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RESPONSE OF GUAR PLANT (*Cyamopsis tetragonoloba* L.) TO DUAL and/or SINGLE INOCULATION WITH VA *Mycorrhiza* and *Azotobacter chroococcum*.

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ABSTRACT

The interaction between inoculum of *Azotobacter chroococcum* and/or VA *Mycorrhiza* in association with Guar plant [*Cyamopsis tetragonoloba* L.] was investigated in a pot experiment. In clay soil, data obtained showed that a dual inoculation with *Azotobacter chroococcum* and VA *Mycorrhiza* increased significantly fresh weight of Guar plant after 75 day compared to control. Also, dry weight recorded a highly significant increase. Likewise, in sandy soil, fresh weight had also achieved highly significant increases after 50 and 75 days due to the dual inoculation process. Concerning NPK contents of Guar plant, *Azotobacter chroococcum* inoculum showed significant increasing in N content of Guar in both clayey and sandy soils compared to control. Whereas, P content of the tested plant was increased significantly in the presence of *Mycorrhiza* inoculum. Microbial inoculation of Guar plant showed increase in numbers of total viable bacteria and *Azotobacter* spp., as well as, the *Mycorrhiza* colonization in the rhizosphere soil was in the same trend. On the other hand, the supernatant of *A. chroococcum* showed high growth promoting substances and minor growth inhibitors, in which the net units of promotion substances was 11.4 and the auxin-like substance, recorded 1.14 mg/ml.
Keywords: Guar plant, *Mycorrhiza*, *Azotobacter* spp., promoting substances.

INTRODUCTION

Guar (*Cyamopsis tetragonoloba*, L. ATUB), is a leguminous crop. It's an important crop in both of agricultural and industrial utilization. Agriculturally, it's grown for animal feeding and provides edible pods, as well as, it's grains is quite nutritive, rich in protein, fat and minerals. It is also used as a green manure crop in newly cultivated areas. Industrially, Guar gum, which is extracted from its seeds and ranged from 11.23 - 26.23% is important because of it's highly mucilaginous, used in cosmetics, explosives, paper, reconstituted tobacco and stabilizer of stiffener in foods and various other products (Ghalab *et al*., 2000 and Omar, 2005). In Egypt, increasing the forage production during the summer season, is one of the major targets of the government to solve production problem of livestock, in order to face human needs (Ghonem, 1990). Consequently, the use of chemical fertilizers is urgent demand for solve this problem, in which they are nutritive for plants, however, it's expensive, as well as, cause environmental pollution. Therefore, the attention is paid toward alternative procedures such as biofertilizers. Some bacteria and fungi have been reported to play a vital role in nitrogen fixation and solubilizing phosphorus element, as well as, produce plant growth substances such as auxins, gibberellins and cytokinines (Chiarini *et al.*, 1998 and Abotaleb *et al.*, 2007). Bacteria such as i.e. *Bacillus polymyxa* and *Azospirilla* were found to be associated with different non-legumes, grown in various soils, in which they have positive significant contributions to N-status of plant-soil system (Mona *et al.*, 2000). Generally, *Azotobacter* spp. are an important member in plant growth promoting rhizobacterial group (PGPR), that participate in interactions with plants to contribute yield increases by both nitrogen fixation and secretion growth promoting substances (Ivan *et al.*, 2004). Another study of Behl *et al.* (2003) pointed out, that co-inoculation of *Azotobacter chroococcum* with VA *Mycorrhiza* led to an increase in viable count of *Azotobacter* spp. in wheat rhizosphere and resulted in improving plant growth. The present study is designed to determine the effect of *Azotobacter chroococcum* and vesicular arbiscular *Mycorrhiza* in Dual and/or single inoculation on the fresh and dry weights, as well as, nutrient content of Guar plants either in clayey or sandy soils.

MATERIALS AND METHODS

A pot experiment was conducted during the summer season of 2007 at Agricultural Research and Experimental station, Faculty of Agriculture, Mansoura University, Dakhlia Governorate, Egypt. This experiment was carried out to study the effect of Guar plant inoculation with *Azotobacter chroococcum* and VA *Mycorrhiza* either in a single and/or a dual inocula in presence of activated doses of N fertilizer compared to control [full dose of N], and these pot contain 10 kg clayey and/or sandy soil.

Grains surface sterilization:

Guar seeds (*Cyamopsis tetragonoloba* L.) kindly provided by Horticulture Dept., Fac. Agric., Minia Univ., were used in this experiment. Seeds were surface sterilized with ethanol (1 minute) followed by 30% H_2O_2 for 5 minutes and subsequently washed with distilled water.

Seeds inoculation:

Bacterial inoculum:

Azotobacter chroococcum was isolated from soil by selective medium (Hegazi and Neimela, 1976) in the Dept. of Microbiology, Soils Water and Environ. Res. Inst. (ARC), Giza, then, it was grown and maintained through the same medium. A broth culture contains approximately 10^8 cell mL^{-1} from *Azotobacter* was added to each pot at planting over the seed (about 10 ml/seed) one week later.

VA *Mycorrhiza* inoculum:

VA *Mycorrhiza* were obtained from Fac. of Sci., Mansoura Univ., for inoculation with VA *Mycorrhiza* to increase the availability of phosphorus in soil. Five ml of spores suspension [about 180 spores/ml] were added on the grains and then covered with some of the same soil.

Mineral fertilization:

Mineral fertilizers were added at the rates of 100 Kg/fed^{-1} superphosphate (15.5% P_2O_5), 50 Kg K_2O fed^{-1} as potassium sulphate (48% K_2O) and nitrogen 80 Kg fed^{-1} as ammonium sulphate (20.5% N), in which it used as control, the activated dose used with inoculation was 15% of the nitrogen recommended dose.

Plant growth conditions:

Ten kg, portions of clayey and sandy soil were used separately in pots, which were collected from the farm of Mansoura University and Kalbsho region, Dakhlia Governorate, respectively. Seeds were distributed at the rate of five seeds/pot, then thinned out to three seedlings/pot after three weeks of sowing. Four experimental treatments were included in this study [clayey soil]. One experiment included a full dose of nitrogen (control). Three experiments included activated doses of nitrogen and microbial inoculation. Similarly, another four experimental treatments were carried out in sandy soil. All pots were irrigated with equal volume of tap water whenever needed. After 50 and 75 days, plants were uprooted and both plants and soils were analyzed. In all experiments the treatments were replicated three times.

Microbiological analysis:

Samples from rhizosphere soil of Guar plants were taken after 50 and 75 days from sowing to determine total bacterial number (Allen, 1969). *Azotobacter chroococcum* was determined by most probable number (Cochran, 1950) using the same medium used in isolation.

Chemical analysis:

After 50 and 75 days from planting, inoculated and non-inoculated representative plants [3 plants/replicate] were uprooted. Total fresh and dry weights of plant were determined. The oven dried plants (70°C) up to a constant weight were powdered and mineralized by sulfuric-perchloric acid (Piper, 1950). Total nitrogen, P content and K were determined (as a percent) by the method of Jackson (1973).

Plant growth substances:

Auxin-like substances were estimated colorimetrically at 530 mu by using P.dimethylamine benzeldehyde (Ehritch reagent) according to Larsen *et al.* (1962). Meanwhile, the promoters and inhibitors substances were determined according to the method of Hartman *et al.* (1967).

Statistical analysis:

Differences between treatments were determined using the statistical procedures for agricultural research (Gomez and Gomez, 1984) and the significance of differences among treatments was tested at 5% probability level.

RESULTS AND DISCUSSION

Fresh and dry weights:

The response of Guar plant [fresh and dry weights] to inoculum of *Azotobacter chroococcum* and/or VA *Mycorrhiza* in clayey and/or sandy soils is presented in Table (1). Results showed that in clayey soil, a dual inoculation of *Azotobacter chroococcum* and VA *Mycorrhiza* increased significantly fresh weight of tested plant after 75 day of cultivation compared to control (a full dose of N), as well as, the dry weight was highly significant increased. Likewise, in sandy soil, fresh weight was highly significant increased after 50 and 75 days in presence of a dual inoculum. These data are corresponding to the finding of other studies that showed the kinetic effect of microorganisms on plants by producing growth plant hormones, auxin-like substances and nutrient cycling (Ahmed *et al.*, 2008). Also Ivan *et al.* (2004) reported that the azotobacters, which were inoculated to seedlings, increased significantly the average root biomass up to 98.2%, the root length by 48.45%, the leaf area by 277.86%, the shoot biomass by 29.49% as compared to controls and they also increased the levels of total chlorophylls and carotenoids up to by 151.0% and 158.73%, respectively.

Table (1): Effect of microbial inoculation and N fertilization on fresh and dry weights of guar plant (g/plant)

Treatments	Clayey soil				Sandy soil			
	50 (day)		75 (day)		50 (day)		75 (day)	
	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight
Control (full N dose)	4.28	1.93	11.57	1.99	2.62	1.14	4.50	1.35
Azotobacter chroococcum + activated N dose	3.15	1.61	9.96	2.08	3.73	1.42	4.44	1.77
VA *Mycorrhiza* + activated N dose	2.52	1.20	10.66	1.97	2.07	1.46	3.35	1.54
Azotobacter chroococcum+ VA *Mycorrhiza* + activated N dose	3.74	1.86	12.76	2.40	3.31	1.61	4.97	1.80
L.S.D. at 5%	0.12**	0.11*	1.33*	0.01**	0.11**	0.14*	0.16**	0.17*

- Activated N dose = 15% of the recommended N dose.

Mineral content:

The effect of microbial inoculation and N fertilization on nutrient content (%) of Guar plants are summarized in Table (2). Data obtained showed significant increases in N content (%) of Guar in both clayey and sandy soils as affected by *Azotobacter chroococcum* compared to control. Phosphorus content (%) of the tested plants increased significantly in the treatment inoculated by VA *Mycorrhiza*, whereas, K content (%) was significantly increased in some treatments. The positive effect of microbial inoculation on N, P and K content in Guar plant is probably due to beneficial association between biofertilizers and partner, which improves the plant quality, and these results are in harmony with the finding of Hauka *et al.* (1990). On the other hand, VA *Mycorrhiza* are able to increase N-content in plant by (a) an direct P-supply (b) a direct uptake of N-compounds from soil by hyphae (c) an direct effect on nitrate reductase activity in plants (Barea *et al.*, 1986).

Table (2): Effect of microbial inoculation and N fertilization on nutrient content (%) of guar plant

Treatments	Clayey soil						Sandy soil					
	50 (day)			75 (day)			50 (day)			75 (day)		
	N %	P %	K %	N %	P %	K %	N %	P %	K %	N %	P %	K %
Control (full N dose)	2.03	0.219	1.47	2.31	0.266	1.40	1.61	0.182	0.96	1.26	0.207	0.63
Azotobacter chroococcum + activated N dose	2.66	0.242	0.92	2.80	0.296	1.45	1.96	0.204	1.17	2.03	0.192	1.38
VA *Mycorrhiza* + activated N dose	2.10	0.374	1.31	2.38	0.336	1.17	1.89	0.242	0.97	1.89	0.222	0.86
Azotobacter chroococcum + VA *Mycorrhiza* + activated N dose	2.38	0.329	1.33	2.66	0.344	1.61	1.75	0.288	0.98	1.96	0.187	1.51
L.S.D. at 5%	0.06**	0.015**	0.03**	0.21*	0.013**	0.07**	0.10*	0.011**	0.02**	0.12**	0.007**	0.02**

- Activated N dose = 15% of the recommended N dose.

Microbial count:

The effect of microbial inoculation in presence of activated N dose on total bacteria and *Azotobacter* counts as compared to full N dose is summarized in Table (3). Results obtained showed that total viable bacteria and *Azotobacter* are correlated with microbial inoculation to both clayey and sandy soils. This finding could be due to the active rhizosphere of plant (Hauka *et al.*, 1990). Data obtained also showed an increase of total viable count in the presence of a dual inoculum of *Azotobacter chroococcum* and VA *Mycorrhiza* compared to other treatments. These results are in agreement with those recorded by Sushma (2003) who tested the chemotactic responses of the plant-growth-promoting rhizobacteria *Azotobacter chroococcum* to roots of vesicular-arbuscular mycorrhizal (*Glomus fasciculatum*) in tomato plants, he found that the significant (P = 0.05) greater number of bacterial cells of wild strains was attracted towards vesicular-arbuscular mycorrhizal tomato roots compared to non-vesicular-arbuscular mycorrhizal tomato roots. Substances exuded by roots served as chemoattractants for these bacteria. *Azotobacter chroococcum* showed a stronger response towards sugars than amino acids, but the response was weakest towards organic acids.

Table (3): Total bacterial and *Azotobacter spp* counts of clayey and sandy soils as influenced by microbial inoculation and N fertilization.

Treatments	Clayey soil				Sandy soil			
	50 (day)		75 (day)		50 (day)		75 (day)	
	T. count x 10^6	Azoto. x 10^6	T. count x 10^6	Azoto. x 10^6	T. count x 10^6	Azoto. x 10^6	T. count x 10^6	Azoto. x 10^6
Control (full N dose)	20	0.04	22	0.14	3	0.04	3	0.12
Azotobacter chroococcum + Activated N dose	140	0.72	60	0.68	45	0.47	15	0.41
VA *Mycorrhiza* + activated N dose	50	0.26	39	0.25	37	0.24	14	0.16
Azotobacter chroococcum + VA *Mycorrhiza* + activated N dose	290	0.76	68	0.69	51	0.54	28	0.37

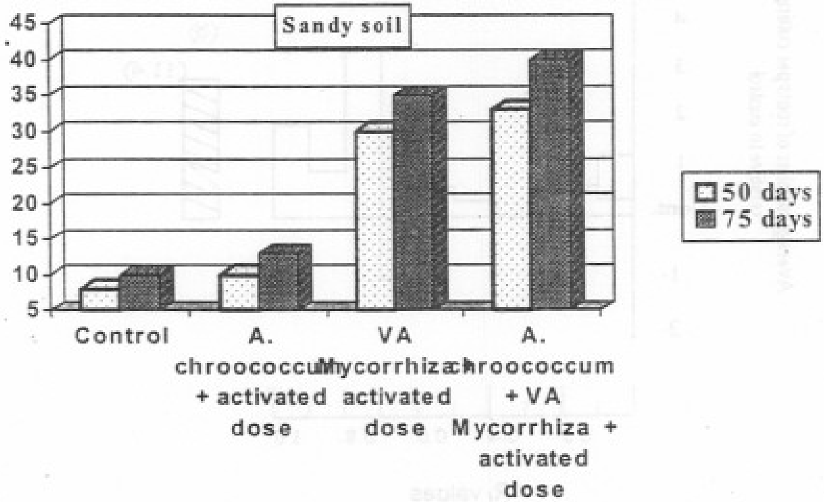
- Activated N dose = 15% of the recommended N dose.

Mycorrhizal colonization:

Mycorrhizal colonization of guar plants either into clay or sandy soil as influenced by microbial inoculation is shown in Fig. (1), which indicates that the VA mycorrhizal colonization increased with increasing the plant age in which, the 60% mycorrhizal colonization formed into roots after 75 days in clay soil inoculated with (VA), meanwhile compared to (17%). In sandy, soil the highest percentage of root colonization (40) was achieved after 75 days from sowing due to inoculation with *Azotobacter chroococcum* and VA *Mycorrhiza*. These findings were similar to those obtained by Gutierrez-Miceli *et al.* (2008) who proved that (VA) colonization increased when diazotrophic bacteria and vermicompost were applied.

Plant growth substances:

The biological activity of *Azotobacter chroococcum* and its effect on the growth of Guar plant is presented in Fig. (2). However, histogram showed that the supernatant of *A. chroococcum* has biological activity on Guar plant including promoters and/or inhibitors substances, in which the chromatographical analysis of the supernatant showed a high content of growth promoters and minor growth inhibitors accumulated in the supernatant. Moreover, the net units of promoters substances being 11.4, meanwhile, the auxin-like substances being 1.14 mg/ml. These data are in accordance with the finding of Ahmed *et al.* (2008), who studied the promoting substances produced by *Azotobacter* isolates and they found that 80% of the isolates produced indole acetic acid (IAA), 74.4% of the isolates produced phosphate-solubilizing substances and 10 - 12.77% produced siderophore substances.



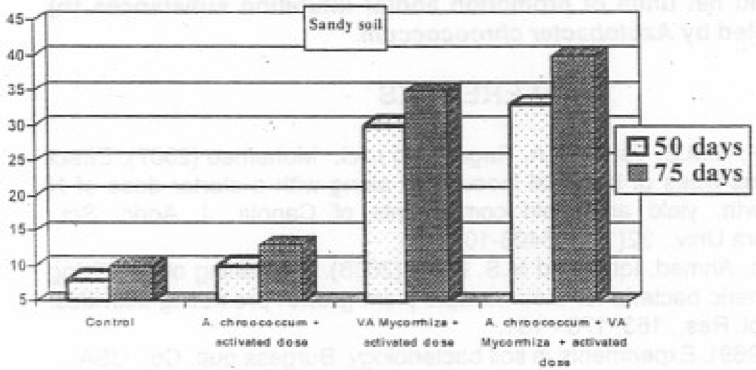
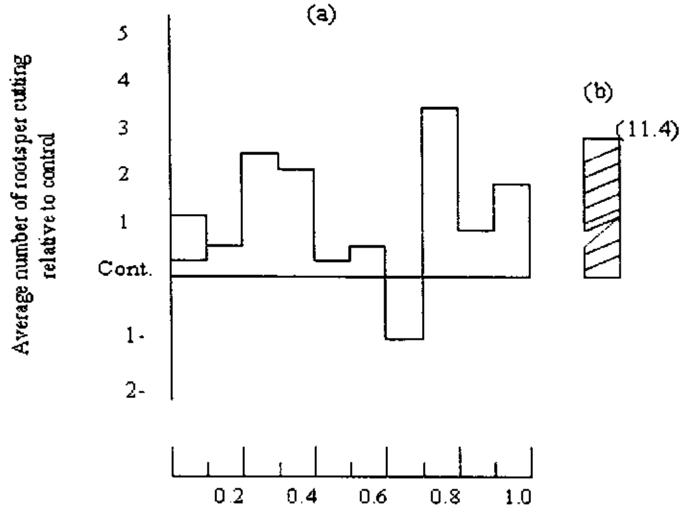


Fig. (1): Percentage ratio of mycorrhizal colonization as affected by microbial inoculation and N fertilization.



R_f values

1 0.3 2.6 2.1 0.3 0.6 1- 3.3 0.6 1.6

Fig. (2): Histogram showing the biological activity at different R_f values (a) and net units of promotion and/or inhibition substances (b) secreted by *Azotobacter chroococcum.*

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دراسة تأثير التلقيح الحيوى بإستخدام فطر الميكروهيزا وبكتيريا الأزوتوباكتر على نبات الجوار

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أجريت هذه الدراسة بهدف إستخدام التلقيح الحيوى بكلا من فطر الميكروهيزا *VA Mycorrhiza* وبكتيريا الأزوتوباكتر *Azotobacter chroococcum* كلا على حدة أو معاً فى تلقيح ثنائى بإضافة ١٥% من السماد النيتروجينى الموصى به كجرعة منشطة مقارنة بالسماد المعدنى ٨٠ كجم/فدان (الكنترول)، وتمت هذه الدراسة في كلاً من تربة طينية Clayey soil وتربة رملية Sandy soil (حيث تم تجميعها من منطقة قلابشو – محافظة الدقهلية).

أوضحت النتائج ما يلى:

وجد أن التلقيح الثنائي باستخدام كلاً من البكتيريا والفطر فى التربة الطينية أدى إلى زيادة معنوية فى الوزن الخضرى لنبات الجوار وذلك بعد ٧٥ يوم من الزراعة، بالإضافة إلى الزيادة المعنوية فى الوزن الجاف، أيضاً وجد زيادة معنوية فى الوزن الخضري fresh weight لنبات الجوار فى التربة الرملية وذلك بعد ٥٠، ٧٥ يوم من الزراعة بإستخدام التلقيح الثنائي.

على الجانب الآخر وجد أن بكتيريا الأزوتوباكتر أعطت زيادة معنوية فى المحتوى النيتروجينى لنبات الجوار مقارنة بالكنترول فى كلاً من التربة الطينية والرملية.

علاوة على ذلك فإن محتوى الفوسفور زاد زيادة معنوية فى حالة إستخدام لقاح *VA Mycorrhiza*.

أيضاً فإن محتوى التربة من بكتيريا الأزوتوباكتر والعد البكتيري الكلى وفطر الميكروهيزا زاد فى كل المعاملات الملقحة ميكروبياً.

على الجانب الأخر أظهرت النتائج من خلال إجراء التحليل الكروماتوجرافى لمستخلص النمو لبكتيريا الأزوتوباكتر وجود محتوى عالي من منظمات النمو أن مواد التنشيط تبلغ ١١,٤ فى حين أن المواد المشابه للأوكسين auxin-like substance تبلغ ١,١٤ مللیجرام/مل.