Effect of Nitrogen Sources, Bio-Fertilizers and Their Interaction on the Growth, Seed Yield and Chemical Composition of Guar Plants

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Abstract: A field experiment was carried out during two successive seasons, 2011 and 2012 aiming to study the effect of utilizing different sources of nitrogen (ammonium nitrate NH_4NO_3 or ammonium sulphate $(NH_4)_2SO_4$ with or without adding bio-fertilizers (biogein at 2 kg/fed., nitrobein at 2 kg /fed., or biogen at 1 kg /fed., + nitrobein at 1 kg /fed.,) as well as their interaction on the plant growth, seed yield, total protein and total guaran content in seeds as well as some chemical contents in leaves. Results revealed that different sources of nitrogen or bio-fertilizers increased the growth parameters; i.e., plant height, number of branches per plant and dry weight of aerial part and leaves per plant, as well as number of pods/plant, weight of seeds (gm/ plant or kg/ fad.,), and chemical constituents such as guaran content, total chlorophyll (a+b), total carbohydrate, total protein and N, P, K compared to untreated plants. Fertilizing plants with ammonium sulphate was the most effective in raising the productivity of seeds and the content and yield of guaran and chemical composition than ammonium nitrate. Treating plants by bio-fertilizer (mixture of biogein+nitrobein) was the most effective in this concern followed by nitrobein and then biogein. The interaction treatment of ammonium sulphate at 60 kg N/fed., + bio-fertilizer (biogein at 1 kg/fed., + nitrobein at 1 kg/fed.,) gave the best result in this concern with significant differences if compared to the control and the other treatments under study in both seasons.
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1. Introduction

Cyamopsis tetragonoloba or cluster bean (Guar) belongs to the family *Fabaceae* (*Leguminaceae*) (Gillet, 1958), is a coarse, upright, bushy, a drought-tolerant summer annual legume and it is cultivated as a feed crop for human and livestock consumption. It is a native plant of India and Pakistan (Rahman and Shafivr, 1967; Patel and McGinnis, 1985).It is grown principally as a seed crop for export and as a vegetable crop for local market in Pakistan. It is grown in tropical Asia, Africa and America. The major world suppliers are India, Pakistan and the United States, with smaller acreages in Australia and Africa (Undersander *et al.*, 2006).

Cyamopsis tetragonoloba plant's growth characteristics make it beneficial for arid production areas. *C. tetragonoloba* bean is commercially grown for its seeds as a source of natural polysaccharide (galactomannan), commercially known as guar gum. Guar gum has a number of uses in food (Khalil, 2001) and other industries, such as paper, textiles, oil well drilling and pharmaceuticals (Anderson, 1949; Whistler and Hymowitz, 1979). *C. tetragonoloba* is a well-known traditional plant used in folklore medicine. It acts as an appetizer, cooling agent, digestive aid, laxative, and is useful in dyspepsia and anorexia Anti-ulcer, anti-secretory, cytoprotective, hypoglycemic, hypolipidemic and anti-hyperglycemic effects (Mukhtar *et al.*, 2006). In addition, Guar beans are potentially high sources of additional phytochemicals (Wang and Morris, 2007).

Guar contains many important nutrients and phytochemicals such as saponin and flavonoids and is well-known traditional plant used in folklore medicine (Mukhtar *et al*., 2006). Many researchers have shown the relationship between legume consumption and health benefits, such as protection from cardiovascular disease, breast cancer, colon cancer, other cancers and diabetes (Kushi *et al.*, 1999; Messina, 1999; Mathers, 2002), anti-inflammatory (Khare, 2004), arthritis (Katewa *et al.*, 2004), anti-oxidant and laxatives effect.

Guar meal is a by-product produced by isolating the guar gum from guar bean containing saponin (Curl *et al.*, 1986; Hassan *et al.*, 2010), 33 to 47.5% crude protein (Bakshi, 1966; Ambegaokar *et al.*, 1969) and can be used as a feed ingredient in animal and poultry nutrition (Lee *et al.*, 2003a, b, 2005; Gutierrez *et al.*, 2007; Hassan *et al.*, 2008).

Plant nutrition is one of the most important factors that increase plant production. Nitrogen is most recognized in plants for its presence in the structure of the protein molecule. In addition, nitrogen is found in such important molecules as purines, pyrimidines, porphyrines, and coenzymes. Purines and pyrimidines are found in the nucleic acids RNA and DNA, which are essential for protein synthesis. The porphyrin structure is found in such metabolically important compounds as the chlorophyll pigments and the cytochromes, which are essential in photosynthesis and respiration. Coenzymes are essential to the function of many enzymes. Accordingly, nitrogen plays an important role in synthesis of the plant constituents through the action of different enzymes (Robert and Francis, 1986). The most inorganic nitrogen sources are NO_3^- and NH_4^+.

Organic farming strategy is growing rapidly all over the world to conserve human health and the environment. Bio-fertilizers are formulations of beneficial microorganisms, which upon application can increase the availability of nutrients by their biological activity and help to improve the soil health for increasing soil fertility with objective of increasing the number of such microorganisms and to accelerate certain microbial processes. The main idea behind organic also secrete various plant growth and health promoting substances (Pandya and Saraf, 2010). Bio-fertilizers are low cost, effective and renewable source of plant nutrients to supplement chemical fertilizers (Boraste *et al.,* 2009). In addition to their role in enhancing the growth of the plants, biofertilizers can act as biocontrol agents in the rhizosphere at the same time. This synergistic effect, when present, increases the role of application of bio-fertilizers in the sustainable agriculture.

Bio-fertilization is generally based on altering the rhizosphere flora, by seed or soil inoculation with certain organisms (microbial inoculants), capable of inducing beneficial effects on a compatible host (El-Haddad *et al.,* 1993). These organisms may affect their host plant by one or more mechanisms such as nitrogen fixation, production of organic acids, enhancing nutrients uptake, synthesis of vitamins, amino acids, auxins and gibberellins which stimulate growth, or increasing the resistance against plant pathogens. (Sprenat, 1990). Bio-fertilizers are produced and practiced in different countries as well as in Egypt under commercial names. Many trials have been conducted in this concern for raising the productivity of medicinal and aromatic plants. Among these trials, the use of nitrogen fixer's microorganisms to reduce or replace the nitrogen fertilizer, needed by plants. Biogein contains *Azotobacter* sp. a nitrogen fixing bacteria, and nitrobein contains Azospirillum, nitrogen fixing bacteria, were obtained from General Organization for Agriculture Equalization Fund (G.O.A.E.F.) Ministry of Agriculture, Egypt.

It is well known that mineral fertilization particularly nitrogen is used to promote growth, oil production and productivity of the plants. Production of medicinal plants using the technique of bio-fertilizers became an essential process to ensure the safety, not only for human but also for the environment in which we live. Bio-fertilizers are one of the most important materials required to substitute of chemical fertilizers for healthy and cheap production. Therefore this study was carried out to investigate the effect of different sources of nitrogen (ammonium nitrate or ammonium sulphate) with or without biofertilizers (biogein, nitrobein or biogein + nitrobein) and their interaction on the plant growth, seed yield and some chemical contents determine the suitable treatments for the maximum production of guar seeds containing more guaran.

2. Materials and Methods

A field experiment was carried out during the two successive seasons of 2011 and 2012 at the Experimental Farm of the National Research Centre in El-Nubaria district, El-Buheira Governorate, Egypt to study the effect of different sources of nitrogen with or without bio-fertilizers and their interaction treatments on the growth, yield and chemical constituents (total guaran content and total protein) of guar plants. The soil texture of the experimental site was sandy in both seasons (Table, 1). Soil and water analysis were shown in Tables (1 and 2), Black *et al.* (1982).

Table (1): Soil physical and chemical analysis.

Soil depth	Soil texture	pH 1:2.5	EC dSm^{-1} 1:5	Soluble cations (meq/100 g soil)				Soluble anions (meq/100 g soil)		
				Na^+	K^+	Ca^{++}	Mg^{++}	HCO^{-3}	Cl^-	SO^{-4}
0-15	Sandy	8.22	0.20	0.90	0.20	0.60	0.50	0.60	0.75	0.85
15-30	Sandy	7.94	0.20	0.80	0.10	0.50	0.30	0.40	0.70	0.60
30-45	Sandy	8.00	0.15	0.60	0.01	0.20	0.20	0.20	0.60	0.21

Table (2): Analysis of irrigation water used in the experiment.

pH 1:2.5	EC dSm^{-1}	Soluble cations (meq/l)				Soluble anions (meq/l)		
		Na^+	K^+	Ca^{++}	Mg^{++}	HCO^{-3}	Cl^-	SO^{-4}
7.55	0.50	2.50	0.20	1.50	0.60	1.20	2.80	0.80

The seeds of guar (*Cyamopsis tetragonoloba*) plant were obtained from Medicinal and Aromatic Research Dep., A.R.C., Ministry of Agriculture. The seeds were sown on 10^{th} April in the two successive seasons. The experimental unit area (plot) was 9.6 m^2 consisting of 4 rows, 0.60 m width and 4 m length and the distance between plants 30cm,every plot containing 50 plants. Irrigation system is surface drip and the irrigation process was done every 3 days.

The experiment included 12 treatments which were the combination of three nitrogen sources; i.e., (control, ammonium nitrate NH_4NO_3 and ammonium sulphate $(NH_4)_2SO_4$ at the rate of 60 kg N/faddan and four bio-fertilizers (control, biogein at 2kg/fad., nitrobein at 2kg /fad., or biogein at 1 kg /fed + nitrobein at 1 kg /fad.,). These treatments were arranged in a split plot design with three replicates. Nitrogen sources were randomly in distributed in main plots, while the bio-fertilizers were randomly arranged in the sub main plots.

The quantity of nitrogen fertilizer at the rate of 60 kgN / feddan was divided into three equal portions as side dressing at 30, 60 and 90 days after planting beside plants. All agricultural practices were carried out as usually recommended for guar production in Egypt.

Biogein contains *Azotobacter* sp. (nitrogen fixing bacteria) and nitrobein contains *Azospirillum* sp. (nitrogen fixing bacteria). Biogein and nitrobein were obtained from General Organization for Agriculture Equalization Fund (GOAEF) Ministry of Agriculture, Egypt. The used bio-fertilizers added as inoculated the seeds and sowing. The bio-fertilizers, biogein and nitrobein was supplied for both mixed with wet seeds by adding 20% Arabic gum solution before planting at the rate of one kg/100 seeds.

The following data were recorded:

On 15^{th} of August a random samples were taken to determine.

A. Vegetative growth:

1. Plant height (cm)
2. Number of branches/plant
3. Dry weight of aerial part (gm/plant)
4. Dry weight of leaves (gm/plant)

B. Yield components:

At harvesting stage of 15^{th} October in the two seasons the following data we recorded:

1. Number of pods/plant.
2. Weight of seeds (gm/ plant).
3. Weight of seeds (kg/ feddan).

C. Chemical constituents:

1. Guaran percentage and content: Guaran percentage was determined only in guar seeds according to the method described by Anderson (1949), while the guaran content was calculated by multiplying guaran percentage by weight of seeds per plant (gm/plant) and faddan (kg/feddan).
2. Plant pigments: Total chlorophyll (a+b) as (mg/gm) of the fresh leaves was extracted from representative samples of the fresh leaves using acetone 85%. The concentrations of chlorophyll a, b and carotenoides were determined by using spectrophotometer and calculated by using Wettstein formula (Wettstein, 1957).
3. Total protein content: Total nitrogen in seeds / plant was determined according to the methods of Champman and Pratt (1961). The calculated total nitrogen percentage was multiplied by the factor 6.25 to obtain the percentage of total protein. Total protein content (gm/plant), was calculated by multiplying protein percentage by weight of seeds per plant.
4. Carbohydrate content: Carbohydrate percentage was determined in leaves according to Herbert *et al.* (1971). Total carbohydrate content (gm/plant), was calculated by multiplying total carbohydrate percentage by weight of leaves per plant.
5. Nitrogen, phosphorus and potassium contents: Nitrogen, phosphorus and potassium percentages in leaves were determined according to the methods of Champman and Pratt (1961). Nitrogen, phosphorus and potassium contents/plant were calculated.

Statistical Analysis

The data of the experiment were statistically analyzed according to Snedecor and Cochran (1981).

3. Results

1. Plant growth parameters and yield and its components

1.1. Effect of nitrogen sources

Tables (3, 5) summarize the growth parameters and yield components during two growth seasons. Generally, nitrogen fertilization at 60 Kg N/ fed., in different forms (ammonium nitrate and ammonium sulphate) increases significantly the growth parameters and yield components compared to control. Also, the results indicated that the different nitrogen forms had a significant effect on plant growth and yield in the two seasons. Results of this study showed that the highest value of plant height, branch number/plant, dry weight of aerial parts and leaves (g/plant) and yield components i.e., number of pods/plant, weight of seeds/plant and /fed., was obtained from ammonium sulphate fertilizer followed by ammonium nitrate fertilizer, and the lowest value from control plants during both seasons (Tables3, 5).

1.2. Effect of biofetilizers

Data in Tables 3 and 5 revealed that bio-fertilizers (biogen, nitrobein and mixture of biogen + nitrobein) treatments had a considerable effect on growth and yield of guar plants during both seasons compared with untreated plants. It can be noticed that both of bio-fertilizers treatment (biogen, nitrobein and mixture of biogen + nitrobein) each alone increases growth characters compared to control. Difference between with and without bio-fertilizer treatments was significant in the two seasons. The treatment of biogen + nitrobein gave the highest values of growth characters i.e., plant height, branch number/plant, dry weight of aerial parts and leaves (g/plant), and yield components i.e., number of pods/plant, weight of seeds/plant and /fed., followed by nitrobein and then biogen treatment compared to untreated plants during both seasons.

1.3. Effect of interaction between nitrogen sources and bio-fertilizers

It is clearly to notice that the combination treatment between nitrogen fertilization sources at 60 Kg N / fed., and bio-fertilizers application (biogen, nitrobein and mixture of biogen + nitrobein) significantly increased growth characters i.e., plant height, branch number/plant, dry weight of aerial parts and leaves (g/plant), and yield components i.e., number of pods/plant, weight of seeds/plant and /fed., comparison to control plants. The treatment of ammonium sulphate with mixture of biogen + nitrobein gave the maximum values of plant height and number of branches/plant and yield components i.e., number of pods/plant, weight of seeds/plant and /fed., followed by the same nitrogen form combined with nitrobein (Tables 4 and 6). On the other hand the highest value of dry weight of aerial parts and leaves (g/plant) was obtained as a result of the combination treatment between ammonium sulphate fertilization at 60 Kg N/ fed., and application with mixture of biogen + nitrobein followed by the treatment of ammonium nitrate fertilization with mixture of biogen + nitrobein in both seasons (Tables 4, 6).

2. Chemical constituents

2.1. Effect of nitrogen sources

Data presented in Tables (5-10), indicated that guaran content (%) as shown in Table (5) and yield (g/plant or kg/fed.,) in Table (7) were significantly increased as a result of nitrogen fertilization. In both seasons, the maximum guaran content (%) and yield (g/plant or kg/fed.,) was obtained with fertilization with 60 Kg N/ fed., as ammonium sulphate followed by 60 Kg N / fed., as ammonium nitrate and unfertilized plants gave the lowest data.

Data in Tables (7, 9) show that total protein, total chlorophyll (a+b), total carbohydrate and nitrogen, phosphorus, potassium content was significantly increased with nitrogen fertilization. The highest values of total protein, total chlorophyll (a+b), total carbohydrate and nitrogen, phosphorus, potassium content content in the first and second seasons were detected in the plants that received nitrogen fertilization (60 Kg N / fed., as ammonium sulphate).

2. 2. Effect of biofetilizers

Concerning the effect of bio-fertilizers on guaran content (%), Table 5 and yield (g/plant or kg/fed.,) in Table 7, it can be noticed that application treatment with (biogen, nitrobein and mixture of biogen + nitrobein) significantly increased guaran content and yield (Tables, 5 and 7) during two seasons. The mixture of biogen + nitrobein gave the highest values for guaran content (%) and yield (g/plant or kg/fed.,) followed by nitrobein and then biogen treatment, whereas, untreated plants gave the lowest values during both seasons.

From Tables (7, 9), it is clear that application of bio-fertilizers leads to more total protein, total chlorophyll (a+b), total carbohydrate and nitrogen, phosphorus, potassium contents compare to none bio-fertilizers application. The maximum values of total protein, total chlorophyll (a+b), total carbohydrate and nitrogen, phosphorus, potassium were observed as a result of treating with mixture of biogen + nitrobein treatment for both seasons.

Effect of these application treatments on nutrients content was observed in Table 9. Generally, these treatments increased nutrients content (N, P and K) compared with control treatment. The highest value of (N, P and K) was resulted from the treatment of mixture of biogen + nitrobein followed by nitrobein and then biogen, while the control treatment gave the lowest values of N, P and K during the two seasons.

2. 3. Effect of interaction between nitrogen sources and bio-fertilizers

Tables 6, 8 and 10 describe the accumulation of guaran content (%) and yield (g/plant or kg/fed.,), total protein, total chlorophyll (a+b), total carbohydrate and nitrogen, phosphorus, potassium in guar.

Concerning the interaction of nitrogen fertilization combined with application bio-fertilizers on guaran content (%) in Table (6) and yield (g/plant or kg/fed.) in Table 8, the data in Table 6 and 8 showed that application of ammonium sulphate combined of bio-fertilizers treatments was the best than ammonium nitrate application combined with the same bio-fertilizers treatments. The highest value of guaran content (%) and yield (g/plant or kg/fed.,) was obtained with nitrogen fertilization at the level of 60 Kg N/ fed., as ammonium sulphate combined with mixture of biogen + nitrobein followed by the treatments (60 Kg N/ fed., ammonium sulphate combined with nitrobein) and (60 Kg N/ fed., as ammonium nitrate combined with mixture of biogen + nitrobein), respectively).

Also, data tabulated in Tables 8 and 10 revealed that the combination treatments had a pronounced effect on total protein, total chlorophyll (a+b) and total carbohydrate content. It is clear that the maximum values of total protein, total chlorophyll (a+b) and total carbohydrate content in the first and second seasons were obtained as a result of the combination treatment between nitrogen fertilizer (ammonium sulphate) at 60 Kg N/ fed., and application biogen + nitrobein.

Results in Table 10 showed that, the effect of the interaction between nitrogen fertilization and bio-fertilizers application treatments on nutrients content (N, P and K). All treatments increased nutrients content compared with the control treatment. Concerning the effect of these treatments on (N, P and K) content, it can be observed that the combination treatment between ammonium sulphate of nitrogen fertilizer (60Kg N / fed.,) and biogen + nitrobein followed by the same mixture of biogen + nitrobein with another form of nitrogen (ammonium nitrate) gave the maximum value of (N, P and K) content during both seasons.

On the other hand, the combination between ammonium sulphate form of nitrogen fertilization and bio-fertilizers application with (biogen + nitrobein) gave the maximum values of total protein, total chlorophyll (a+b) and total carbohydrate content followed by the same form of nitrogen with nitrobein during both seasons (Tables 8, 10).

Table 3: Effect of nitrogen sources and bio-fertilizers on the growth parameters of guar plant during two seasons (2011 and 2012)

Treatments	Plant height(cm)		Number of branches/plant		Dry weight of aerial parts (gm)		Dry weight of leaves /plant (gm)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Nitrogen sources								
Control	135.02	137.54	13.56	14.25	55.30	57.68	22.36	23.36
AN	151.96	154.44	15.80	16.55	62.12	64.66	24.87	26.16
AS	166.19	169.26	18.47	18.11	66.15	69.25	28.25	29.75
L.S.D. at 5 %	0.91	0.90	0.65	0.60	0.56	0.67	0.76	0.76
Biofertilizers								
Control	137.51	140.93	13.33	14.27	53.08	56.06	20.56	22.05
B	148.02	149.76	15.11	15.71	59.03	61.83	23.68	24.96
N	154.26	156.80	16.42	16.75	63.93	66.19	26.23	27.18
B+N	164.43	167.49	18.92	18.48	68.71	71.37	30.16	31.50
L.S.D. at 5 %	1.06	1.04	0.75	0.70	0.65	0.78	0.87	0.87

AN: ammonium nitrate, AS: ammonium sulphate, B: Biogein, N: Nitrobein

Table 4: Effect of the interaction between nitrogen sources and bio-fertilizers on the growth parameters of guar plant during two seasons (2011 and 2012)

Nitrogen sources	Biofertilizers	Plant height(cm)		Number of branches/plant		Dry weight of aerial parts (gm)		Dry weight of leaves /plant (gm)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	Control	122.37	126.70	11.06	11.96	47.25	50.17	16.64	18.90
	B	132.94	133.46	13.21	13.52	52.85	55.12	20.80	21.00
	N	137.97	140.40	13.92	14.90	57.75	59.71	24.26	25.20
	B+N	146.81	149.58	16.06	16.64	63.35	65.72	27.73	28.35
AN	Control	139.53	141.61	13.21	14.56	53.55	56.18	20.80	22.05
	B	148.72	150.97	14.99	16.29	60.20	63.60	23.22	24.15
	N	155.13	158.60	17.13	16.98	65.45	67.48	26.00	26.95
	B+N	164.45	166.57	17.85	18.37	69.30	71.37	29.46	31.50
AS	Control	150.62	154.47	15.71	16.29	58.45	61.83	24.26	25.20
	B	162.41	164.84	17.13	17.33	64.05	66.78	27.04	29.75
	N	169.69	171.42	18.21	18.37	68.60	71.37	28.42	29.40
	B+N	182.03	186.33	22.85	20.45	73.50	77.02	33.28	34.65
L.S.D. at 5 %		1.83	1.81	1.30	1.22	1.13	1.35	1.51	1.52

AN: ammonium nitrate, AS: ammonium sulphate, B: Biogein, N: Nitrobein

Table 5: Effect of nitrogen sources and bio-fertilizers on number of pods per plant, weight of seeds (per plant and feddan) and guaran percentage of guar plant during two seasons (2011 and 2012)

Treatments	Number of pods / plant		Weight of seeds (gm/ plant)		Weight of seeds (kg/ feddan)		Guaran (%)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Nitrogen sources								
Control	111.16	112.75	16.95	18.37	395.54	428.70	25.56	27.04
AN	124.41	126.08	18.96	20.28	442.60	473.19	27.21	28.61
AS	132.66	134.08	21.71	23.14	506.69	539.92	29.38	30.71
L.S.D. at 5 %	0.76	0.74	0.59	0.51	13.83	12.09	0.52	0.54
Biofertilizers								
Control	112.88	115.55	15.22	16.87	355.15	393.65	25.42	26.87
B	121.33	122.33	18.08	19.06	421.91	444.88	26.46	27.57
N	125.44	126.44	20.77	22.07	484.66	514.98	28.19	29.31
B+N	131.33	132.88	22.77	24.38	531.39	568.91	29.46	31.41
L.S.D. at 5 %	0.88	0.86	0.68	0.59	15.97	13.98	0.61	0.62

AN: ammonium nitrate, AS: ammonium sulphate, B: Biogein, N: Nitrobein

Table 6: Effect of the interaction between nitrogen sources and bio-fertilizers on number of pods per plant, weight of seeds (per plant and feddan) and guaran percentage of guar plant during two seasons (2011 and 2012)

Nitrogen sources	Biofertilizers	Number of podes/ plant		Weight of seeds (gm/ plant)		Weight of seeds (kg/ feddan)		Guaran (%)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	Control	98.33	100.66	13.39	15.60	312.42	363.99	23.92	25.47
	B	109.66	110.33	16.13	17.33	376.51	404.43	24.96	26.17
	N	115.00	116.33	18.71	19.41	436.59	452.97	26.34	27.22
	B+N	121.66	123.66	19.57	21.14	456.62	493.41	27.04	29.31
AN	Control	115.66	119.00	15.45	16.29	360.49	380.17	25.30	26.52
	B	123.33	124.33	17.51	18.72	408.56	436.79	26.34	27.22
	N	126.00	127.00	19.91	21.84	464.63	509.59	28.08	29.31
	B+N	132.66	134.00	23.00	24.26	536.73	566.21	29.12	31.41
AS	Control	124.66	127.00	16.82	18.72	392.53	436.79	27.04	28.61
	B	131.00	132.33	20.60	21.14	480.66	493.41	28.08	29.31
	N	135.33	136.00	23.69	24.96	552.75	582.39	30.16	31.41
	B+N	139.66	141.00	25.75	27.73	600.82	647.10	32.24	33.50
L.S.D. at 5 %		1.53	1.49	1.18	1.03	27.66	24.21	1.07	1.08

AN: ammonium nitrate, AS: ammonium sulphate, B: Biogein, N: Nitrobein

Table 7: Effect of nitrogen sources and bio-fertilizers on guaran content (per plant and feddan), total protein content and total chlorophyll content of guar plant during two seasons (2012 and 2012)

Treatments	Guaran content (gm/ plant)		Guaran content (kg/ feddan)		Total protein content (gm/ plant)		Total chlorophyll (a+b) content (mg/gm)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Nitrogen sources								
Control	4.36	4.99	101.83	116.63	4.09	4.52	3.41	3.50
AN	5.20	5.86	121.43	136.75	4.72	5.16	3.55	3.63
AS	6.44	7.17	150.42	167.41	5.80	6.33	3.67	3.84
L.S.D. at 5 %	0.22	0.21	5.15	4.92	0.17	0.14	0.05	0.03
Biofertilizers								
Control	3.88	4.55	90.61	106.25	3.67	4.15	3.36	3.46
B	4.81	5.27	112.30	123.11	4.53	4.86	3.51	3.60
N	5.88	6.50	137.41	151.87	5.32	5.75	3.60	3.70
B+N	6.76	7.70	157.90	179.82	5.97	6.59	3.71	3.87
L.S.D. at 5 %	0.25	0.24	5.75	5.68	0.20	0.16	0.05	0.05

AN: ammonium nitrate, AS: ammonium sulphate, B: Biogein, N: Nitrobein

Table 8: Effect of the interaction between nitrogen sources and bio-fertilizers on guaran content (per plant and feddan), total protein content and total chlorophyll content of guar plant during two seasons (2011 and 2012)

Nitrogen sources	Biofertilizers	Guaran content (gm/ plant)		Guaran content (kg/ feddan)		Total protein content (gm/ plant)		Total chlorophyll (a+b) content (mg/gm)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	Control	3.20	3.97	74.81	92.82	3.02	3.65	3.17	3.31
	B	4.03	4.53	94.06	105.86	3.83	4.20	3.40	3.50
	N	4.93	5.28	115.05	123.30	4.60	4.83	3.50	3.53
	B+N	5.28	6.19	123.38	144.56	4.92	5.41	3.57	3.63
AN	Control	3.90	4.32	91.14	100.86	3.76	4.02	3.40	3.47
	B	4.61	5.08	107.72	118.73	4.34	4.70	3.53	3.60
	N	5.58	6.39	130.38	149.30	4.98	5.55	3.60	3.67
	B+N	6.70	7.63	156.46	178.10	5.82	6.39	3.67	3.80
AS	Control	4.53	5.36	105.89	125.08	4.24	4.80	3.50	3.60
	B	5.79	6.20	135.13	144.73	5.42	5.69	3.60	3.70
	N	7.14	7.84	166.79	183.01	6.39	6.88	3.70	3.90
	B+N	8.30	9.29	193.87	216.80	7.16	7.97	3.90	4.17
L.S.D. at 5 %		0.44	0.42	10.30	9.84	0.34	0.28	0.11	0.09

AN: ammonium nitrate, AS: ammonium sulphate, B: Biogein, N: Nitrobein

Table 9: Effect of nitrogen sources and bio-fertilizers on total carbohydrate, nitrogen, phosphorus, potassium contents in leaves of guar plant during two seasons (2011 and 2012)

Treatments	Total carbohydrate content (gm/ plant)		Total nitrogen content (mg/ plant)		Total phosphorus content (mg/ plant)		Total potassium content (mg/ plant)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Nitrogen sources								
Control	2.11	2.36	586	628	119	128	547	584
AN	2.51	2.75	688	733	149	161	636	680
AS	3.30	3.63	812	885	180	195	760	821
L.S.D. at 5 %	0.08	0.09	25	23	3.9	3.9	21	19
Biofertilizers								
Control	1.86	2.13	529	578	11.10	12.40	493	537
B	2.40	2.68	637	688	13.70	14.90	597	648
N	2.86	3.08	731	784	15.80	16.70	685	724
B+N	3.45	3.76	883	944	19.00	20.50	815	871
L.S.D. at 5 %	0.09	0.10	29	27	5.8	5.8	23	21

AN: ammonium nitrate, AS: ammonium sulphate, B: Biogein, N: Nitrobein

Table 10: Effect of the interaction between nitrogen sources and bio-fertilizers on total carbohydrate, nitrogen, phosphorus, potassium contents in leaves of guar plant during two seasons (2011 and 2012)

Nitrogen sources	Biofertilizers	Total carbohydrate content (gm/ plant)		Total nitrogen content (mg / plant)		Total phosphorus content (mg/ plant)		Total potassium content (mg / plant)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	Control	1.27	1.69	401	472	75	95	374	436
	B	1.92	2.08	537	556	108	111	503	524
	N	2.41	2.60	644	694	132	139	605	634
	B+N	2.84	3.06	762	789	159	167	707	741
AN	Control	1.92	2.14	544	581	114	126	493	531
	B	2.25	2.43	625	664	135	145	583	624
	N	2.66	2.87	728	768	159	167	679	713
	B+N	3.21	3.56	853	918	187	205	789	853
AS	Control	2.38	2.56	642	680	144	151	613	646
	B	3.04	3.54	749	844	168	190	705	795
	N	3.50	3.77	822	889	185	195	771	825
	B+N	4.29	4.64	10.33	11.26	223	243	950	1020
L.S.D. at 5 %		0.17	0.18	50	47	9.8	9.8	41	37

AN: ammonium nitrate, AS: ammonium sulphate, B: Biogein, N: Nitrobein

4. Discussion

Many investigators studied the effect of different sources of nitrogenous fertilizers on different plant species. El-Sayed (1991) on pot marigold and Abd-Alazeem (1992) on (*Tagetes minuta* L.) concluded that ammonium sulphate andammonium nitrate were efficient in enhancingplant height, number ofboth branches and leaves , plant dry weight , and seed weight of plant. Omran *et al.* (1998) on peanut found that, ammonium sulphate application yielded the highest dry weight of plant and grains than ammonium nitrate or the control. Sharife and Keshda (2000) on canola plants found that, nitrogen addition in the forms of ammonium sulphate significantly increased plant height, number of branches, seed yield and oil yield if compared with urea or calcium nitrate as a source of nitrogen. Bardisi and Abdel Bary (2000) on lettuce plant showed that, fertilizing with ammonium sulphate increased the dry weight of leaves and plant if compared with ammonium nitrate or calcium nitrate. Badran *et al.* (2001) on guar plants, found that , all of the vegetative parameters including plant height, number of branches/plant, dry weight of leaves and whole plant increased with all sources of nitrogen, but the high rate of ammoniumnitrate and ammonium sulphate were the most effective concerning the seed yield, the most effectivetreatment was ammonium sulphate. Habib (2005) on *Oreodoxa regia* seedlings showed that, all nitrogen sources significantly increased the plant height and the ammonium sulphate resulted in the tallest seedlings and the fresh weight of foliage. The contents of phosphors and potassium were the highest with the treatment of ammonium sulphate.

Said-Al Ahl (2005) reported that plant height, number of branches, plant fresh and dry weights, umbels number and fruits yield increased with nitrogen and/or bio-fertilizer treatment r in *Anethum graveolens*, with the high dose of nitrogen producing the highest values. Hassan *et al.*(2012) reported that bio-fertilizer treatments, enhanced plant height, branch number/plant, plant dry weight, Pods number/plant, Pods dry weight, seed index ,seed number/plant and seed yield / plant & / feddan,nitrogen, phosphorus, potassium, protin percentage and Alkaloids percentage and Alkaloids content/plant(g).The bifertilizers stimulate the growth, yield and chemical constituents. These results in harmony with those obtained by, Swaefy *et al.* (2007) on peppermint plant, El-Shafie *et al*. (2010) on khella plants. Hellal *et al.* (2011) on dill plant indicated that applying biofertilizer treatment alone or in combination with chemical N fertilizer increased the growth, yield and chemical constituents of dill plant compared to the untreated control. The highest values of vegetative growth, oil yield, chlorophyll content and NPK percentages were recorded by the treatment of bio-fertilizer plus two third of recommended dose of nitrogen fertilizer. Also, Abdel-Kader *et al.* (2012) on roselle and guar. El Gendy *et al.* (2013) on *Cymbopogon Citratus* showed that, nitrogen as soil application and biofertilizers had a significant effect on vegetative growth characters. On the other hand, the interaction treatments between of N and biofertilizers led to significant increment for yield of essential oil compared to control during both seasons. Applications of biofertilizers with nitrogen fertilizers resulted in a significant effect of polyphenol and flavonoid content in both seasons. Ghilavizadeh *et al.* (2013) on ajowan showed that seed yield, essential oil content and essential oil yield were obtained by using the biofertilizer.

The favorable effect of biogein on plant growth and chemical contents of other medicinal and aromatic plants. Abd El-Latif *et al.* (2002) mentioned that the maximum value of plant height, number of branches /plant, fresh and dry weight of *Mataricaria chamomilla* L. plantwhen received treatments of 4 kg biogein/fed. Eissa (2004) on sage found that plant height, number of branches per plant, the greatest herb yield per faddan was produced from plant inoculated by biogein bio-fertilizers, also N,P, K and total carbohydrate per plant.

Concerning the effect of nitrobein on medicinal and aromatic plants, Paramaguru and Natarajon (1995) found that Azospirillum as a seed treatment and soil application combined with 56 kg N/ha., increased plant height, number of branches and number of lateral roots *of Capsicum annum* plants grown under semi-arid conditions and Abd El-Salam (2007) on roselle plants.

Effect of biogein and nitrobein, Amin (1997) on coriander, fennel and caraway plants showedthat, the growth characters were positive influenced by seed inoculation (*Azotobacter* sp. and *Azospirillum* sp.) with half dose of inorganic fertilizers, Saleh *et al.* (1998) showed that the growth of datura and its content of alkaloids were greatly improved by inoculation with Azotobacter and Azospirillum. El-Sawy *et al.* (1998) with *Ammi visnaga* reported that inoculation with mixture of *Azotobacter* and *Azospirillum* amendment with full dose of inorganic N-fertilizer remarkably increased plant growth and production of Khellin. Harridy and Amara (1998) found that plant growth and yield of roselle plant showed a positive response especially with Azotobacter with 0 and 40 kg N/ fed. It was found that total N content in plant leaves was affected according to the addition of different strains of bacteria and inorganic N rate. Eid and El-Ghawwas (2002) on marjoram and Eissa (2004) on sage plants, they mentioned that nitrobein application caused a positive effect on growth characters, seed yield and the chemical constituents of plants. Kandeel *et al.* (2002) on *Ocimum basilicum* found that plant height, number of branches , fresh and dry weight of herb, essential oil (percentage and content), and (N,P,K percentage) were increased by inoculation (*Azotobacter* sp. and *Azospirillum* sp.) with levels nitrogen fertilizers. Badran and Safwat (2004) on fennel showed that the growth characters, yield components and oil yield were positive influenced by inoculation organic manure and bio-fertilizers. Badawi *et al.* (2005) on sweet fennel found that interaction treatments between bio-fertilizers, nitrogen sources and nitrogen levels increased vegetative growth, chemical contents (N, P, K content) and essential oil yield and Abd El-Salam (2007) on bottle gourd plant.

Application of bio-fertilizer and nitrogen treatments to guar plants increased plant height. This may be due to the increase of N in the root zone and the synergistic effect of these microorganisms on the physiological and metabolic activities of the plant. This enhancing effect may induce exudate of some hormonal substances like cytokinins and auxins, which encourage plant height. The nutrients available in the soil increased with increasing nitrogen application and bio-fertilizers. This may be attributed due to more atmospheric nitrogen fixed in the soil, which was probable due to mobilization of bacteria, providing favorable conditions (Rajput and Singh, 1996). The increase in nitrogen uptake enhanced physiological activities of plants and thereby increased the growth and yield (Rajput *et al.,* 1995). The ability of *Azotobacter* to produce growth substances and antifungal substances in addition to fixed nitrogen made available to plants was probably the reason of higher yields (Mishutin and Shilnikova, 1971). Similarly, *Azospirillum* is reported to produce indole acetic acid, gibberellins and cytokinins like substances along with nitrogen fixation (Tein *et al.,* 1979). Nitrogen stimulates the meristimatic activity for producing more tissues and organs. Abd-El-Fattah and Sorial (2000) ensured that increasing nitrogen levels increased of cytokinins and gibberellins which enhance cell division and cell enlargement and thus increased vegetative growth. Meanwhile, (Subb-Rao, 1984) who stated that, the favourable effect of bio-fertilizers on growth parameters might be ascribed to its important role in fixing atmospheric N as well as increasing the secretion of natural hormones namely IAA, GA3 and cytokinins, antibiotics and possibly raising the availability of various nutrients.

Thus, it can be concluded that treating guar plants with nitrogen and bio-fertilizer increased the formation of branches. The increase in number of branches may be due to the increase in nitrogen content in the soil and biological fixation nitrogen as well as growth promoting substances such as indole acetic acid, cytokinins and gibberellins produced by the organisms used. The increase in the level of nitrogen fertilizer and biological fixation nitrogen as well as growth promoting substances were responsible for increased number of branches, causing higher photosynthesis and assimilation rates, metabolic activity and cell division which were responsible for increase in the growth characters, yield attributes and yield of guar(Chauhan *et al.,* 1996).

In conclusion the increment in plant fresh weight may be attributed to a greater proliferation of root biomass resulting in the higher absorption of nutrients and water from the soil leading to production of higher vegetative biomass (Taylor and Klepper, 1978; Hamblin, 1985). The increase in plant fresh weight may be due to the increase of N in the root zone as a result of nitrogen application and fixed N by bacteria. Also, the solubilization of mineral nutrients synthesis of vitamins, amino acids and gibberellins, which stimulate growth and yield (Sprenat, 1990). The stimulation effects of applying nitrogen on vegetative growth characters may be due attributed to the well known functions of nitrogen in plant life, being a part of protein; it is an important constituent of protoplasm. Also, enzymes, the biological catalytic agents, which speed up life processes, have N as their major constituents. Moreover, nitrogen involves in many organic compounds of plant system. A sufficient supply of various nitrogenous compounds is therefore, required in each plant cell for its proper functioning (Mengel and Kirkby, 1987). Generally, the enhancing effect of N-fertilization and bio-fertilizers on plant growth may be due to the positive effects of N-element on activation of photosynthesis and metabolic processes of organic compounds in plants which in turn, encourage the plant vegetative growth (Gardener *et al.,* 1985). The increase in plant growth ascribed to contributing some hormone substances, such as gibberellins, auxins and cytokinins (Tien *et al.,* 1979; Bouton *et al.,* 1985; Cacciari *et al.,* 1989). These phytohormones may stimulate the cell elongation and division and hence plant growth (Paleg, 1985).

The superiority of bio-fertilizers and nitrogen application alone or together for stimulating plant dry weight exhibited the same trend owing to The favorable effect of nitrogen fertilizer and bio-fertilizers on plant growth and yield attributes might be due to the improved nutrition and production of growth-promoting substances by micro-organisms (Dhillo *et al.,* 1980). The superiority of the application of nitrogen + bio-fertilizers than the other treatments may be due to the increase in available amounts of nutrients (Salem, 1986); also, microbiological processes can change unavailable forms of nutrients into available ones that can be easily assimilated by plants (Subb-Rao, 1981; Alaa El-Din, 1982). In other words, the positive interactions between the applied N-fertilizer levels and biofertilizers on plant vegetative growth may be due to the promoting effects of both N-element and biofertilizers together on the established plant roots and nutrient uptake (Wange, 1995). Increasing N fertilizer increase the population of bacteria and this in turn increase nitrogen fixation and release of phytohormones and trace elements for that interaction increase plant growth.

The increment of the number of pods per plant could be explained through the effective role of previous fertilization treatments in increasing the number of branches per plant. The necessity of N, as a plant nutrient is emphasized by the fact that it is a main constituent of many organic compounds in plant (Tyler *et al.,* 1988). The increase in pods number may be due to the existence of some phenolic compounds which proved to be effective in promoting flowers (Dakhly *et al.,* 2004). However, Dai *et al.* (1995) showed that a high leaf N content prior to the physiological differentiation stage was a key factor for flower bud formation.

The increase in seeds yield may be due to the increase in the number of pods per plant. Such effects of fertilization treatments under study on seeds yield may be attributed to the important rule of these fertilization treatments on the synthesis of metabolites used in seed formation. The increase in yield with nitrogen treatment is probably due to a deficiency of this element in the soil (Prakasa-Rao, 1992). Nitrogen application exerted beneficial effect on yield-attributing characters, which in turn increased the seed yield (Shivran *et al.,* 1995).

The stimulatory affect of nitrogen was observed and discussed by Bali (1988) on dill found the increase in the yield could be attributed to the beneficial effects of N on plant height, number of branches/plant, number of umbels and seed weight/plant. Also, the increase in fruits yield could be due to bio-fertilizer inoculation which helped meet nitrogen requirement besides nitrogen fertilizer and other plant growth substances resulting in good yield (Sawarkar and Goydani, 1996). Also, Abd-El-Fattah and Sorial (2000) on squash stated that increasing the fruits yield by the application of N levels may be due to the enhancement effect of nitrogen to vegetative growth and leaves area/plant which create a large surface available for photosynthesis and thus in turn increased the weight of fruits and total yield. Besides, nitrogen is an important constituent of chlorophyll, which increases photosynthesis. The increase in number of grains/row may be attributed to the effect of nitrogen on increasing pollen grains/tassel and silking maturity period as well as to the longer ear of maize plant. (El-Rewainy and Galal, 2004). Also, Dakhly *et al.* (2004) on squash showed that both N application and bio-fertilizers increased in sex ratio which reflects the important of equilibrium between male and female flowers that increase a good pollination and high fruit setting percentage. The considerable increase in number of female flowers which reflected the increase in fruit yield may be primarily attributed to the increase in microbial production of cytokinins within the rhizosphere (Noel *et al.,* 1996). On the other hand, Shifriss (1985) found that the female expression of squash plants could be converted into monoecouism by spraying with GA_3. Moreover, Chailakhyon (1979) stated that the sex expression in plants is regulated by gibberellins, which are synthesized in leaves, and cause male sex expression and by cytokinins synthesized in roots and cause female sex expression. Since, lau *et al.* (1995) reported that N and biofertilizer application had significant effects on traits affecting the male function of squash plants (staminate flower production pollen, production per flower and pollen grain size).

Said-Al Ahl (2005) showed that nitrogen fertilizer and/or bio-fertilizer treatment led to an increase in the total carbohydrate, total chlorophyll and N, P, K contents in *Anethum graveolens*.The stimulation effects of applying nitrogen may be attributed to the well known functions of nitrogen in plant life, as described previously. Generally, the enhancing effect of N fertilization may be due to the positive effects of nitrogen on activation of photosynthesis and metabolism of organic compounds that encourage plant development. Accordingly, nitrogen plays an important role in the synthesis of plant constituents through the action of different enzymes. A sufficient supply of various nitrogenous compounds is, therefore, required in each plant cell for proper functioning. Previous studies report that nitrogen application affects the growth, yield, essential oil and chemical composition Abdul Al-Kiyyam *et al.* (2008) on marjoram and Said-Al Ahl *et al.* (2009) on oregano.

The increment in plant growth parameters reflect the vital importance role of nitrogen as a plant nutrient, since it is an integral part of the chlorophyll molecule, nucleic acids, proteins, and other important substances (Delvin and Witham, 1983). The increase in chlorophyll content may be due to available nitrogen but also the increase in trace elements in the soil caused by the organic acids produced by microoganisms leading to a decrease in the pH of the soil (Subb-Rao, 1981).

The increment in total carbohydrates may be due to the increase of photosynthesis as a result of the increase in photosynthetic pigments content in the leaves. The necessity of N as a plant nutrient is emphasized by the fact that it is a main constituent of many organic compounds in plant (Tyler *et al.,* 1988). (Aloni *et al.*, 1991) indicated that, changes in the leaf starch and soluble sugars concentrations occurred as a function of N supply and leaf age. In the roots, low N led to lower sucrose and higher levels of soluble sugars and starch. More sucrose was transported and accumulated into leaf veins of low N tissue.The increment in nitrogen percentage in both seasons may be due to fertilized with nitrogen and fixed nitrogen by fixers used as a source of nitrogen. As well as the increase of root size, which led to augmentations in nitrogen uptake from the soil? The application of nitrogen increased the concentration of N in plant tissue and also the total fresh herbage yield, which ultimately led to the increased uptake of N (Singh *et al.,* 1997).The increment of P% in plant tissues as a result of bacteria which secrete organic acids leading to transfer fixed phosphate to available phosphate. In addition, the increment available P in the root zone, which increases P uptake (Burger *et al.*, 1997). The increase of root size as a result of the increment in root length may explain these increases in potassium percentages in both seasons at both stages. Also, pH of the soil has an important role in potassium absorption from the soil, the suitable pH in soil for potassium absorption is between (5 and 7) (Schroeder, 1984). Lopez Camelo *et al.* (1995) studied the effect of urea (0 or 75 kgN/ha) on coriander observed that total accumulation of N, P and K was higher in flowers than in seeds. No significant diffierences in nutrient accumulation due to N fertilizer was apparent. An association between acetic acid-extrable P and K and total concentrations of these elements in tissues.

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