Effect of Two Species of *Cyanobacteria* as Biofertilizers on Characteristics and Yield of Chickpea Plant

Sanaa, j. Burjus*[1], Abdul latif M. Jawad[1], Nabeel K. Al-Ani[2]
[1]Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.
[2]Department of Biotechnology. College of Science, AL-Nahrain University Baghdad, Iraq.

Abstract

The *cyanobacteria* strains were used in bioreactors to produce biomass. The total biomass after one month in suitable conditions such as efficient gas exchange, powerful light source and suitable medium composition was 7.99 mg/ml for *N. commune* and 5.83mg/ml for *A. circinalis*. These algae were applied alone or mixed in two rates (5 or 10 ml/ 100 g compost); *Azotobacter chroococcum* was used before 7 days of harvesting or with other *cyanobacteria* species. The total Nitrogen showed 1.84% with *Azotobacter*, however the nitrogen in mix culture was 1.80% mean while, the control treatment was 1.49%.. These results indicate that we can reduce chemical fertilizers by 1/4 or may 1/2 dose of normal requirement on growth and yield of chickpea plant.

Keyword: *cyanobacteria*, biomass, bio- fertilizer.

تاثير نوعين من الطحالب الخضر المزرقة كاسمدة حيوية على صفات وانتاجية نبات الحمص

سناء جميل برجس*[1]، عبد اللطيف محمد جواد[1]، نبيل خلف العاني[2]
قسم علوم الحياة، كلية العلوم، جامعة بغداد، بغداد، العراق[1]
قسم التقانات الاحيائية، كلية العلوم، جامعة النهرين، بغداد، العراق[2]

الخلاصة :

تم استخدام انواع من السيانوبكتريا في المفاعل الحيوي لانتاج الكتل لحيوية. الحصيلة النهائية للكتل الحيوية التي تم الحصول عليها بعد مده شهر واحد من حضنها بالمفاعل لنووي المزود بالتبادل الغازي ومصدر للاضاءة مع الوسط الغذائي الملائم وكانت 7.99ملغم/مل *N. commune* و 5.83 ملغم/مل *A. circinalis* اضيفت نوعين من السيانوبكتريا بصورة مفردة او مع بعضها بمستويين 5 مل و 10 مل لكل 100 غم من الماده العضوية وباستخدام مستوى واحد من الرطوبة هو 1:2 (ماء : مادة عضوية) وقبل 7 ايام من انتهاء فترة الحضن اضيفت بكتريا *Azotobacter chroococcum* بصورة مفردة او مع انواع من السيانوبكتريا لوحظ ان المحتوى العضوي ارتفع بزيادة 1.84 % في معاملة السيانوبكتريا المرتبطة ببكتريا (*A. chroococcum*) يتبعها معاملة السيانوبكتريا المرتبطه مع بعضها بزياده 1.80 % عن المعاملة الغير ملقحه والتي كانت 1.49 % . لقد اظهرت النتائج ان استخدام الاسمدة الاحيائية يقلل من استخدام 1/4 الى 1/2من التوصيه السمادية للاسمدة الكيميائية في نمو وانتاج نبات الحمص.

Introduction

Algae especially nitrogen fixer *cyanobacteria* have been used in agriculture for many years, and their ability to carry out both photosynthesis and nitrogen fixation confers on them an ecological and

*Emial:sana_br_alani67@yaoo.com

agricultural advantage as a renewable natural resource of biological nitrogen. Researchers have clearly shown that one of the most effective nitrogen-fixing biological systems in the rice fields are certain blue-green algae that contribute about 25-30 kg N/ha/season [1]. High-density of photoautotrophic algal cell suspensions in a system designed photobioreactor (PBR) which has been, constructed, and implemented to achieve high photosynthetic rates. This unit has designed for efficient oxygen and biomass production rates, and it also can be used for the production of secreted products [2].

Azotobacter chroococcum, a free-living diazotroph has also been reported to produce beneficial effects on crop yield through a variety of mechanisms including biosynthesis of biologically active substances, stimulation of rhizospheric microbes, modification of nutrient uptake and ultimately boosting biological nitrogen fixation [3-5]. The better alternative for chemical fertilizers is organic farming, the method of cultivating land and raising crops in such a way to keep the soil fertile by use of organic wastes. With live components of beneficial *cyanobacteria* inoculants organic fertilizers are 100% natural from wheat waste. Algal cultures were applied to the tested compost in two rates 5 and 10 % v/w (5 or 10 ml of each algal culture was added to 100 g of mature compost); except the mixed culture that applied at rate (10 % v/w) only, where each one ml of each algal culture contained 5 mg (as dry wt. algae). Aims of this study are as follows:

1-Studying the changes in compost properties (total nitrogen) in addition of two cyanobacterial strain *Nostoc commune* and *Anabaena circinalis* either alone or mix in different level and *Azotobacter chroococcum* to the compost either alone or mix with cyanobacterial strain.

2- Investigated the effect of nitrogen- biofertilizers on growth and yield of chickpea plant.

Materials and methods

The algal species (filamentous *cyanobacteria*); *Nostoc commune* and *Anabaena circinalis* employed throughout the current study were isolated from rice station in Alnagaf-Alashraf. Bioreactor parts sterilized then were ready to use, the body was filled with generally 500 ml of sterilized liquid JM medium. The filters were connected to a dual timer and a compressor with solenoid valves or air pumps to regulate gas exchange then incubated in illuminated incubator 2500 lux and maintained until the exponential phase to be reached after 13 days [6]. The gas supplied was a mixture of nitrogen, oxygen and carbon dioxide, whose compositions could be controlled. The biomass obtained after three weeks of incubation with media supplied [7]. *Azotobacter chroococcum* was obtained from organic national center for organic farming (Ministry of agriculture/ Iraq), which was grown on sucrose mineral salt liquid culture medium, After three days of incubation a suspension was made (10^6 cells in 1 ml) to determinate the antagonism between *A. chroococcum* and either *N. commune* or *A. circinalis* by using dual culture method in petridish. The compost heap was collected from wheat wastes in national center for organic farming, in thirteen plastic containers contained 2000gm of uniform compost. Algal cultures of *A. circinalis* and *N. commune* were applied to the tested compost in two ratios 5 and 10 % v/w, except the mixed culture of these algal species that applied at rate (10% v/w).

Each of treatment was exposed to one level moisture liquid: solid 1:2 ratio. Determined total nitrogen compound using kejldhal method [8] at the end of month [6]. Ten seeds of chickpea were placed in each pot and thinning was made after two weeks to maintain five plant /pot. Regular watering was done to maintain optimum soil moisture. The compost containers were given the following treatments in four replicate:

T1 Compost of organic only, **T2** Compost of chemical fertilizer only 0.5 gm added in each replicate as recommended dos (27: 27: 27), **T3** Inoculated 100 g compost 5 ml of *N. commune*, **T4** Inoculated100 g compost 1ml of *A. chroococcum* and 5 ml of *N. commune*, **T5** Inoculated 100 g compost 10 ml of *N.commune*,**T6** Inoculated 100 g compost 1ml of *A. chroococcum* and 10 ml of *N. commune*,**T7** Inoculated 100 g compost 5 ml of *A. circinalis*, **T8** Inoculated 100 g compost 1ml of *A. chroococcum* and 5 ml of *A. circinalis*, **T9** Inoculated 100 g compost 10 ml of *A. circinalis*.**T10** Inoculated 100 g compost 1ml of *A. chroococcum* and 10 ml of *A.circinalis*,**T11** Inoculated 100 g compost 10 ml mix of *A. circinalis* and *N. commune*,**T12** Inoculated 100 g compost 1 ml of *A.chroococcum* and10 ml mix of *A. circinalis* and *N. commune.*

The standard Kjeldahl method for determining total N in plants was followed involving the digestion of the plant sample with a catalyst in hot sulfuric acid, converting the organic N, to NH_4. N contents of the dried shoots were determined using Kjeldahl analysis The oven dried plant samples were ground to fine powder and used for estimating the of NPK. Root and shoot portions were separated from plants and dried by air. The shoot and root dry weights were recorded separately and

expressed in gram per plant, the plant samples were dried in oven at 75 $\overset{o}{C}$ to constant weight [9] . Five plants were randomly taken at 76 days for chickpea after sowing from each pot and the following data were achieved:

Morphological characters

1- Shoot length (cm). `

2 Number of branches chickpea

3- Number of pod for chickpea.

Fresh weights

1. Fresh weight root / plant (g)
2. Fresh weight shoot /plant (g)

Dry weight

Plant was dried at 75 $\overset{o}{C}$ until constant weight and the following data were determined:

1. Dry weight root / plant (g).
2. Dry weight of shoot / plant (g).

Chemical composition determination

Determination the total percentage of Nitrogen, phosphorus and potassium contents in plants. The methods as described by [8]. After extracting the samples total Nitrogen% was determined by titrating against standard 0.01 (N) H_2S0_4, total phosphorus% by spectrophotometer and total potassium % by using flame photometer.

Total chlorophyll contents

Total leaf chlorophyll content was determined on fresh leaf samples in mature leaves selected from the middle of each new shoot from each replicate. According to the method described by [10] using a Minolta SPAD 502 chlorophyll meter model.

Leaf area

The leaf area (mm^2) of each plant was measured by Area meter (AM300).

Yield and its components

Weight of 1000 seeds was recorded in addition to grain yield.

Statistical analysis

All obtained data in the whole experiments were subjected to proper statistical analysis of variance according to [11] and means separation were done according to LSD at 0.05 % level with C.R.D analysis.

Results and Discussion

Identification of isolates was done on the basis of trichomes /filaments shape, size, cell dimensions, akinetes/ heterocyst, if present etc. A measuring ocular calibrated to the different magnifications (10x, 40x and 100x magnitude oculars) was used to calculate cell sizes. All microscopic observations were performed frequently to monitor growth of the two algal species, and to check that cultures were free of any contaminants.

Cyanobacteria can be cultivated photoautotrophic methods (where algae require light to grow and create new biomass). The cultures were grown axenic is difficult to achieve without more time consuming maintenance of the species and demands a specialized growth system or bioreactor due to ability of *cyanobacteria* to produce exo-polysaccharides that help bacteria feed on the nutrition these provide and many actually live as epiphytes on species producing polysaccharide sheets. to keep the number of bacteria at a low level under biomass production, the strategy was to keep the algae in the exponential growth phase. This would limit the number of dead algal cells and other waste products produced under un favorable conditions. Second, most of the bacterial population could be removed trough under optimal speed of centrifugation. After this treatment, the algae would greatly outnumber the resisting bacterial biomass, and the extract could be regarded as suitable for further screening.

Results showed that at the end of growth, it was easier to harvest the biomass but the general productivity of the reactor is decreased due to the long residence time of the algae. A trade off has to be made where harvesting costs are least and productivity is greatest and this agreed with [12]. There is no single best method of harvesting microalgae. The choice of preferable harvesting technology depends on algae species, growth medium, algae production, end product, and production cost benefit.

Algal strains were identified according to [13]. Algal species, which exanimate under compound microscope (40x) were represented in the figure-1 ,2:

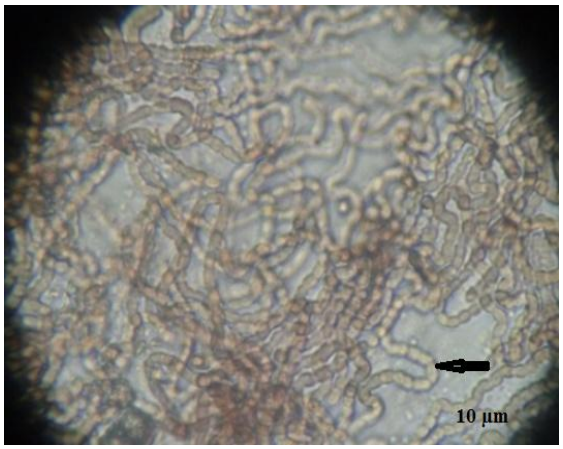

Figure 1- *N. commune* (40x)

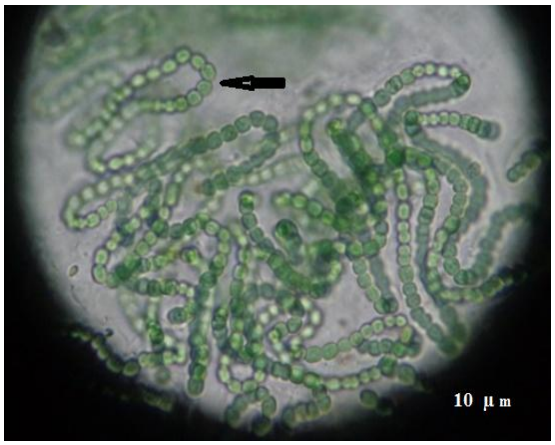

Figure 2- *A. circinalis* (40x)

Results showed that algal growth in Jaworskis medium (JM) even after 13 days and the longer growth period was likely to be needed to indicate the full extent of the growth in 5 ml (JM) agreed with previous study [14]. The algal biomass was alive during the incubation period, and the highest algal growth was observed nearly after three weeks from the beginning, then turned to be constant and declined as a normal pattern of *cyanobacteria* growth and this agreed with [15], Shifted the color culture from either light (green or yellow) to the dark (green or yellow) to increase biomass figure-3.

Figure 3- *A. circinalis* grown in the bioreactors

Biomass of *cyanobateria* was expressed on fresh and dry weight (mg/ml) basis table-1, which was in the end point of growth. Maximum fresh weight was observed in *N. commune* 8.33mg/ml then *A.circinalis* and recorded 6.08 mg/ml, when oven was used, the dry weight (mg/ml) was observed in *N. commune* 0.33mg/ml followed by then *A.circinalis* and recorded 0.25mg/ml. Total biomass was calculated by [16].

Table 1- Fresh and dry biomass estimation (mg/ml) after one month of incubation period:

Cyanobacterial species	Fresh weight mg/ml	Dry weight mg/ml
N. commune	8.33	0.33
A. circinalis	6.08	0.25

Growth condition: light intensity- 2500 lux; inoculum- 30 ml/l; room temperature- 28 ± 2 $\mathring{C}$ and Initial PH 8.2.

Data in table-2 showed that after addition of either one or two *cyanobacteria* species to compost materials, significant difference between the treatments was observed in availability of compost nitrogen. Applying dose level (5 ml) of each, *N. commune* 1.71% increased 13.25% *A. circinalis* 1.60 increased 7.28%, while the level (10 ml) of *N. commune* 1.80% increased 19.21% and *A. circinalis* 1.74% increased 15.23% over control 1.51% after one month of incubation under the same conditions at liquid: solid ratio of 1:2. The highest significant increasing with combination of both *cyanobacteria* species *N. commune* and *A. circinalis* in (5 ml) level for one combined with *A. chroococcum* resulted in nitrogen content 1.84% increased 21.85% followed by combination of both *cyanobacteria* species together 1.82% increased 20.53%.

Addition of either one or two algal species to compost materials, significant difference between the treatments was observed in availability of compost nitrogen due to different concentrations of *cyanobacteria* species biomass as a result showed each ml of *N. commune* contained 7.99 mg. while, *A. circinalis* contained 5.83 mg. The highest total nitrogen of algal activity were recorded due to dehydrogenase enzyme activity in the beginning of all compost treatments was low then increased until reached the maximum after one month [17] as a result of compost addition to the soil causes the algal and bacteria to move to the analysis of these compost, therefore, they consume the nitrogen chain to itself to grow and multiply, and after the end of this stage begins in the analysis of compost and nitrogen production.

Table 2- percentage of total nitrogen content in mature compost:

Treatments	Inoculums size (ml)	Time (week)	Liquid: solid ratio	Total Nitrogen%
TI	-	-	-	1.51
T2	5 ml	4	1:2	1.71
T3	5ml+20 ml	1	1:2	1.66
T4	10 ml	4	1:2	1.80
T5	10 ml+20ml	1	1:2	1.77
T6	5 ml	4	1:2	1.62
T7	5 ml+20 ml	1	1:2	1.65
T8	10 ml	4	1:2	1.74
T9	10 ml+20 ml	1	1:2	1.79
T10	10 ml	4	1:2	1.82
T11	10 ml+20 ml	1	1:2	1.84
T12	20 ml	4	1:2	1.81
LSD at 0.05				0.08

T1= Control (compost without inoculation), T2= *Nostoc commune* (5 ml) T3= *Nostoc commune* (5 ml) combined with *Azotobacter chroococcum* T4= *Nostoc commune* (10 ml) T5 = *Nostoc commune* (10 ml) combined with *Azotobacter chroococcum* T6=*Anabaena circinalis* (5 ml), T7=*Anabaena circinalis*(5 ml) combined with *Azotobacter chroococcum*, T8= *Anabaena circinalis* (10 ml), T9=*Anabaena circinalis*(10 ml) combined with *Azotobacter chroococcum*,T10 =Mix *cyanobacteria* (10 ml),T11=Mix *cyanobacteria* (10 ml) combined with *Azotobacter chroococcum*,T12= *Azotobacter chroococcum.*

For chickpea plant all treatments were supplied with compost, the only difference being the control having compost without microbial inoculants. The result showed plant height of chickpea plant highly significantly increased in T12 was observed (mix *cyanobacteria* combined with *A. chroococcum*) 43.93cm followed by T10 (*A.circinalis* (10 ml) combined with *A. chroococcum*) 43.85cm and T11 (mix *cyanobacteria* (10 ml)) 43.4cm compared with T2 (chemical fertilizer treatment) 45.15cm. The percentage over T1 (control) 39.2cm was increased 12.07%, 11.86%, 10.71% and 15.19%, respectively, table-3 Inoculation compost with both different *cyanobacteria* level and *A. chroococcum* individual or combination to cultivated chickpea crop enhances stem height due to increased absorption of nutrients which resulted in increase in the synthesis of carbohydrates and produce different types of secondary metabolites such as auxins, auxin like substances, gibberellin like substances, cytokinins and abscisic acid [18-21], which in turn stimulated the efficiency of nutrient uptake and thus lead to increased plant height.

The evaluation of algal biomass chlorophyll is presented in table-3. Total chlorophyll of plant leaves responded positively to *cyanobacteria* inoculation, highly significantly increased in T12 (mix of *cyanobacteria* combined with *A.chroococcum*) 53.85 followed by T10 (*A.circinalis* (10 ml) combined with *A. chroococcum*) 53.80, (mix *cyanobacteria* (10 ml)) 53.77 and T9 (*A.circinalis* (10 ml)) 53.45 compared with T2 (chemical fertilizer treatment) 53.98. The percentage over T1 (control) 49.80 was increased: 8.13%, 8.03%, 7.97%, 7.33% and 8.39%, respectively. Cyanobacterial suspension contain a special set of biologically active including plant growth regulators, which can decrease senescence and transpiration and increase the content of leaf chlorophyll in response to the different fertilization treatments may have accumulated chlorophyll in leaves agreed with [22]. These results are in good agreement with those of [23] who observed a significant increase in chlorophyll may be due to the increase in pigment biosynthesis.

The result showed that the highly significantly increase in leaf area, T12 (mix of *cyanobacteria* combined with *A. chroococcum*)1270 mm² followed by T10 (*A. circinalis* (10 ml) combined with *A.chroococcum*) 1170 mm² and T11 (mix *cyanobacteria* (10 ml)) 1132 mm² compared with T2 (chemical fertilizer treatment) 1310 mm². The percentage over T1 (control) 848 mm² was increased: 49.76%, 37.97%, 33.49% and 54.78%, respectively. The lowest value was recorded in T3 (*N. commune* (5 ml)) 865 mm², which was increased over control but that was not significant, table-3. Also, the increase in leaf area in response to the different fertilization treatments may have accumulated chlorophyll in leaves. Significantly higher in the treatment T12 (mix of *cyanobacteria* combined with *A. chrococcum*) at all the growth stages this could be due to the beneficial effect of inoculants, which helped the plants to get more nitrogen which is the component of chlorophyll molecule.

The highly significant increase in number of branches was shown in T12 (mix of *cyanobacteria* combined with *A. chroococcum*) 5.562 followed by T10 (*A.circinalis* (10 ml) combined with *A. chroococcum*) 5.250 and T11 (mix *cyanobacteria* (10 ml)) 5.06 compared with T2 (chemical fertilizer treatment) 5.938, table-3. The percentage over T1 (control) 3.25 was increased: 71.14%, 61.54%, 55.69% and 82.71%, respectively. The lowest value was recorded in T3 (*N. commune* (5 ml)) 3.56, T4 (*N. commune* (5 ml) combined with *A. chroococcum*) and T7 (*A. circinalis* (5 ml)) 3.63, which increased over control but that was not significant. Synthesis of plant growth regulators by *cyanobacteria* and *A. chroococcum* increased the number of branches which made the ability for plants to absorbed nutrient elements agreed with [24].

Table 3- Effect of some biofertilizers on chlorophyll, plant height, leaf area and number of branch of chickpea plant.

Treatments	Plant height (cm)	Chlorophyll (spad)	Leaf area (mm^2)	Number of branch
T1	39.20	49.80	848	3.25
T2	45.15	53.98	1310	5.94
T3	40.45	51.15	865	3.56
T4	41.24	52.33	988	3.63
T5	42.89	53.10	1120	4.69
T6	43.25	53.67	1131	4.94
T7	41.54	51.38	925	3.63
T8	42.19	52.47	1065	4.69
T9	43.20	53.45	1128	4.75
T10	43.85	53.80	1170	5.25
T11	43.40	53.77	1132	5.06
T12	43.93	53.85	1270	5.56
T13	42.04	52.45	1055	4.00
L S D at 0.05	0.709	1.30	74.51	0.57

T1= Control (compost without inoculation), T2= chemical fertilizer T3= *Nostoc commune*(5ml) T4= *Nostoc commune*(5ml) combined with *Azotobacter chroococcum* T5= *Nostoc commune*(10 ml) T6 = *Nostoc commune* (10ml) combined with *Azotobacter chroococcum* T7=*Anabaena circinalis* (5ml),T8=*Anabaena circinalis*(5ml) combined with *Azotobacter chroococcum*, T9= *Anabaena circinalis*(10 ml), T10=*Anabaena circinalis*(10 ml) combined with *Azotobacter chroococcum*,T11 =Mix cyanobacteria (10 ml),T12=Mix cyanobacteria (10 ml) combined with *Azotobacter chroococcum*,T13= *Azotobacter chroococcum.*

Data analysis for fresh and dry weight of shoot and root at the beginning of flowering stage (74 days) showed that inoculation treatments significantly increased over uninoculated treatment therefore, application of *cyanobacteria* species and bacterial treatments resulted a significant improvement in grain yield and plant biomass as compared with control.

Highly increase fresh biomass of shoot in table-4 was observed in T12 (mix of *cyanobacteria* combined with *A. chroococcum*) 14.15 g followed by T10 (*A.circinalis* (10 ml) combined with *A. chroococcum*) 13.82 g and T11 (mix *cyanobacteria* (10 ml)) 13.75 g compared with T2 (chemical fertilizer treatment) 15.35 g. The percentage over T1 (control) 10.10 g was increased: 40.1%, 36.83%, 36.14% and 51.98%, respectively. The lowest value was recorded in T3 (*N. commune* (5 ml)) 10.10 g, T4 (*N. commune* (5 ml) combined with *A. chroococcum*)) 11.04 g and T7 (*A. circinalis*(5 ml)) 11.40 g. Highly increase of fresh biomass of root was observed in T12 (mix of *cyanobacteria* combined with *A. chroococcum*) 5.60 g followed by T10 (*A.circinalis* (10 ml) combined with *A. chroococcum*) 5.42 and T11 (mix *cyanobacteria* (10 ml)) 5.31 g compared with T2 (chemical fertilizer treatment) 5.67 g, table-4.The percentage over T1 (control) 2.26g was increased: 147.79%, 139.82%, 134.96% and 150.88%, respectively. The lowest value was recorded in T3 (*N. commune* (5 ml)) and T4 (*N. commune* (5 ml) combined with *A. chroococcum*)) 3.66 g which increased over control but that was not significant.

Highly increase in dry biomass of shoot was observed in T12 (mix of *cyanobacteria* combined with *A. chroococcum*) 4.280 g followed by T10 (*A.circinalis* (10 ml) combined with *A. chroococcum*) 4.260 g and T5 (*N. commune* (10 ml)) 4.200 g compared with T2 (chemical fertilizer treatment) 4.870 g. The percentage over T1 (control) 3.150 g was increased: 35.87%, 35.24%, 33.33% and 54.60%, respectively. The lowest value was recorded in T4 (*N. commune* (5 ml) combined with *A. chroococcum*)) 3.6g, T7 (*A. circinalis* (5 ml)) 3.650g, T3 (*N. commune* (5 ml)) 3.7 g, and T8 (*A. circinalis* (5 ml) combined with *A. chroococcum*) 3.77 g, which increased over control but that was not significant. table-4. Highly increase in dry biomass of root was observed in T6 (*N.commune* (10

ml) combined with *A. chroococcum*) 3.290 g followed by T12 (mix of *cyanobacteria* combined with *A. chroococcum*) 3.20 g and T5 (*N. commune* (10 ml)) 2.930 g compared with T2 (chemical fertilizer treatment) 4.490 g. The percentage over T1 (control) 1.210 g was increased: 171.90%, 164%, 142.15% and 271.07%, respectively. The lowest value was recorded in T7 (*A. circinalis* (5 ml)) 1.260 g T3 (*N. commune* (5 ml)) 1.630 g, T8 (*A. circinalis* (5ml) combined with *A. chroococcum*) 1.670 g T4 (*N. commune* (5 ml) combined with *A. chroococcum*)) 1.870, and which increased over control but that was not significant. table-4.

Fresh biomass increase was also one of the key parameters improved by using of inoculated compost in chickpea. These increases could be attributed to the nitrogenase enzymes well as nitrate reductase activities of the algae associated with the surface; or may be to the amino acids and peptides produced in the algal suspention and other compounds that stimulate growth of crop plants. These results are in good agreement with [25], also [26] who studied the effect of BGA as biofertilizer on chickpea and they found that it enhanced all the morphological characters and biomass of the chickpea. Increased weight due to *cyanobacteria* and *Azotobacter* inoculation but decreased the dry wet weight is not accurate as the amount of water varies from one plant to another depending on the irrigation of these plants. These results are in concordance with most similar previous studies [27].

Table 4- Effect of some biofertilizers on fresh weight of shoot and root of chickpea plant.

	Fresh weight (g/plant)		**Dry weight (g/plant)**	
Treatments	Shoot	Root	Shoot	Root
T1	10.10	2.26	3.150	1.210
T2	15.38	5.67	4.870	4.490
T3	10.10	3.66	3.700	1.630
T4	11.04	3.66	3.600	1.870
T5	12.52	4.81	4.200	2.930
T6	12.79	5.10	4.000	3.290
T7	11.40	3.81	3.650	1.260
T8	12.44	4.73	3.770	1.670
T9	12.76	5.06	4.150	2.560
T10	13.82	5.42	4.260	2.890
T11	13.75	5.31	4.170	2.840
T12	14.15	5.60	4.280	3.200
T13	12.37	4.16	4.120	2.368
L S D at 0.05	1.67	1.527	0.660	0.855

T1= Control (compost without inoculation), T2= chemical fertilizer T3= *Nostoc commune*(5ml) T4= *Nostoc commune*(5ml) combined with *Azotobacter chroococcum* T5= *Nostoc commune*(10 ml) T6 = *Nostoc commune* (10ml) combined with *Azotobacter chroococcum* T7=*Anabaena circinalis* (5ml),T8=*Anabaena circinalis*(5ml) combined with *Azotobacter chroococcum*, T9= *Anabaena circinalis* (10 ml), T10=*Anabaena circinalis*(10 ml) combined with *Azotobacter chroococcum*,T11 =Mix Cyanobacteria (10 ml),T12=Mix cyanobacteria (10 ml) combined with *Azotobacter chroococcum*,T13= *Azotobacter chroococcum.*

The results showed that total protein in table-5, which calculated depend on concentration of total nitrogen present multiplied by 6.25. The highest increase of total protein content was recorded in T12 (mix of *cyanobacteria* combined with *A. chroococcum*) 14.69 % followed by T10 (*A. circinalis* (10 ml) combined with *A. chroococcum*) 13.13 % and T11 (mix *cyanobacteria* (10 ml)) 12.94% compared with T2 (chemical fertilizer treatment) 16.44 %. The percentage over T1 (control) 9.88 % was increased: 48.68 %, 32.89 %, 30.97 % and 66.40 %, respectively. The lowest value was recorded in T3 (*N. commune* (5 ml)) 10.44 %.

Highly increase in total nitrogen content was recorded in table-5 T12 mix of *cyanobacteria* combined with *A. chroococcum*) 2.35 % followed by T10 (*A. circinalis* (10 ml) combined with *A. chroococcum*) 2.1 % and T11 (mix *cyanobacteria* (10 ml)) 2.07 % compared with T2 (chemical fertilizer treatment) 2.63 %. The percentage over T1 (control) 1.58 % was increased: 48.73%, 32.91%,

31.01% and 66.40%, respectively. The lowest value was recorded in T3 (*N. commune* (5 ml)) 1.67%. The increase in protein was related to the increase of N % content [27], this might be due to increased availability of nitrogen and its uptake and storage in grain. Nitrogen being the essential constituent that makes up to 16% by weight of protein .Increase in N content might be due to *cyanobacteria* and *Azotobacter* inoculation single or together causing relatively greater utilization of available N by plants in presence of *cyanobacteria* and *Azotobacter* compared with un inoculated However, the decreased amount of N at the later period of crop growth might be due to dilution effect arising from the increased biomass production .This results are in concordance with most similar previous studies [28].

Highly increase in total percentage of phosphor was recorded in T6 (*N. commune* (10 ml) combined with *A. chroococcum*) 0.43% followed by T12 mix of *cyanobacteria* combined with *A. chroococcum*) 0.40%, T5 (*N. commune* (10 ml)) 0.39% compared with T2 (chemical fertilizer treatment) 0.47% in table-5 The percentage over T1 (control) 0.30% was increased: 43.33%, 33.33%, 30% and 56.67%, respectively. The lowest value was recorded in T3 (*N. commune* (5 ml)) and T7 (*A. circinalis* (5 ml)) 0.32%. Leguminous crops have a high phosphorus requirement than other crops to attain optimum growth and productivity [29]. Phosphor content was increased, these effects might be due to that in bio-organic farming system a set of soil microorganisms, processing the ability of mobilizing the unavailable forms of nutrient elements to available forms, has been successfully agreed with [30] and might be explained by the synergistic relationship among themselves resulting in greater absorption of phosphorus by chickpea plant, helps to increase P uptake in the soil. These results are agreed with previous study [31].

Highly increase in total percentage of Potassium was recorded in T6 (*N. commune* (10 ml) combined with *A. chroococcum*) and T2 (chemical fertilizer treatment) 2.54% followed by T12 mix of *cyanobacteria* combined with *A. chroococcum*) and T5 (*N. commune* (10 ml)) 2% in table-5. The percentage over T1 (control) 1.50% was increased: 69.33% and 33.33%, respectively. The lowest value was recorded in T3 (*N. commune* (5ml)) 1.53%. Inoculation with single inoculum *cyanobacteria* results in enhanced assimilation of mineral nutrients especially K in plants, but such assimilation of K in plants might be further enhanced with their dual inoculation resulting from their strong synergistic relationships. These results are in concordance with most similar previous studies [32].

Table 5- Effect of some biofertilizers on total percentage of protein, Nitrogen, Phosphor and Potassium of chickpea plant.

Treatments	Total N%	Total P%	Total K%
T1	1.58	0.30	1.50
T2	2.63	0.47	2.54
T3	1.67	0.32	1.53
T4	1.79	0.35	1.55
T5	1.98	0.39	2.00
T6	2.00	0.43	2.54
T7	1.92	0.32	1.59
T8	1.96	0.38	1.50
T9	2.00	0.37	2.54
T10	2.10	0.38	1.55
T11	2.07	0.38	1.56
T12	2.35	0.40	2.00
T13	1.94	0.36	2.54
LSD at 0.05	0.32	0.06	0.52

T1= Control (compost without inoculation), T2= chemical fertilizer T3= *Nostoc commune*(5ml) T4= *Nostoc commune*(5ml) combined with *Azotobacter chroococcum* T5= *Nostoc commune*(10 ml) T6 = *Nostoc commune* (10ml) combined with *Azotobacter chroococcum* T7=*Anabaena circinalis* (5ml),T8=*Anabaena circinalis* (5ml) combined with *Azotobacter chroococcum*, T9= *Anabaena circinalis*(10 ml), T10=*Anabaena circinalis*(10 ml) combined with *Azotobacter chroococcum*,T11 =Mix cyanobacteria (10 ml),T12=Mix cyanobacteria (10 ml) combined with *Azotobacter chroococcum*,T13= *Azotobacter chroococcum.*

The number of pods per plant was increased by applying biofertilizer, table -6. Highly increase in number of pods recorded in T11 (mix of *cyanobacteria* (10 ml)) 12.19 followed by T12 mix of *cyanobacteria* combined with *A. chroococcum*) 9.31 compared with T2 (chemical fertilizer treatment) 13.19. The percentage over T1 (control) 6.44 was: 89.29%, 44.56% and 104.81%, respectively. Lower number of pods in the main branch due to drought stress can be attributed to protein decomposition and it's on to peptides and amino acids. Translocation of carbon and nitrogen into shoots is also retarded under drought stress [33]. The increased number of pods per plant when they treated it with biologic organic fertilizer, BGA reduced the number of days for crop maturity by almost 30 days in BGA treatment, however, it was witnessed that, the incidence of leaf-spot disease very low, giving rise to healthy plants with a dense growth accompanied by healthy pods. These results agreed with [34].

The favorable effect of BGA application on seed yield characters may be due to their influence on plant growth features agreed with [35] Highly increase in 1000-seed weight showed with table-6 was recorded in T12 (mix of *cyanobacteria* (10 ml) combined with *A. chroococcum*) 560.9g followed by T10 (*A.circinalis*(10 ml) combined with *A. chroococcum*) 551.1g, T11 (mix of *cyanobacteria* (10 ml)) 540. 4g and T6 (*N. commune* (10 ml) combined with *A. chroococcum*) 513.6g compared with T2 (chemical fertilizer treatment) 583.3g.The percentage over T1 (control) 409 g was increased: 37.14%, 34.74%, 32.13%, 25.57% and 42.62%, respectively. The increase in the chickpea seed may also be attributed to the higher absorption of N, P and K which might have favorably affected the chlorophyll content of leaves resulting increased synthesis of carbohydrates and build up of new cells. These results are in confirmation with the reports of [36] and may be the drought as an abiotic stress reduces photosynthesis and limits growth and seed yield agreed with [37].

Table 6- Effect of some biofertilizers on number of pods /plant and 1000-Seed weight (g) in chickpea plant.

Treatments	Number of pods /plant	1000-Seed weight(g)
T1	6.44	409.0
T2	13.19	583.3
T3	7.94	442.2
T4	7.94	472.7
T5	9.12	512.7
T6	8.25	513.6
T7	7.56	478.3
T8	6.56	494.9
T9	8.81	513.3
T10	8.12	551.1
T11	12.19	540.4
T12	9.31	560.9
T13	7.06	479.3
L.S.D at 0.05	1.11	

T1= Control (compost without inoculation), T2= chemical fertilizer T3= *Nostoc commune*(5ml) T4= *Nostoc commune*(5ml) combined with *Azotobacter chroococcum* T5= *Nostoc commune*(10 ml) T6 = *Nostoc commune* (10ml) combined with *Azotobacter chroococcum* T7=*Anabaena circinalis* (5ml),T8=*Anabaena circinalis*(5ml) combined with *Azotobacter chroococcum*, T9= *Anabaena circinalis*(10 ml), T10=*Anabaena circinalis*(10 ml) combined with *Azotobacter chroococcum*,T11 =Mix *cyanobacteria* (10 ml),T12=Mix *cyanobacteria* (10 ml) combined with *Azotobacter chroococcum*,T13= *Azotobacter chroococcum*.

Partial or total replacement of chemical fertilizers will be useful in Iraq soil to overcome the harmful effects of chemical fertilizers and to maintain soil fertility and groundwater. Finally obtaining fewer amounts of healthy products with less environmental disturbances is preferred over obtaining higher amount of non-healthy products with more environmental disturbances.

This study revealed that two cyanobacterial species *Nostoc commune* and *Anabaena circinalis* isolated from Iraqi water in Alnagaf-Alashraf southern Iraq have the ability to fix atmospheric nitrogen with highly productivity for growth and yield of crops. For the mass culture of cyanobacterial species, open pond system has mainly been the dominating systems until now. However, closed system of light-distributing in plate design known as photobioreactors, are now increasingly finding a

new application both for high value product in agriculture. The outcome of the above experiment proved that biomass obtained from cultivation of *N.commune, Anabaena circinalis* in bioreactor to inoculated mature compost was the best way of cultivation of nitrogen fixing *cyanobacteria* with low cost. The application of *cyanobacteria* in growth and yield of chickpea plant reduces the need of chemical fertilizers about 30 %-50 % and subsequently reduces environmental pollution compared with other mineral chemical fertilizers.

References

1. Venkataraman, G. S. **1979.** Blue-green algae in rice cultivation, an evaluation. *Glimpses Plant Res.* 4:74-81.

2. Pulz, O. **(2001).** Photobioreactors: production systems for phototrophic microorganisms. Applied *Appl. Microbiol. Biotechnol*. (57), p: 287-293.

3. Lakshmann, M. **2000** *Azotobacter* inoculation and crop productivity. In: Narula N (Eds.) *Azotobacter in Sustainable Agriculture*. CBS Publishers, New Delhi, India, pp. 109-116

4. Paul, S., Verma, O.P. and Rathi, M. S. **2002**. Potential of homologus and heterologus *Azotobacter chroococcu*m strains as bio-inoculants for cotton. *New Botanist* 29 p: 169-174.

5. Somers, E., Vanderleyden, J. and Srinivasan, M. **2004**. Rhizosphere bacterial signaling: A love parade beneath our feet. *Critical Reviews in Microbiology* 30 p: 205-240.

6. Minoo, J. and Bernhard, O. P. **1991**. High-density photoautotrophi algal cultures: design, construction, and Operation of a novel photobioreactor system. *Biotechnology and Bioengineering*, 38 1182-1189.

7. Jackson, ML **1973.**: *Methods of Soil Chemical Analysis*. Prentice Hall of India Pvt. Ltd., New Delhi.

8. 8.Valenciano, B. J., Miguélez-Frade, M. M. and Marcelo,V**.2009.** Short communication. Response of chickpea (*Cicer arietinum* L.) to soil zinc application. *Spanish Journal of Agricultural Research* 7(4), 952-956.

9. Yadva**,** U.L. **1986.**A rapid and non –destructive method to determine chlorophyll in intact leaves .*Hort. Sci.*, 21, p: 1449-1450.

10. Snedecor, G.A. and W.G. Cochran, **1980**: *Statistical Methods*, 11th. Ed., the Iowa State Univ. Press, Ames, Iowa, U. S. A., p: 172-334. 305p.

11. Christophere**,** J. **2008.**Algae derived products and technologies University of Bath137. W. J. Oswald, in Micro-Algal Biotechnology ed. M. A. Borowitzka, University Press, Cambridge, Editon edn., 1988, pp. 357-394.

12. Atle Uldahl, S. **2006.** Microalgae bioprospecting spore swelling and germination as a bioassay for the rapid screening of crude algae extracts for antifungal activity. Degree of cond. Thesis, Institute of Biology. University of Bergen**.**

13. Desikachary, T.V. **1959.** *Cyanophyta*. Academic press. New York.

14. Oldare, M., Nehrenheim, E., Ribe,V., Thorin, E., Gavara, M. and Grube, M. **2011.** Cultivation of algae with indigenous species-potentials for regional biofuel production. *J. Applied Energy* 88, P: 3280–3285

15. Stal**,** J.S., **1995**. Physiological ecology of cyanobacteria in microbial mats and other communities. *New Phytol*., 131-132.

16. El-Gamal, A.H.M. **2011.**Impact of algal addition to mature compost as affected by differrent moisture levels. *Aust. J. Basic & Appl. Sci.*, 5(9): 729-737.

17. Abou-Hadid, A.F.; Hussein, M.S. and El-Saied, H.M. **1993:** Herb and heavy metals Composition of leafy vegetables as influenced by different nitrogen sources. *Egypt. J. Hort*., 20 (2).

18. Serdyuk, O. P., Smolygina, L. P., Kobzar, E. V. and Gogotov, I. N. **1992.** Phytohormones formed by the nitrogen fixing association of Anabaena–Azollae. *Doklady Biochem*. 325:149–151.

19. Marsalek, B., Zahradnickora, H., Hronkova, M. **(1992).**Extracellular abscisic asid produsedby cyanobacteria under salt stress. *J. Plant physiol*, 139, p: 506-508.

20. Esch, H.; Hundeshagen, B.; Schneiderpoetsch, H. and Bothe, H. **1994**: Demonstration of abscisic acid in spores and hyphae of the arbuscular mycorrhizal fungus Glomus and in the nitrogen fixing cyanobacterium *Anabaena voriabilis*. *Plant Sci.* 99, pp: 9-16.

21. Ordog, V. **(1999).** Beneficial effects of microalgae and cyanobacteria in plant/soil systems, with special regard to their auxin and cytokinin like activity. International Workshop and Training course on Microalgal Biology and Biotechnology, Mosonmagyarovar, *Hungary. June*, pp: 13-26.

22. Younis, M. E., El-Shahaby, O. A., Abo-Hamed, S. A. and Haroun, S. A. **1991.** Plant growth, metabolism and adaptation in relation to stress conditions. XI. Modification of osmotic-stress-induced metabolic effects by GA_3 or IAA in *Pisum sativum* plants. *Acta Agron. Hung*. 40, pp:367-375.

23. Govedarica, and M, Jarak, M. **1995**. Mikrobiologija zemljišta. Poljoprivredni fakultet, inistitute za ratarstvo I povertarstvo, Novi Sad.

24. Shahzad, S. M., Khalid, M. ,Arshad, M., Khalid, M. and Mehboob ,I. **2008.** Integrated use of plant growth promoting bacteria and P-Enriched compost for improving growth, yield and nodulation of Chickpea *J.Bot.,*40 (4):1735-1441.

25. 25. Jagannath, S.B.A., Umapati-Dengi; Eshwarlal-Sedamakar **2002.** Algalization studies on chickpea (Cicer arietinum L). *Biotechnology of microbes and sustainable utilization*. p: 145-150.

26. Abd El-Gawad, A.M and Zeinab, T. El-Sayed, **2006.** Evaluation the Response of Wheat to Bio-Organic Agriculture under Siwa Oasis Conditions. *Journal of Food Agriculture and Envirnoment* 4, pp:1-6.

27. Wang, S.M.; Wang, Q.L. and Li SH, Zhang, J.R. **1991.** A study of treatment of spring wheat with growth promoting substances from nitrogenfixing blue green algae. *Acta Hydrob Sin* 15, pp:45–52.

28. Qureshi, A., Ahmad, M.J. Naveed, M., Iqbal, A., Akhtar, N. and Niazi, K.H. **2009.** Co-inoculation with *Mesorhizobium ciceri* and *Azotobacter chroococcum* for improving growth, nodulation and yield of chickpea (*Cicer arietinum* L.). *Soil & Environ*. 28(2),pp: 124-129.

29. Gitari, J.N. and Mureithi, J.G. **2003.** Effect of phosphorus fertilization on legume nodule formation and biomass production in Mount Kenya Region. *East Afr Agric Fory J*. 69:pp 183-187.

30. Saber, M.S.M. **1994.** Bio-organic farming systems for sustainable agriculture. Inter-Islamic Network on Genetic Engineering and Biotechnology, INOGE Publ. 3, Cairo, Egypt.

31. Das DK, **(2004).** *Introductory Soil Science*. Kalyani Publishers , Ludhiana, India, pp. 349-350.

32. Pany, B.K. **2003.** Lecture delivered in the Winter School on Characterization and sustainable management of acid soils of eastern India" at OUAT, Bhubaneswar. 18 November - 8 December.

33. Lodeiro, A.R., Gonzalez, P., Hernandez, A. L., Balague J. and Favelukes,G. **(2000).** Comparison of drought tolerance in nitrogen-fixing and inorganic nitrogen-grown common bean. *Plant Science*, 154, pp:31- 41.

34. El Kramany, M.F., Bahar A., Mohamad, F. and Kabesf, M.O. **2007.** Utilization of bio-fertilizer in field crops production 16-groundnut yield, its components and seed contents as affected by partial replacement of Chemical fertilizers by bio-organic-fertilizers .Department of Field .Research National Research Center. Dokki, Cairo, Egypt. *Journal of Applied Science Research,* 3(1),pp:25-29.

35. Amal, Z. H.; Soha, S. M. M. and Hamdino, M. I. A. **2010**. Influence of different cyanobacterial application methods on growth and seed production of common bean under various levels of mineral nitrogen fertilization *Nature and Science*; 8 (11).

36. Shashidhara, G. B., **2000.** Integrated nutrient management for chilli (*Capsicum annuum* L.) in Alfisops of Northern Transition Zone of Karnataka. M. Sc. (Agri.) Thesis, Univ.Agric. Sci., Dharwad, Karnataka, India.

37. Bao, A., Wang S., Wu, G., Xi, J., Zhang, J. and Wang, C. **2009.** Over expression of the Arabidopsis H+-P Phase enhanced resistance to salt and drought stress in transgenic alfalfa (Medicago sativa L.). *Plant Science*, 176,pp:232-240.