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RESPONSE OF SOYBEAN PLANTS TO INOCULATION WITH RHIZOBIA AND CYANOBACTERIA

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ABSTRACT: *Field experiments were carried out on a clayey alluvial soil to evaluate the effect of (Bradyrhizobium japonicum (B.j), ,Nostoc muscorum, Anabaena oryzae, B.j. +Nostc, B.j.+Anabaena, and B.j.+Nostc+Anabaena , on soybean (Glycine max L.) vegetative growth, root nodulation status , yield and its components. Also, microbial activity in soybean rhizosphere soil and technology characters of seeds (protein, oil, and carbohydrate content) were determined. Results indicated that, inoculation with Brady rhizobium or cyanobacteria improved plant growth parameters, i.e., plant height, number of branches, number and dry weight of pods above the control (60kgN) . However, co-inoculation of Brady rhizobium with the two cyanobacteria significantly enhanced those parameters, where they scored significant increases in number and dry weight of root nodules, as well as nitrogenase activity of the root system, as compared with the single inoculation with Brady rhizobium alone or the control. The mixed inoculation improved dehydrogenase activity in the rhizosphere soil, as well as the seed content of oil, protein and carbohydrate were achieved. Also, the highest yield and its components were also recorded with the mixed inoculation.*

***Key words**: Glycine max, N_2-Fixation, Brady rhizobium Nostoc, Anabaena, plant growth parameters.*

INTRODUCTION

Soybean (*Glycine max*) is one of the most important oil seeds in the world. It contains 18-22 % oil, 40-45% protein, 30% carbohydrate and total sugar about 10%. Moreover, it is also a good source of calcium, phosphorus, copper, potassium, magnesium and thiamine. It is used in food industry for flour, oil, cookies, candy, milk, vegetable chees, lecithin and many other products, (Masuda and Goldsmith, 2009). However, soybean oil can be converted to biodiesel and glycerol. Soybean production in Egypt has increased, since it started in 1976, and its yield had reached to about 966 ha. about 2895 metric ton per hectare (El - Agroudy *et al.,* 2011). Mineral fertilizers are essential components of modern Agriculture. Intensive crop cultivation requires the use of nitrogen fertilizers. However, fertilizers are short supply, expensive and environmentally hazardous. Therefore, it is important to explore the possibility of supplementing nitrogen in the form of biofertilizers derived from microbial origin. .Biofertilizers are safe environmentally, cheaper and at the same time satisfy the nutrient demands of crop plants (Badawy *et al*., 1996).

Ecological interactions between rhizobia and other soil bacteria have been of interest in recent years because of their agronomical implications. Many papers have reported enhancement of nodulation and growth and yield of legumes, Hungria and Nogueira (2013) found that, co-inoculation with rhizobia and *Azospirillum* increased the yield of soybean by 16-19% above control. Also, Kumar *et al*. (2016) found that, inoculation of *Phaseolus valgaris* with mixed inocula was more effective, compared with single inoculation and increased plant height, nodulation, number of pods per plant, seed per pod and grain yield in pot and field experiments. Likewise, Elsayed *et al.* (2016) observed that, using non-invasive spectral detection, coinoculation of peanut with *Bradyrhizobium* and PGPR increased yield of two cultivars, compared to single inoculation.

In recent years, more worldwide and more attention has been paid to use cyanobacteria that occur in almost every environment on the earth and several species are known to form symbiotic associations with bacteria, as well as with eukaryote (Bergman *et al*. 2007). Many cyanobacteria are capable of N_2 fixation and taking up and assimilating organic compounds, which may play an important role in secondary metabolism (Prasanna *et al*.2004). Moreover, some cyanobacteria may produce plant growth –promoting substances and play an important role in building up soil fertility (Sergeeva *et al*. 2002).

Cyanobacteria strains belonging to the genera *Nostoc* and/or *Anabaena* can be used as alternative to increase soil productivity and improve plant growth in a sustainable agriculture regime. There is currently great interest in developing new associations with many plants. Aref and Al-Kassas (2006) study the effect of cyanobacterial inoculation as a nitrogen source may substitute partially on maize plants. They found that, using cyanobacteria significantly increased maize yield above the control.

The aim of this work was to investigate the effect of single and dual inoculation with cyanobacteria and rhizobia on growth, nodulation status, crop yield, yield components and microbial activity in rhizosphere soil of soybean cultivated inalluvial clay soil.

MATERIALS AND METHODS

Two field experiments were carried out in a private farm at Ashmon, Meinufiya Governorate, Egypt, during two successive summer seasons, i.e. 2012/2013 and 2013/2014 to study the effect of inoculation with cyanobacteria alone or coinoculation with rhizobia . Treatments carried out were: control (60kgN/fed.), *Nostoc muscorum*, *Anabaena oryzae, Bradyrhizobium japonicum* (B.j.), (B.j.), +*Nostc*, (B.j.)+ *Anabaena*, and (B.j.)+ *Nostc*+ *Anabaena* each with 30 kg N/fed., on soybean vegetative growth, nodulation status , nitrogenase activity, yield and yield components, as well as dehydrogenase activity in the rhizosphere soil of plants. Biochemical constituents of seeds (protein, oil, and carbohydrates) were also determined. The soil of the experimental field plots was clayey in texture, having pH 7.70, organic matter 2.2 % , total N 0.21 %, available P 0.03 %, according to Page *et al.* (1982). The field was prepared by ploughing and puddling, then divided into 21plots (3mx3m), 7 treatments each with three replicates, in a randomized block design. Soybean seeds variety “Giza 111”, were inoculated with cyanobacteria inoculum, *Nostoc muscorum*, *Anabaena oryzae* and *Brady rhizobium* sp. either of individually or mixed. All strains were kindly supplied by the Microbial. Dept., Soils, Water & Environ Res. Inst. Agric. Res. Center (ARC), Giza, Egypt.

Analytical procedures:

- Plant growth criteria:

Plant height, number of branches, shoot dry weight, number of pods and pod dry weight, were determined after 75 days of planting , as well as number of root nodules, dry weight of nodules, nitrogenase and rhizosphere dehydrogenase activity, were also determined.

Contents of seed constituents:

- Oil was determined by Soxhlet extraction as described by A.O.A.C.(1990).
– Protein was determined as nitrogen by micro Kjeldal method, according to A.O.A.C. (1990), then calculated by multiplying N % in the factor 6.25 to get the protein content .

- Carbohydrate was determined by phenol-sulfuric acid, according to Dubois *et al* . (1956).
- Dehydrogenase activity : colourimetrically, for the 2,3,5- triphenyl formazan (TPF) produced from the reduction of 2,3,5- triphenyl tetrazolium chloride (TTC), using acetone for extraction (Thalmann.,1967).
- Nitrogenase: by means of gas liquid chromatograph for ethylene produced from the reduction of acetylene (Hardy *et al.,* 1973).

At maturity, a sample of five guarded plants were uprooted to measure number of branches ,number of pods , number of seeds /pod , seed index (100 seed weight) and seed yield /plant, then the four inner rows were harvested to calculate seed yield /fed . Analyses of variance were computed, according to Gomez and Gomez (1984) using the least significant difference at 0.05 level to compare the differences among means.

RESULTS AND DISCUSSION

Effect of microbial inoculation on growth of soybean plants

Data in Table (1) indicate that, cyanobacterial inoculation and rhizobia, either individually or mixed improved the vegetative parameters measured.

Results showed that the mixed microbial inoculation showed the highest records of almost all parameters under study, compared to the other treatment. The treatment with (B.j.) +*Nostc+Anabaena* + 30 kg N/fed, was mostly the best significantly. Shoots dry weight increased with 15% by using the mixture of inocula, as compared with other treatments.

The addition of cyanobacteria singlely plus half dose of mineral fertilizer could not compensate the full dose, of nitrogen fertilizer. Previous results obtained (Sekina Tantawy and Nagwa atef, 2010) who found that, coinoculation with cyanobacteria plus *Azospirillum* or *Azotobacter* provoked germination and increased growth parameters in *Lupinus termis*. Earlier reports had shown that, inoculation with rhizobia stimulated the growth of plants by providing not only fixed N but also by increasing microbial diversity and structure in the plant rhizosphere (Trabelsi *et al.,* 2012). Likewise, coinoculation including cyanobacteria scored the heights figures, being due to the contribution of cyanobacteria not only in N_2 fixation , but also in increasing organic matter content in soil. Karthikeyan *et al.* (2007) reported that, cyanobacteria excrete IAA, amino acids and other growth-promoting compounds into their immediate environment and stimulate microbial population in the rhizosphere soil .

Table (1): Effect of cyanobacteria and rhizobial inoculation on growth parameters of soybean plants.

Treatments	Plant Height (Cm)			Shoot dry weight (g/plant)			Number of branches		
	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean
Control (60kgN)	78.70	76.25	77.47 b	27.44	29.40	28.42c	1.67	2.00	1.83 dc
Nostoc+30kgN	70.21	67.25	68.94 d	24.30	25.11	24.70 e	1.00	1.33	1.66 e
Anabaena+30kgN	63.94	67.88	65.91 e	24.13	24.42	24.28 e	1.00	1.67	1.33de
B. japonicum +30kgN	74.74	73.74	47.24 c	26.41	27.91	28.42 c	1.67	2.00	1.83 dc
B.j.+No.+30kgN	73.58	68.78	71.18 d	29.61	30.51	28.64 c	2.33	2.67	2.50ab
B.j.+Ana.+30kgN	78.40	78.63	78.51ab	27.99	29.29	30.06 b	1.67	2.33	2.00 bc
B.j.+No.+Ana.+30kgN	80.48	80.88	8.62 a	31.52	33.49	32.50 a	2.67	2.67	2.67 a
L.S.D. at 0.05	2.71			1.24			0.57		

S1: First season , S2: Second season

Effect of microbial inoculation on nodulation status of soybean plant root.

Data presented in Table (2) indicated that, all the tested treatments stimulated the nodulation status of plant roots. Cyanobacteria singly or combined with rhizobia caused significant increases in number and dry weight of nodules, as well as nitrogenase activity. Mixed inoculation of all strains achieved the highest increase that reached 311%, 251% and 115.37 % above the control, for number and dry weight of nodules and nitrogenase activity, respectively. These results are in accordance with the findings of Dashti *et al* ., (1998) who found that, co-inoculation of soy bean with *B. japonicum* and PGPR increased nodules number and hastened the N_2 fixation. Also, Thilak *et al*., (2006) showed that coinoculation with *Rhizobium-Azospirillum* or any PGPR could affect the growth and nitrogen fixation by increasing the infection sites that were later occupied by rhizobia and enhancing the occupancy of introduced *Rhizobium* in nodules of legume. Similar finding, confirming that the co-inoculation increased nodulation of legume roots, was observed by El-Howeity (2004) and Abdel-Wahab *et al.* (2006) whom reported that co-inoculation of some legumes with rhizobacteria exerted beneficial effects on nodulation of faba bean and peanut plant roots.

Co-inoculation of common bean (*P. vulgaris* L.) with *Rhizobium tropici*-CIAT 899, *P. polymyxa*-DSM36 and *P. polymyxa-Loutit* strains resulted in greater growth compared to inoculation with Rhizobium alone. Furthermore, co-inoculation exhibited greater nodulation (number and biomass) and nitrogen content compared to drought-stressed plants inoculated with only *Rhizobium* (Figueiredo *et al.,* 2008). Moreover, El-Howeity *et al*.(2009) and Zhang *et al*. (2016) reported that, coinoculation with *Rhizobium* and PGPR resulted in significant increases in number and dry weight of root nodules , and nitrogenase activity, as compared with control of faba bean and peanut plants under field conditions.

Table (2): Effect of cyanobacteria and rhizobial inoculation on nodulation status of (*Glycin Mix*.L) plants under field conditions

Treatments	Number of nodules/ plant			Dry weight of noules(g/plant)			Nitrogenase activity (ARA) (n moles ethylene/g soil/h)		
	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean
Control (60kgN)	26	27	26.50 f	0.55	0.58	0.56 g	24.70	21.89	23.29 f
Nostoc+30kgN	54.67	57.33	56.00 e	0.69	0.77	0.73 c	33.44	27.56	30.50 e
Anabena+30kgN	59.00	64.00	61.50 d	0.78	0.86	0.82 e	41.48	33.01	37.24 d
B. japonicum +30kgN	68.67	69.00	68.83 c	1.13	1.22	1.17 d	46.69	38.70	42.69 b
B.j.+No.+30kgN	80.33	84.67	82.5 b	1.36	1.45	1.40 c	47.20	47.06	47.13 b
B.j.+Ana.+30kgN	83.33	83.67	83.50 b	1.70	1.83	1.76 b	47.10	44.63	45.86 b
B.j.+No.+Ana.+30kgN	108.67	109.67	109.16a	1.91	2.04	1.97 a	50.75	49.58	50.16 a
L.S.D. at 0.05	5.17			0.065			2.08		

S1: First season, S2: Second season

Effect of microbial inoculation on yield and some of its attributes of soybean crop:

Data presented in Table (3) showed that single inoculation by cyanobacteria or rhizobia and their coinoculation improved the number of soybean pods /plant, dry weight of pods/ plant and not clear with 100 seed weight. Mixed inoculation of rhizobia + *Nostoc* + *Anabaena* scored the highest value of number of pods/plant when gave an increase 17.63% above the control. Also, the increase of dry weight of pod reached 26.73%. Increases in the number of pods, dry weight of pods and 100 seeds weight were certainly referred to the microbial coinoculation which improved soil structure and porosity via secretion of polysaccharides and mucilage (Nain *et al.,* 2010).

Effect of microbial inoculation on some biochemical constituents of soybean seeds:

Contents of oil, protein and carbohydrate in the seeds of soybean, subjected to different microbial inoculants, i.e. *Nostoc, Anabaena, Rhizobium* , either singly or in combination are presented in Table (4). The highest oil content was recorded by using the combined inoculation of the used N2 fixers , revealing the order : *B.j.+Nostoc* > B.j. +*Anabaena* > *B.j.* + *Anabaena* ,respectively . Protein content was also improved with the mixed inoculation, wheres, rhizobial inoculation was more effective than the single cyanobacterial strains, but coinoculation with all strains significantly increased protein percent to 3.06% approximately. Likewise, coinoculation resulted in a similar action on carbohydrate contents in soy bean seeds, as compared with the other treatments.

Table (3): Effect of cyanobacteria and rhizobial inoculation on yield of soybean plants under field conditions

Treatments	Number of pods/ plant			Dry weight of pod (g/plant)			100 seed weight (g)		
	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean
Control (60kgN)	64.67	67.67	66.16 c	20.77	21.07	20.91 d	15.56	15.53	15.54 ab
Nostoc+30kgN	54.00	58.33	56.16 d	19.05	19.79	19.42 e	15.19	15.27	15.23 de
Anabena+30kgN	53.00	56.33	54.66 d	17.29	18.28	19.42 f	15.30	15.33	15.31 cd
B. japonicum +30kgN	62.33	66.67	64.50 c	20.20	21.98	21.08 d	15.24	15.65	15.44 bc
B.j.+No.+30kgN	69.00	71.33	70.16 b	23.25	24.60	23.92 b	15.34	15.30	15.32 cd
B.j.+Ana.+30kgN	68.33	70.33	69.33 b	21.57	24.16	22.86 c	15.01	15.19	15.10 e
B.j.+No.+Ana.+30kgN	77.00	78.67	77.83 a	25.65	27.35	26.50 a	15.80	15.52	15.66 a
L.S.D. at 0.05	2.01			0.74			0.19		

S1: First season , S2: Second season

Table (4): Effect of cyanobacteria and Brady rhizobial inoculation on biochemical constituents of soybean seeds:

Treatments	Oil %			Protein %			Carbohydrate %		
	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean
Control (60kgN)	19.73	20.93	20.33 b	39.62	40.01	39.81 bc	33.20	33.07	33.13 abc
Nostoc+30kgN	20.23	21.05	20.63 b	40.22	40.71	40.46 ab	32.64	33.18	32.91 bc
Anabena+30kgN	19.80	20.22	20.01 b	39.01	40.15	39.64 c	32.40	32.72	32.56 c
B. japonicum +30kgN	22.16	23.11	22.63 a	40.56	41.46	41.01 a	33.88	33.06	33.46 ab
B.j.+No.+30kgN	22.06	23.74	22.89a	40.02	40.84	40.43 ab	34.38	32.93	33.65 a
B.j.+Ana.+30kgN	22.40	22.77	22.58 a	39.22	40.06	39.64 bc	34.14	33.11	33.62 a
B.j.+No.+Ana.+30kgN	22.52	23.18	22.84 a	40.33	41.33	41.03 a	33.70	33.30	33.49ab
L.S.D. at 0.05	1.07			0.83			0.65		

S1: First season , S2: Second season

Effect of microbial inoculation on yield and some of its attributes of soybean crop:

Data resulted in Table (5) declare the effect of using single and /or mixed inoculation with rhizobia and cyanobacteria plus half dose of recommended nitrogen fertilizer, in a comparison with full dose of the mineral nutrient, on the seed and straw of soybean yields . The obtained results showed that, the single inoculation by cyanobacteria improved seed yield of the crop, but not significantly with the control. Microbial combination significantly increased seed yield compared with the control or the single inoculation. The full dose of nitrogen fertilizer scored the highest straw yield referring to the other treatments.

It is worthy to note that, using cyanobacteria or rhizobia as a single inoculant gave lower straw yield. The full dose of nitrogen fertilizer (60 kg N) scored the highest straw yield, but not significant with the mixed inocula of all strains. Similar finding, confirming that the coinoculation increased yield of legume, was observed by El-Howeity *et al*. (2009), Sanchez *et al.* (2014) and Zimmer *et al.* (2016) who reported that , coinoculation with rhizobia and PGPR increased yield and protein content of *Phaseolus vulgares* and soybean.

Effect of microbial inoculation on microbial activity in rhizosphere soil of soybean plants:

Microbial activity in the rhizosphere soil of soybean was evaluated by dehydrogenase activity (DHA) and presented in Fig. (1). Results exerted that, the application of microbial inoculation significantly increased DHA. Coinoculation treatments gained higher values of DHA. These increases of dehydrogenase activity might be due to the support of cyanobacteria, either singly or mixed with rhizobia. Such contribution encouraged all endogenous microorganisms in the soil and also stimulated the root growth of the soybean plants, since cyanobacteria represented a source of organic matter which favours the activity of the majority of soil microorganisms. Dehydrogenase activity depends on the metabolic state of soil microorganisms (Frankenberger and Dick

1983). Nain *et al.,* (2010) found that, inoculation of wheat with cyanobacteria increased dehydrogenase activity under field conditions.

Finally, the present results confirm the benefits of the combined microbial inoculation, including cyanobacteria together with the specific rhizobia, for legume cultivation, particularly soybean.

Table (5): Effect of cyanobacteria and rhizobial inoculation on seed and straw yields of soybean plants

Treatments	Seed yield (ton/fed.)			Straw yield (ton/fed.)		
	S1	S2	Mean	S1	S2	Mean
Control (60kgN)	1.55	1.62	1.58 cd	3.64	3.65	3.64 a
Nostoc+30kgN	1.54	1.51	1.52 d	3.59	3.62	3.60 ab
Anabaena+30kgN	1.51	1.56	1.53 d	3.47	3.57	3.52 c
B. japonicum +30kgN	1.62	1.65	1.63 c	3.55	3.59	3.57 abc
B.j.+No.+30kgN	1.69	1.75	1.72 b	3.57	3.55	3.55 bc
B.j.+Ana.+30kgN	1.74	1.77	1.75 b	3.50	3.55	3.52 c
B.j.+No.+Ana.+30kgN	1.82	1.90	1.86 a	3.62	3.64	3.63 a
L.S.D. at 0.05	0.06			0.07		

S1: First season , S2: Second season

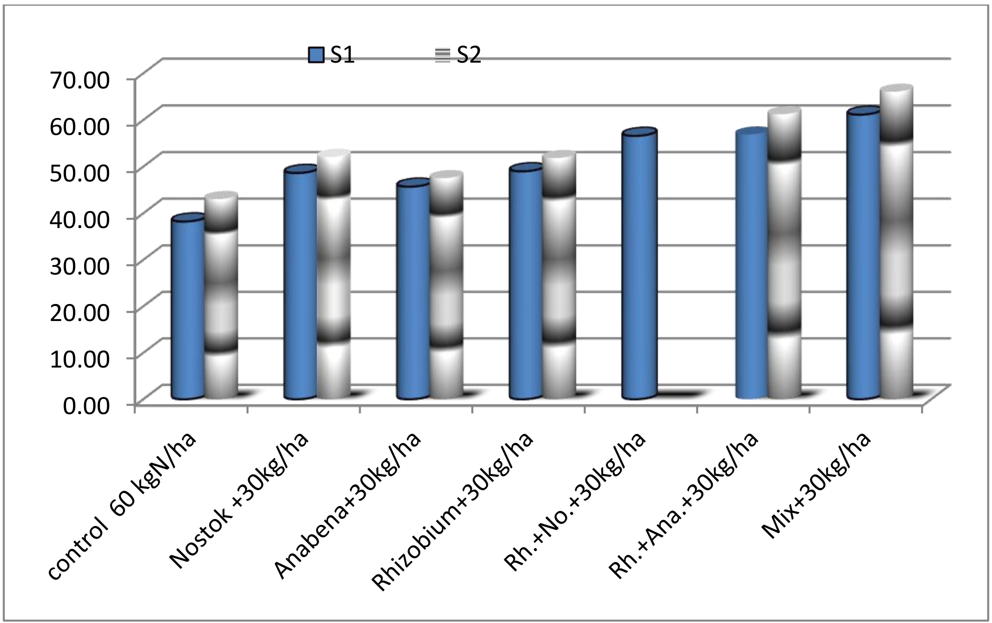

Fig. (1): Effect of cyanobacteria and *B. japonicum* inoculation on dehydrogenase activity "DHA" in rhizosphere soil of soybean plants.

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استجابة نباتات فول الصويا للتلقيح المشترك بين الريزوبيا والسيانوبكتيريا

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الملخص العربى

تم زراعة تجربة حقلية لموسميين متتالين لنبات فول الصويا عامى 2013/2012– 2014/2013 ، فى ارض رسوبية طينية وذلك لتقييم اثر المعاملات الاتية :

1– كنترول (60 وحدة ازوت) للفدان

2– تلقيح بسلالة السيانوبكتيريا *Nostoc muscorum* +30 وحدة ازوت

3– تلقيح بسلالة السيانوبكتيريا *Anabaena oryzae* + 30 وحدة ازوت

4– تلقيح بالريزوبيا المتخصصة (البرادى ريزوبيم) + 30 وحدة ازوت

5– تلقيح مشترك بين كل من : البرادى ريزوبيم والسيانوبكتيريا (*Nostoc muscorum* + 30 وحدة ازوت)

6– تلقيح مشترك بين كل من : السيانوبكتيريا و البرادى ريزوبيوم (*Anabaena oryzae* + 30 وحدة ازوت)

7– تلقيح مشترك بين كل السلالات : البرادى ريزوبيم والسيانوبكتيريا (*Anabaena* + *Nostoc muscorum* *oryzae* + 30 وحدة ازوت)

وتم تقييم الصفات الخضرية للنباتات ، وهى تحديدا : طول النبات ، والوزن الجاف وعدد الاشطاء وكذلك حالة التعقيد للجذور (عدد العقد ووزنها) وانزيم النيتروجينيز والمحصول الكلى ، وأيضا تم تقدير الصفات البيوكيميائية للبذور مثل(نسبة الزيت – البروتين –و الكربوهيدرات)، كذلك النشاط الحيوى فى ريزوسفير النباتات (بتقدير انزيم الديهيدروجينيز).

وأظهرت النتائج زيادة معنوية للصفات الخضرية مع التسميد الحيوى لكل المعاملات الميكروبية مقارنة بباقى الاضافات والكنترول . وقد تفوق التسميد المشترك معنويا مقارنة بالتسميد المنفرد . وحقق استخدام السيانوبكتيريا مشتركة مع الريزوبيا زيادة معنوية فى التعقيد وزيادة معدل تثبيت النيتروجين . ارتفاع المحصول الكلى من البذور مقارنة بباقى المعاملات .كذلك زاد النشاط الكلى للكائنات الحية فى منطقة الريزوسفير متمثلا فى انزيم الديهيدروجينيز ز هذا مما يؤكد فعالية استخدام السيانوبكتيريا فى التسميد الحيوى والحد من الاضافات المتزايدة من التسميد الكيماوى والحفاظ على البيئة .