Productivity of Pepper Plants Grown Under Different Nitrogen Levels As Influenced By the Use of Different Cyanobacteria Forms

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Abstracts: The present work was conducted at the Experimental Farm of El-Kassasin Horticultural Research Station (30º 37´ 36.869″ N for Latitude and 31º 56´ 22.227″E), Ismailia Governorate, during two seasons of 2012 and 2013, to study the effect of cyanobacteria inoculation applied with different methods (Dry, Soaking and Billets) in combination with reduced nitrogen rate of 75 % N compared to full nitrogen rate (100% N) on both yield and quality of sweet pepper crop (*Capsicum annuum* L.) (cv. Marconi) M some soil chemical and soil rhizosphere biological activity. Results revealed that the use of cyanobacteria with different forms along with 75 % N improved the pepper yield and its components, its plants N, P and K concentration and content in both tested seasons. The pepper yield obtained by the treatment of Dry + Soaking + Billets + 75% N was not significantly differed from those recorded due to the control treatment (100 % N). As well as, the use of cyanobacteria with different forms along with 75 % N enhanced the soil available N, P and K status and the soil rhizosphere pepper plants biological activity in terms of total cyanobacteria count**,** total bacterial count, dehydrogenase activity and CO_2 evolution amount. In conclusion, the use of cyanobacteria with different forms along with 75 % N has the possibility to save 25% of the mineral nitrogen required for the sweet pepper cultivation although this phenomena need to be executed and tried for other vegetable crops in different soil types and location to be recommended.

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1. Introduction

The use of conventional chemical farming methods, which is substantially increased crop production, was once regarded as a kind of agriculture revolutions, which would solve all problems relating to producing sufficient food for the ever growing world population. However, this belief was later over-shadowed by the emergence of numerous environmental and social problems associated with the heavy use of agrochemicals in intensive farming systems.

Conventional farming methods are generally associated with degradation of the environment. Among other things, soil degradation is one of the most serious problems, which affect crop production.

Increasing prices of agrochemicals especially nitrogen, often leaves the marginal farmers with low profits. Uncertain availability of those agrochemicals, especially in the developing countries such as Egypt, is often a serious constraint for the farmers in their attempt to increase crop production. Such problems have directed the attention of the agriculturalists world-wide to seek alternative methods for farming.

In attempting to develop productive, profitable and sustainable agriculture systems, several agriculturalists turn to farming methods, which are based on technologies. One of the several to achieve this goal is the use of the biological nitrogen fixation through cyanobacteria in order to improve soil fertility and crop productivity. The use of nitrogen fixing cyanobacteria ensure entirely or partially the mineral nitrogen and/or enhances the availability of soil nutrients **(Myint, 1999).**

Due to sweet pepper (*Capsicum annuum* L.) is one of the most popular and favorite vegetable crop that cultivated in Egypt for local market and exportation. High cash crops such as sweet pepper have occupied an important rank in Egyptian and world agriculture due to high profit and nutritional values for human. Also, pepper requires a great, amount of nitrogen for good production in both fields and greenhouses, therefore. There is a great deal of interest in using biofertilizer technology to reduce the excessive use of costly and non eco-friendly mineral nitrogen. The heterocystous cyanobacteria are characterized by their ability to form tight association with the roots epidermis and cortical intracellular space **(Gantar *et al.*, 1995).** Cyanobacteria excrete a great number of substances that influence plant growth and development. These microorganisms have been reported to benefit plants by producing growth-promoting regulators (the nature of which is said to resemble gibberellins and auxins), vitamins, amino acids, polypeptides, antibacterial and antifungal substances that exert phytopathogen biocontrol and polymers, especially exopolysaccharides, that improve soil structure and exoenzyme activity **(Zaccaro, 2000). Moustafa and Omar (1990)** reported that inoculation of tomatoes with a mixture inoculum of *Azospirillum lipoferum* and cyanobacteria, formally called blue-green algae (a mixture of different cyanobacteria strains) and/or cyanobacteria alone as biofertilizer led to increase significantly as improved the quality of tomato fruits. Also, **Kotb *et al.* (1990)** showed that inoculation with *Azorpirillum* and /or algae gave significant positive differences for fresh weight of tomato fruits and plants dry weight when compared to the control plants without inoculation. **Zeenat and Sharma (1990)** reported that the inoculation of tomato with cyanobacteria in presence of reduced chemical nitrogen fertilizer (75 % N) improved tomato plants growth and increased significantly the yield compared to control treatment without inoculation. They suggested that cyanobacteria secreted considerable amounts of growth–promoting substances into the surrounding medium, thereby increasing the growth and yield of tomato**. Lopez- Cortes and Delgado (2003)** reported that inoculation of chilli pepper with the cyanobacterium *Scytonema* increased the yield by 30% over the uninoculated control.

Recently, several investigators reported that, it is possible to reduce the amounts of chemical fertilizers by using biofertilizers **(Yadav *et al.,* 2003, Mohsen, 2006 and Ali *et al*., 2009).**

So, the aim of this work is to study the effect of cyanobacteria inoculation applied with different methods (dry, soaking and billets) in combination with reduced nitrogen rate of 75 % N compared to full nitrogen rate (100% N) on both yield and quality of sweet pepper crop (*Capsicum annuum* L.) (cv. Marconi) as well as studying same effect on some soil chemical and biological characters.

2. Materials and Methods

The present work was conducted at the Experimental Farm of El-Kassasin Horticultural Research Station (30º 37′ 36.869″ N for Latitude and 31º 56′ 22.227″E), Ismailia Governorate, during two seasons 2012 and 2013, to study the effect of cyanobacteria inoculation applied with different methods (Dry, Soaking and Billets) in combination with reduced nitrogen rate of 75 % N compared to full nitrogen rate (100% N) on both yield and quality of sweet pepper crop (*Capsicum annuum* L. cv. Marconi) as well as studying same effect on some soil chemical and biological characters. The soil of the experimental field was sand in texture and the physical and chemical analyses of soil are shown in Table (1).

Pepper seeds were sown in nursery on 10th June in foam trays and seedlings transplanted (with 3-4 true leaves about 40 days) on 21st and 22nd July in both seasons. Cyanobacteria were provided by Agric. Microbiol. Dept., Soils, Water & Environ. Res. Inst., ARC, Giza, Egypt. Cyanobacteria were applied as culture filtrate that contains a mixture of different cyanobacteria strains, i.e., *Nostoc muscorum* and *Anabaena oryzae*. To obtain the cyanobacteria culture filtrate, each cyanobacterium strain was grown and propagated for 5 weeks on the free nitrogen BG 11_0 medium described by **Allen and Stanier (1968)**. The developed cyanobacteria cultures were centrifuged (3000 rpm min^{-1}) and the supernatant were used as cyanobacteria filtrate by mixing the supernatant for each strain together to have the cyanobacteria culture filtrate (**Aref *et al*., 2009)**. The filtrate was used in soaking treatment for pepper seedlings before transplanting. As well as, these cyanobacteria strains were prepared as soil based inoculum as described by **Vennkataraman (1972)** to be used as dry cyanobacteria inoculum (1kg ha^{-1}) for pepper plants at 10 days before transplanting.

Table (1): Some physical and chemical properties of the experimental soil (Page *et al.*, 1982)

Property	Value	
	Season 2012	Season 2013
Particle size distribution (%)		
Coarse sand	05.40	05.50
Fine sand	79.50	79.70
Silt	09.10	09.00
Clay	06.00	05.80
Texture grade	Sandy	Sandy
$CaCO_3$ (%)	01.68	01.73
Saturation percent (SP) %	23.50	23.80
pH (soil paste)	08.10	08.05
E.C in paste extract (dS m^{-1}, 25°C)	00.88	00.93
Soluble cations (meq/L) :		
Ca^{++}	03.90	04.12
Mg^{++}	02.70	02.65
Na^{+}	01.85	01.91
K^{+}	00.55	00.60
Soluble anions (meq/L) :		
$CO^{=}_3$	00.00	00.00
HCO^{-}_3	01.75	01.86
Cl^{-}	04.10	04.23
$SO^{=}_4$	03.15	03.19
Total-N (%)	00.02	0.020
Total soluble- N (mg kg^{-1})	26.00	23.60
Available- P (mg kg^{-1})	04.60	04.65
Available-K (mg kg^{-1})	185.50	191.20
Organic matter (%)	00.36	00.35
DTPA-extractable (mg kg^{-1}):		
Fe	05.50	05.30
Mn	03.10	03.21
Zn	01.10	01.23
Cu	00.04	00.05

Samples of the soil were obtained from 25 cm soil surface.
*Soluble-N: K_2SO_4 extract, Avai-P: Na-bicarbonate extract, Avai-K: NH_4OAc extract.
** DTPA: Di-ethylene tri-amine penta acetic acid.

Immobilized cyanobacteria sodium alginate billets inoculum (250 kg ha^{-1}) was prepared from both strains as described by **Musgrave *et al.* (1982).** Sodium alginate was prepared in $BG11_0$ (nitrogen free) medium by warming the solution in a water bath. Three ml of 21-day-old mixed culture of *N. muscorum* and *A. oryzae* were added after the solution cooled down to room temperature. The solution was mixed thoroughly and using a syringe canula. The mixture was added drop wise into 100 ml of 1% Ca Cl_2 solution. Calcium alginate beads formed in the Ca Cl_2 solution were left in the same solution for hardening at 4^oC for one hour. The beads were harvested and washed with sterile distilled water and then $BG11_0$.

Nitrogen fertilizer amount is applied as described in the treatments in the form of ammonium sulphate (20.5 % N) and added in four equal doses added during soil preparation, one and two months after transplanting and at flowering stage, while Phosphorus was added at the rate of 300 kg ha^{-1} super phosphate (15% P_2O_5) in two equal split doses, the first during soil preparation and the second at two months after transplanting and potassium was applied at the rate of 100 kg potassium sulphate (48% K_2O) in two equal split doses, the first during soil preparation and the second at flowering stage.

The experiment included eight treatments as follows:
1- Dry + 75 % N.
2- Billet + 75 % N.
3- Soaking + 75 % N.
4- Dry + billet + 75 % N.
5- Dry + soaking + 75 % N.
6- Soaking + billet + 75 % N.
7- Dry + billet + soaking + 75 % N.
8- 100 % N traditional recommended dose.

These treatments were arranged in a complete randomized block design with three replications. The experimental unit area was 21 m^2 (4.2 x 5 m) and each unit contained six rows with 5 m length for each and 70 cm width of them, the distance between seedlings was 25 cm, four inner rows were possessed for yield determination, whereas the two outer rows were for determination of plant growth characters.

Data recorded:

A. Plant Growth:

A random sample of three plants from each plot was taken at age of 80 days and the following data were recorded:

Plant height (cm), number of leaves/plant, number of branches/plant and dry weight of aerial parts (stems + leaves).

A random sample of other three plants from each plot was taken and dried at 70^oC till a constant dry weight and the dry weight of stem + leaves was determined.

B. Fruit Yield and quality:

Mature fruits were continuously harvested upon reaching suitable maturity stages. The following data were recorded:

1- Fruit weight (g).

2- Number of fruits/plant = $\frac{\text{Total number of fruits/plot}}{\text{Number of plants/plot}}$

3-Fruit yield/plant (g) = $\frac{\text{Total weight of fruits/plot}}{\text{Number of plants/plot}}$

4- Total yield (tons).

Total fruit yield was calculated on the basis of total yield along harvesting at full–ripe maturity stages by summing (the sum of all harvests).

C. Nitrogen, phosphorus and potassium concentration, total NPK contents and crude protein content:

Nitrogen, phosphorus and potassium concentration were determined in pepper plants on the basis of dry weight according to the methods described **by Horneck and Miller (1998), Olsen and Sommers (1982) and Horneck and Hanson (1998)**, respectively. Total NPK plant contents were calculated as NPK content on dry weight basis (kg ha^{-1}). Crude protein was calculated based on total N concentration according to **A.O.A.C. (1990).**

At flowering stage soil samples from each treatment were collected from pepper rhizosphere plants to determine total cyanobacteria count (**Allen and Stanier, 1968),** total bacterial count (**Allen, 1959**), dehydrogenase activity (**Casida *et al*., 1964**) and CO_2 evolution amount (**Pramer and Schmidt, 1964**).

Statistical Analysis:

Data were tested by analysis of variance according to **Gomez and Gomez (1984)** and the means separations were compared by using Least Significant Difference (LSD) at level 5%.

3. Results

1-Vegetative growth:

The effect of cyanobacteria applied with different methods, i. e., Dry, Soaking and /or Billets combined with 75 % compared to the use of 100 % N on pepper vegetative growth in terms of plant height, number of branches and leaves $plant^{-1}$ and dry weight of whole pepper plants are shown in Table (2). Results revealed that, the use of cyanobacteria with different forms along with 75 % N gave values of these parameters not significantly differed from those given by the use of 100% N in both tested seasons. Both Soaking + Billets + 75 % N and Dry + Billets + Soaking + 75 % N treatments recorded values for these parameters that were slightly higher than those recorded by the treatment of 100% N in both seasons. For instance, the highest dry weight of whole pepper plant was 17.54 g for the treatment Dry + Billets + Soaking + 75 % N against 16.93 g for the treatment of 100 % N in 2012 season. These results indicate the use of cyanobacteria either as Soaking + Billets + 75 % N or as Dry + Billets + Soaking + 75 % N was the favorite treatments that can save 25 % of the mineral nitrogen fertilizer required for pepper plants. However, the use of cyanobacteria as dry, billet or soaking in combination was better than the use of any of them as single or coble of them in combination.

Table (2): Effect of inoculation with different forms of cyanobacteria and nitrogen rates on vegetative growth of pepper plants at harvest during the seasons of 2012 and 2013

Character	Plant height (cm)		No. of branches plant^{-1}		No. of leaves plant^{-1}		Dry weight of whole plant (g)	
Treatments	2012	2013	2012	2013	2012	2013	2012	2013
100 % N (Control)	72.29	75.85	5.64	5.82	113.82	117.65	15.97	16.93
Dry + 75 % N	63.06	74.58	5.58	5.65	111.99	115.38	15.81	16.88
Billets + 75 % N	64.70	74.65	5.70	5.80	112.05	116.97	15.77	16.84
Soaking + 75 % N	67.09	73.78	5.96	5.60	111.83	114.88	15.86	16.90
Dry + Billets + 75 % N	69.28	74.90	5.37	5.64	112.49	114.94	15.83	16.64
Dry + Soaking + 75% N	68.05	78.28	5.70	5.91	111.72	116.67	15.77	16.67
Soaking + Billets + 75 % N	70.89	77.76	6.07	6.24	114.58	118.39	16.65	17.36
Dry + Soaking + Billets + 75 % N	71.37	78.75	6.53	6.68	114.89	118.90	16.89	17.54
L. S. D. @ 5%	2.93	1.79	0.60	0.54	1.87	3.84	0.85	0.93

Pepper yield and its components:

Data in Table (3) show the effect of the use of cyanobacteria with different methods, i. e., Dry, Soaking and /or Billets combined with 75 % N compared to the use of 100 % N on the yield of pepper and its components in two examined seasons. In this respect, the use of cyanobacteria with any form of either Dry, Billets and Soaking along with 75 % N or any couple of them in combination with 75 % N gave pepper yield and its component which were not significantly differed from those recorded by the treatment of control (100% N) in both tested seasons. For instance, the fruit yield ha^{-1} recorded 25.45 and 28.89 ton ha^{-1} for the control 100% N against 24.12 and 27.55 ton ha^{-1} (Dry + 75% N), 24.22 and 27.24 ton ha^{-1} (Billets + 75% N), 24.21 + 27.39 ton ha^{-1} (Soaking + 75% N), 24.18 + 27.56 ton ha^{-1} (Dry + Billets + 75 % N) and 24.63 and 27.64 ton ha^{-1} (Dry + Soaking + 75 % N) for the first and the second season, respectively. On the other hand the treatment of Dry + Soaking + Billets + 75 % N scored significantly the highest values for pepper yield and its component compared to the other tested treatments in both seasons. The corresponding significant of these high values were 24.78 and 26.88 fruits (No. of fruits plants^{-1}), 40.63 and 42.13 g (fruit weight), 945.59 and 1060 g (fruit yield plant^{-1}) and 28.60 and 31.23 ton ha^{-1} (fruit yield ha^{-1}) for the first and the second season, respectively. Also the values of pepper yield and its component given in response to the use of cyanobacteria with any form of either dry, billets and soaking along with 75 % N or any couple of them in combination with 75 % N were not significantly differed from each other (Table 3).

Nitrogen, phosphorus, potassium concentration and crude protein content of pepper plants:

Data in Table (4) show the effect of using cyanobacteria with different methods, i. e., Dry, Soaking and /or Billet combined with 75 % compared to the use of 100 % N on the nitrogen, phosphorus, potassium and crude protein concentration of pepper plants. Results revealed that using cyanobacteria with different methods combined with 75 % N achieved concentration percentages of nitrogen, phosphorus, potassium and crude protein of pepper plants that were not significantly different from those achieved by the control treatment (100 % N) in both seasons. For instance, the corresponding nitrogen concentration percentages were 1.90 and 1.93 % (Dry + 75 % N), 1.92 and 1.96 (Billets + 75 % N), 1.95 and 1.98 (Billets + 75 % N), 1.97 and 2.02 (Dry + Billets + 75 % N), 1.99 and 2.05 (Dry + Soaking + 75 % N) and 2.03 and 2.07 % (Soaking + Billets + 75 % N) against 2.08 and 2.10 for the control treatment (100 % N) for the first and second season, respectively. However, the treatment of Dry + Soaking + Billets + 75 % N gave significantly the highest concentration percentages of nitrogen, phosphorus, potassium and crude protein of pepper plants compared to the other tested treatments in both seasons. The relative high significant concentration percentages for these parameters were 2.37 and 2.30 (Nitrogen), 0.74 and 0.71 (phosphorus), 1.50 and 1.54 (potassium) and 14.81 and 14.69 (crude protein) for the first and second season, respectively.

Table (3): Effect of inoculation with different forms of cyanobacteria and nitrogen rates on yield and its components of pepper plants at harvest during the seasons of 2012and 2013

Character	No. of fruits plant^{-1}		Fruit weight (g)		Fruit yield plant^{-1} (g)		Fruit yield (ton ha^{-1})	
Treatments	2012	2013	2012	2013	2012	2013	2012	2013
100 % N (Control)	22.36	24.56	38.86	39.11	878.47	983.87	25.45	28.89
Dry + 75 % N	21.14	23.79	37.66	38.87	870.33	918.43	24.12	27.55
Billets + 75 % N	21.37	23.25	37.87	38.31	829.21	924.64	24.22	27.24
Soaking + 75 % N	21.57	23.36	37.31	38.63	833.50	946.37	24.21	27.39
Dry + Billets + 75 % N	22.12	23.19	37.56	38.86	832.70	953.57	24.18	27.65
Dry + Soaking + 75% N	22.10	23.12	37.73	38.73	830.89	959.85	24.63	27.64
Soaking + Billets + 75 % N	22.24	23.47	37.66	38.94	854.73	962.40	24.84	27.76
Dry + Soaking + Billets + 75 % N	24.78	26.88	40.63	42.13	945.59	1060.45	28.60	31.23
L. S. D. @ 5%	1.16	1.44	1.87	1.75	65.66	77.58	1.97	2.33

Table (4): Effect of inoculation with different forms of cyanobacteria and nitrogen rates on NPK concentration and protein contents of pepper plants at harvest during the seasons of 2012 and 2013

Character	N		P		K		Crude protein	
	(%)							
Treatments	2012	2013	2012	2013	2012	2013	2012	2013
100 % N (Control)	2.08	2.10	0.55	0.57	1.27	1.29	13.00	13.13
Dry + 75 % N	1.90	1.93	0.44	0.50	1.15	1.20	11.88	12.06
Billets + 75 % N	1.92	1.96	0.46	0.53	1.17	1.22	12.00	12.25
Soaking + 75 % N	1.95	1.98	0.48	0.54	1.19	1.23	12.19	12.38
Dry + Billets + 75 % N	1.97	2.02	0.49	0.51	1.22	1.24	12.31	12.63
Dry + Soaking + 75% N	1.99	2.05	0.50	0.53	1.24	1.26	12.44	12.81
Soaking + Billets + 75 % N	2.03	2.07	0.52	0.55	1.25	1.28	12.69	12.94
Dry + Soaking + Billets + 75 % N	2.37	2.3	0.74	0.71	1.50	1.54	14.81	14.69
L. S. D. @ 5%	0.22	0.21	0.13	0.12	0.21	0.23	1.38	1.31

Total nitrogen, phosphorus, potassium contents of pepper plants:

Data in Table (5) show the effect of using cyanobacteria with different methods, i. e., Dry, Soaking and /or Billet combined with 75 % N compared to the use of 100 % N on total nitrogen, phosphorus and potassium content of pepper plants. Obtained results confirmed that all cyanobacteria treatments recorded total nitrogen, phosphorus and potassium content values of pepper plants that were not significantly differed from those recorded by the control treatments (100 % N) except for the treatment of Dry + Soaking + Billets + 75 % N in both seasons. For instance, the corresponding values of nitrogen were 458.28 and 531.72 kg ha^{-1} (Dry + 75 % N), 465.02 and 533.90 kg ha^{-1} (Billets + 75 % N), 472.10 and 543.32 kg ha^{-1} (Soaking + 75 % N), 476.35 and 560.75 kg ha^{-1} (Dry + Billets + 75 % N), 490.14 and 566.63 kg ha^{-1}and 504.25 and 574.63 kg ha^{-1}(Soaking + Billets + 75 % N) against 529.36 and 606.69 kg ha^{-1} for the control treatment (100% N) for the first and second seasons, respectively. On the other respect, the treatment of Dry + Soaking + Billets + 75 % N gave the highest significant values of total nitrogen, phosphorus and potassium content of pepper plants compared to the other tested treatments including the control treatments (100 % N) in both tested seasons. The corresponding high values for total N, P & K contents of pepper plants were 677.82 and 718.29 kg ha^{-1} (total N content), 211.64 and 221.73 kg ha^{-1} (total P content) and 429.00 and 399.24 total K content) for both examined seasons. It is also of worth to notice that all the cyanobacteria treatments gave total N, P & K contents values of pepper plants that were not significantly differed from each other's.

Some soil chemical properties:

Data in Table (6) show the effect of using cyanobacteria with different methods, i. e., Dry, Soaking and /or Billet combined with 75 % compared to the use of 100 % N on some soil chemical properties in terms of the soil reaction (pH), electric conductivity (EC) and soil available N, P and K. Results revealed that all cyanobacteria treatments led to decrease slightly both pH and EC for both tested seasons with priority to the treatment of Dry + Soaking + Billets + 75 % N as compared to the control treatment (100 % N). The corresponding values were 7.93 and 7.83 (pH) and 1.64 and 1.63 dSm^{-1}(EC) against 8.10 and 8.00 (pH) and 1.70 and 1.72 dSm^{-1} (EC) for the control treatment in the first and second seasons, respectively.

Due to the soil available N, P & K, same trend seen in both pH and EC was true, since also all the cyanobacteria treatments caused increases in the soil available N, P & K in both seasons compared to the control treatment. However, the most vigorous impact for cyanobacteria treatment was also due to the treatment of Dry + Soaking + Billets + 75 % N in both seasons. The relative N, P & K values were 175.24 mg kg^{-1} (N), 0.78 mg kg^{-1} (P) and 685.00 mg kg^{-1} (K) in the first season and 182.84 mg kg^{-1} (N), 0.94 mg kg^{-1} (P) and 721.24 mg kg^{-1} (K) in the second season against the least values of 147.00 mg kg^{-1} (N), 0.58 mg kg^{-1} (P) and 642.00 mg kg^{-1} (K) in the first season and 153.00 mg kg^{-1} (N), 0.55 mg kg^{-1} (P) and 632.00 mg kg^{-1} (K) in the second season for the control treatment (100 % N).

Table (5): Effect of inoculation with different forms of cyanobacteria and nitrogen rates on total N, P and K content of pepper plants at harvest during the seasons of 2012 and 2013

Character	N		P		K	
	Kg ha^{-1}					
Treatments	2012	2013	2012	2013	2012	2013
100 % N (Control)	529.36	606.69	139.98	164.67	323.22	372.68
Dry + 75 % N	458.28	531.72	106.13	137.75	277.38	330.60
Billets + 75 % N	465.02	533.90	111.41	144.37	283.37	332.33
Soaking + 75 % N	472.10	543.32	116.21	147.91	288.10	336.90
Dry + Billets + 75 % N	476.35	560.75	118.48	141.02	294.10	342.86
Dry + Soaking + 75% N	490.14	566.62	123.15	146.49	305.41	348.26
Soaking + Billets + 75 % N	504.25	574.63	129.17	152.68	310.50	355.33
Dry + Soaking + Billets + 75 % N	677.82	718.29	211.64	221.73	429.00	399.74
L. S. D. @ 5%	73	89	32	31	45	39

Table (6): Effect of inoculation with different forms of cyanobacteria and nitrogen rates on some soil chemical properties during the seasons of 2012 and 2013

Treatment	Character				
	pH 1: 2.5	EC dSm^{-1}	Available mg kg^{-1}		
			N	P	K
Season 2012					
100 % N (Control)	8.10	1.70	147.00	0.58	642.00
Dry + 75 % N	7.99	1.68	150.00	0.62	650.00
Billets + 75 % N	7.95	1.67	154.00	0.67	662.00
Soaking + 75 % N	7.90	1.68	160.00	0.62	656.00
Dry + Billets + 75 % N	8.00	1.66	163.00	0.69	670.00
Dry + Soaking + 75% N	7.98	1.66	166.12	0.70	675.00
Soaking + Billets + 75 % N	8.00	1.67	169,01	0.75	678.00
Dry + Soaking + Billets + 75 % N	7.93	1.64	175.24	0.87	685.00
Season 2013					
100 % N (Control)	8.00	1.72	153.00	0.55	632.20
Dry + 75 % N	7.95	1.69	158.00	0.58	660.32
Billets + 75 % N	7.95	1.67	161.00	0.62	662.45
Soaking + 75 % N	7.94	1.65	165.00	0.65	660.82
Dry + Billets + 75 % N	7.89	1.69	169.00	0.67	685.02
Dry + Soaking + 75% N	7.91	1.68	172.68	0.72	687.12
Soaking + Billets + 75 % N	7.93	1.65	175.74	0.79	692.85
Dry + Soaking + Billets + 75 % N	7.83	1.63	182.84	0.94	721.24

Soil rhizosphere biological activity of pepper plants:

Data in Table (7) show the effect of using cyanobacteria with different methods, i. e., Dry, Soaking and /or Billet combined with 75 % compared to the use of 100 % N on some soil rhizosphere biological activity of pepper plants in terms of total bacteria count**,** total cyanobacteria count, dehydrogenase activity (DHA) and CO_2 evolution amount. Results pointed out that cyanobacteria application increased the soil rhizosphere biological activity of pepper plants detailed as total bacteria count**,** total cyanobacteria count, DHA and CO_2 evolution amount over those of the control treatments in both examined seasons. However, the couple use of two cyanobacteria forms plus 75 % N gave values of these parameters was better and higher than those recorded by the use of cyanobacteria in single form along with 75 % N. For instance, the couple cyanobacteria treatment of Dry + Billets + 75 % N gave higher values of these parameters than those recorded by either control treatments or the use other couple cyanobacteria treatments of Dry + Soaking + 75 % N or Soaking + Billets + 75% N in both seasons. The corresponding higher values for this treatment were 44.34 and 42.65 cfu g^{-1}soil x 10^6 (total bacteria count) and 8.74 and 9.45 cfu g^{-1}soil x 10^3 (total cyanobacteria count), 918.12 and 934.25 mg CO_2 100 g^{-1}soil day^{-1} (CO_2 evolution) and 70.15 and 56.35 mg TPF g^{-1}soil day^{-1} (DHA) for the treatment of Dry + Billets + 75 % N for first and second season, respectively. While the values of 22.60 and 21.60 cfu g^{-1}soil x 10^6 (total cyanobacteria count), 3.50 and 3.20 cfu g^{-1}soil x 10^3 (total cyanobacteria count), 507 and 492 mg CO_2 100 g^{-1}soil day^{-1} (CO_2 evolution) and 17.63 and 15.84 mg TPF g^{-1}soil day^{-1} (DHA) for the control treatment, and the values of 42.15 and 41.15 cfu g^{-1}soil x 10^6 (total cyanobacteria count), 8.25 and 8.12 cfu g^{-1}soil x 10^3 (total cyanobacteria count), 907.45 and 912.46 mg CO_2 100 g^{-1}soil day^{-1} (CO_2 evolution) and 63.12 and 54.62 mg TPF g^{-1}soil day^{-1} (DHA) for the treatment of Dry + soaking + 75 % N, and the values of 43.45 and 41.45 cfu g^{-1}soil x 10^6 (total cyanobacteria count), 8.45 and 9.15 cfu g^{-1}soil x 10^3 (total cyanobacteria count), 913.12 and 924.18 mg CO_2 100 g^{-1}soil day^{-1} (CO_2 evolution) and 68.64 and 53.12 mg TPF g^{-1}soil day^{-1} (DHA)for the treatment of soaking + Billets + 75 % N, all in the first and second seasons, respectively. On the other hand, the use of single form alone along with 75 % N gave less values of the tested parameters the those achieved by the use of their couple forms (Table 7). However, the values recorded in response to the use of cyanobacteria in single forms were still higher than those scored by the control treatment.

Table (7): Effect of application of different forms of cyanobacteria and different nitrogen rates on some soil rhizosphere biological activities of pepper plants after 100 days from transplanting during the seasons of 2012 and 2013

Character / Treatment	Total bacteria count *cfu g^{-1} soil x 10^6	Total cyanobacteria count cfu g^{-1} soil x 10^3	CO_2 evolution (mg CO_2 100 g^{-1} soil day^{-1})	**DHA (mg TPF g^{-1} soil day^{-1})
Season 2012				
100 % N (Control)	22.60	3.50	507.00	17.63
Dry + 75 % N	38.50	7.80	850.00	48.96
Billets + 75 % N	36.20	7.65	854.00	50.75
Soaking + 75 % N	38.00	7.68	897.85	54.25
Dry + Billets + 75 % N	44.34	8.74	918.12	70.15
Dry + Soaking + 75% N	42.15	8.25	907.45	63.12
Soaking + Billets + 75 % N	43.45	8.45	913.12	68.64
Dry + Soaking + Billets + 75 % N	45.82	9.65	930.25	81.15
Season 2013				
100 % N (Control)	21.60	3.20	492.00	15.84
Dry + 75 % N	36.50	8.15	810.00	35.96
Billets + 75 % N	34.20	8.45	830.00	43.75
Soaking + 75 % N	35.00	8.78	910.85	50.25
Dry + Billets + 75 % N	42.65	9.45	934.25	56.35
Dry + Soaking + 75% N	41.15	8.12	912.46	54.62
Soaking + Billets + 75 % N	41.45	9.15	924.18	53.12
Dry + Soaking + Billets + 75 % N	46.82	10.16	955.28	62.45

***cfu = Colony formed / Unit.** ****DHA= Dehydrogenase activity.**

4. Discussion

Current soil management strategies are mainly dependent on inorganic chemical-based fertilizers, which caused a serious threat to human health and environment. The exploitation of beneficial microbes as a biofertilizer has become paramount importance in agriculture sector for their potential role in food safety and sustainable crop production. The eco-friendly approaches inspire a wide range of application of biofertilizers such as plant growth promoting rhizobacteria (PGPRs), endo- and ectomycorrhizal fungi, cyanobacteria and many other useful microscopic organisms led to improved nutrient uptake, plant growth and plant tolerance to abiotic and biotic stress **(Bhardwaj *et al.*, 2014)**. Also, Biofertilizer enhance the nutrient availability to crop plants (by process like fixing atmosphere N or dissolving P present in the soil); and impart better health to plants and soil thereby enhancing crop yields in moderate way **(Boraste *et al.*, 2009)**. Subsequently, In this study, cyanobacteria is tested as a nitrogen fixing and plant growth promoting biofertilizer applied in different forms, i.e., soil based cyanobacteria inoculum (Dry) and Immobilized cyanobacteria beads inoculum (Billets) and soaking (cyanobacteria filtrate) along with 75 % N compared with the recommended nitrogen rate of 100 % N for pepper plants, in an attempt to reduce the excessive use of the mineral nitrogen consumed by pepper plants. Results revealed that the use of cyanobacteria in different forms combined with 75 % N with priority to the treatment of Dry + Billets + Soaking + 75 % N had mainly enhanced the growth, yield and its components, N, P & K concentration, total N, P & K contents for pepper plants. As well as, they improved the available soil N, P & K and the soil rhizosphere biological activity of pepper plants. These results can be noticed by cyanobacteria that are mainly used under paddy condition in rice soils and they can contribute about 20 % of nitrogen for rice production **(Roger and Kulasooriya, 1980)**. The heterocystous cyanobacterium *Nostoc* sp. is usual among characterized cyanobacteria in its ability to form tight association with wheat roots and penetrate both roots epidermis and cortical intracellular space **(Gantar *et al.*, 1991). Moustafa and Omar (1990)** reported that inoculation of tomatoes with a mixture inoculum of *Azospirillum lipoferum* and blue-green algae (a mixture of different cyanobacteria strains) and/or cyanobacteria alone as biofertilizer led to increase significantly tomato yield as improved the quality of tomato fruits. **Abd El-Rasoul *et al.* (2004)** revealed that cyanobacteria inoculation combined with 96 kg N ha^{-1} (65 % N) was superior to the use of 144 kg N ha^{-1} (100 % N) indicating that 96 kg N ha^{-1} plus cyanobacteria inoculation was more beneficial for rice grain and straw yields. They explained that cyanobacteria have the ability to excrete in their surrounded media plant growth promoting like substances. They, describe these promoting materials to resemble to gibberellins and auxins. **Hassan (2011)** noted that that the use combination of cyanobacteria dry inoculum + cyanobacteria Billets + 75 % N gave values of vegetative growth, vitamin C and leaf chlorophyll content comparable with those achieved due to the use of 100 % N (the control). He also added that this treatment enhanced the rhizosphere soil biological activity in terms of total count bacteria, cyanobacteria count, CO_2 evolution and dehydrogenase activity to be higher than those achieved due to the use of 100 % N (the control treatment). **Zulpa *et al.* (2008)** studied the effect of cyanobacteria products of *Tolypothrix tenuis* and *Nostoc muscorum* on the microbiological activity and the nutrient content of the soil. The biomass and extracellular products of both strains increased the soil microbial activity. *Nostoc muscorum* and *T. tenuis* biomasses increased the soil oxidizable C (15%; 14%), total N (10%; 12%) and available P (22%; 32%), respectively. *T. tenuis* extracellular products increased by 28% oxidizable carbon and *N. muscorum* extracellular products increased by 15% the available phosphorus. These are caused the soil biological activity to be increased also because they are a continuously renewable carbon source. **Caire *et al.* (2000)** established that cyanobacteria can increase the soil enzymatic activity. **Aref and EL- Kassas (2006)** found that cyanobacteria inoculation to maize field enhanced significantly any of total count bacteria, cyanobacteria count, CO_2 evolution, dehydrogenase and nitrogenase activities compared to the control treatment received no inoculation. They explained that biofertilization with cyanobacteria led to increase microorganisms' community and in turn biological activity in soil through increasing the organic matter and microbial activity. **Zeenat and Sharma (1990) and Zeenat *et al.* (1994)** found in tomato experiments that inoculation with cyanobacteria combined with reduced dose of mineral nitrogen led to increase significantly the vegetative growth of tomato plants. Meanwhile, they explained that cyanobacteria do not only fix nitrogen but also release growth promoting-like substances in the surrounded media, which in turn encourage the plant vegetative growth and productivity. **Haroun and Hussein (2003)** postulated that soaking the seeds of *Lupinus termis*, in cyanobacteria filtrate increased chlorophyll a & b contents in leaves. On the other hand, **Ghazal and Sarabana (2010)** reported that the immobilized alginate beads cyanobacteria inoculum (Billets) inoculated to rice was superior than the soil based cyanobacteria inoculum (Dry). The use of cyanobacteria improved the chemical soil properties in both tested seasons. **Saxina and Tilak (1998)** noted that soil inoculation with cyanobacteria decrease the soil pH and helps in reducing the soil alkalinity due the organic acid secreted by cyanobacteria. **Rodriguez *et al.* (2006)** found that cyanobacterial inoculation decreased soil EC of soil in comparison with control 100 % N and owed this effect to that cyanobacteria synthesize and liberate plant growth regulators such as gibberellins and indole acetic acid that could exert natural beneficial effect on salt stress and thus reduce the soil EC. The use of reduced mineral nitrogen rate (75 % of full N) along with cyanobacteria inoculation (Dry and/or Billets) for pepper was comparable in maintaining the soil available NPK) with same effect of the use of 100 % N (Control). Biofertilizers benefit virtually any soil type, clay soil, for example, has tiny, tightly packed particles that hamper the flow of water, nutrients and oxygen. Cyanobacteria as biofertilizer can reconfigure the clay into larger spaces between the particles. The larger spaces between the particles improve the flow of water, oxygen, and nutrients to roots, on the other words, increasing the nutrients availability **(Boraste *et al*., 2009)**. Inoculation with cyanobacteria generally increased the soil biological activity compared to non-inoculated treatments. **Abd El-Rasoul *et al.* (2004)** and **Ghazal *et al.* (2011)** in wheat, EL**-Zeky *et al*. (2005)** in rice and **EL-Gaml (2006)** in maize found that inoculation with cyanobacteria combined with different reduced levels of nitrogen increased the biological soil activity of the post harvest soils in terms of total count bacteria, cyanobacteria count, CO_2 evolution and dehydrogenase activity (DHA) over the control treatment. They explained that cyanobacteria inoculation led to increase the soil microorganisms community in soil because of that they secreted polysaccharides and thus add to the soil organic matter increased the microbial activity, which in turn increased dehydrogenase activity, CO_2 evolution and subsequently increased the soil fertility and plant growth performance. Moreover, **Aref and EL-Kassas (2006)** stated that cyanobacteria are popularly known as blue green algae, constitute the most important inputs in rice cultivation and recently in maize **(Ghazal *et al*., 2013)** and in wheat (**EL-Ayouty *et al*., 2012)**. Cyanobacteria form an inexpensive farm grown input, which helps in a better crop nutrient management, while working in perfect harmony with nature. Cyanobacteria also fix the atmospheric nitrogen in soil under extreme conditions and add organic matter, synthesize and liberate amino acids, vitamins and auxins and exo-polysaccharides reduce oxidizable matter content of the soil, provide oxygen to the submerged rhizosphere, ameliorate salinity, buffer the pH, solubilize phosphates and increase the efficiency of fertilizer use in crop plants and in turn enhance and improve quality and quantity of crop yield and yield attributes **(Kaushik, 2004)**. Maize inoculation with the cyanobacteria resulted in improved maize growth and nitrogen uptake **(Maqubela and Mnkeni, 2009)**. They explained that the improved growth appeared was related to the increase in soil N and favorable mineralization due to the increase of soil carbon and aggregate stability. This led to improve water holding and infiltration capacities of the soil, and potentially the plant water use efficiency from the soil.

Generally, due to the present study. Several reports confirmed the beneficial effects of cyanobacteria inoculation with crops rather than rice, such as barley, oats, tomato, cucumber, carrot, maize, wheat, raddish, chilli, sugar cane and lettuce **(Thajuddin and Subramanian, 2005, EL-Shahat, 2007, Abed *et al*., 2009 and Ghazal, 2013).**

Conclusion

In conclusion, this work led to take in consideration much attention for establishing the technology of cyanobacteria application with different forms to vegetable crops with a view of saving partially some of the costly non-ecofriendly mineral nitrogen fertilizers. Further studies on other vegetable crops rather than pepper need to be carried out for more confirmation.

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