over population and limitation of agricultural land in Egypt, the cultivation of new land (desert land) and increasing its fertility and productivity become a must. Toshky is a national project acting all of these aims. In the present study, soil collected from various situation of Toshky, microbial counts (total bacteria, total fungi and actinomycetes) represented the majority of the microbial load of such soils. Also, it is indicated that growth of all soil microflora was inhibited by increasing soil salinity. In this respect Gilmour (1990) and Atlas & Bartha (1993) reported that at high salt concentration, the hypertonic environment tends to dehydrate non-halotolcent microorganisms. They also indicated that high salt concentrations may lead to denature the essential protein that is necessary for enzyme activities.

Results of clover revealed that all growth parameters of clover were dramatically inhibited and decreased because of higher quantities of soluble salts in soil.

Similar findings were obtained by Bajpai and Gupta (1979) who reported that the higher salinity level delayed the nodulation of clover and even the formed nodules were ineffective. Significant reduction in biological yield, nodulation and nitrogen uptake were also observed. About 50 and 90% yield reduction were observed due to the harmful effect of high salinity.

Mozafar and Oertli (1990) noted that salt stress lead to change the concentration of abscisic acid (ABA) in plant tissues. ABA is known to affect the membrane permeability and the uptake of several nutrients by plant roots. The cultivated clover had not succeeded but it could be ploughed in the soil to increase its fertility.

After soil treated with nemaliss to get rid of nematode and leaching to reduce the salinity, treatment cultivation indicated that inoculation of cereal crops with associative diazotrophs such as *Azospirillum*, *Azotobacter* and *Azorhizobium* significantly increased plant biomass, N-content and wheat plant height (Sabry *et al.,* 1998).Webster *et al.*,(1997) and Sabry *et al.*(2000) found that wheat inoculation with *Azorhizobuium coulinedans* resulted in significant increases in plant dry weight of shoots and N-content compared to uninoculated plants. Results of the present work are in harmony with those obtained by above mentioned workers. *Azorhizobium* was superior among all other diazotrophs and supported plant growth in presence of quarter and half dose of nitrogen. These results are in agreement with those recorded by Gohar *et al,* (1986) and Sabry *et al.,* (1998) who found that associative diozatrophs are beneficial at low doses of N-fertilizer whereas they were significantly better when accompanied with half dose of nitrogen, while higher doses of nitrogen along with the associative N- fixers diazotrophs had inhibited associative diozatrophs.They added that inoculation with associative in presence of half dose of nitrogen had an inhibitory effect on N_2-ase activity (Mertens & Hess, 1984 and Da Silva *et al,* 1993). The increasing effect of inoculation with *Azospirillum* and *Azotobacter* was confirmed by Soliman *et al.*, (1995) who found that inoculation with *Azospirillum* gave better effects on plant growth and nitrogenease activity than inoculation with *Azotobacter.*

In conclusion; this work showed that (1) A microbial structure of Toshky soils is good and active (2) Toshky soils are available for cultivation as they are but some of them need a simple reclamation method like leaching to reduce salinity, also re-cultivation could do that: (3) results of cultivation in the present work are in harmony with those obtained in new cultivated soil with a similar structure of Toshky soils.

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البناء الميكروبي لأراضي توشكى المستصلحة حديثاً وتأثير التلقيح بالبكتريا المثبتة للنيتروجين على محصول ونمو محاصيل القمح والبرسيم

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في تجربة لاكتشاف مدى خصوبة أراضي توشكى المستزرعة حديثا ، تبين أن بعض مناطق توشكى شديدة الملوحة وأخرى متوسطة الملوحة وبعضها خالى من الملوحة، لذلك تم اختيار الصفات الكيماوية والطبيعية لعينات مختلفة من هذه الأراضى مختارة من مواقع مختلفة من منطقة توشكى وكذلك اختبار البناء الميكروبي للأراضى حيث تم عد المجموعات الميكروبية الرئيسية الآتية :

١) البكتريا الكلية ٢) الفطريات ٣) الأكتينوميسينات

و بطبيعة منطقة توشكى ذات الحرارة الشديدة فقد تم عد البكتريا المتحملة للحرارة، كما تم زراعة نوعين من المحاصيل، المحصول الأول هو البرسيم المسقاوى وتم تلقيحه بالريزوبيا حيث لم يتحمل ملوحة التربة (٨,١٧ ملليموز) وانهارت النباتات بعد ٣٠ يوم وقبل أن تصل إلى ٤٥ يوم حيث كان مقدرا أخذ العينة الأولى. وبعد معاملة التربة بالمستحضر الحيوى نيماليس للتخلص من النيماتودا وعمل غسيل للتخلص من الأملاح تم زراعة المحصول الثانى وكان القمح حيث تم تلقيحه بالأزوتوباكتر و الأزوسبيرلم و الأزوريزوبيا ، وهذا أدى إلى زيادة في المحتوى النيتروجينى مع التلقيح والاستغناء عن أكثر من ٥٠% من النيتروجين المعدني .

أهم النتائج يمكن تلخيصها:

١) البناء الميكروبي لأراضي توشكى الجديدة بناء جيد وحيوى .

٢) بعض أراضى توشكى صالحة للزراعة بدون أى معاملات وبعضها يحتاج إلى معاملات استصلاح بسيطة مثل الغسيل وربما تكرار الزراعة قد يؤدى الى استصلاحها.

٣) بعض أراضى توشكى مصابه بالنيماتودا ويستحسن معالجتها قبل الزراعة.

٤) النتائج المتحصل عليها في تجارب الزراعة يقترب من النتائج المتحصل عليها في أراضي جديدة أخرى لها نفس البناء لأراضى منطقة توشكى.