Can cyanobacteria biofertilizer enhance the growth of *phaseolus Vulgaris* plants?

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Abstract

Impact of blue green algal inocula (cyanobacteria biofertilizer) on the growth of kidney bean plants grown on clay-sandy soil (2:1 v / v) was investigated in pots. Application of 10%, 20% and 30 % of cyanobacteria inoculum either by foliar application or soil incorporation for 30 days induced significant increase in root length, shoot length, fresh weight, dry weight and leaf area and significant decreases in electrolyte leakage. The magnitude of response is most pronounced in case of soil incorporation treatment. Transmission electron microscopy of leave sections of xylem and phloem of *Phaseolus* plants showed the presence of cyanobacteria in both xylem vessels and phloem sieve tubes inducing the application and treatment of cyanobacteria either from root to leaves (soil incorporation treatment) or from leaves to roots (foliar spray treatment). The best value was achieved by cyanobacteria inocula at concentration of 20% treatment either by foliar application or by soil incorporation to stimulate the growth of kidney bean plants.

Key words: Biofertilizer, cyanobacteria, growth and kidney bean.

Introduction

The impact of fertilizers is an inevitable and basic requirement of modern intensive vegetable farming because of increase demand of nutrients of the high yield varieties. Of the fertilizer, nitrogen being an essential element deserves the vital position. Physiologically it plays the key role and has been considered as a yield limiting factor .However, increased cost of the fertilizer is being an economical constrains. Moreover, the continuous use of chemical fertilizers causes the ecological and biochemical imbalance in the vegetable yield**) Roger and Kulasooriya ;1980 ,Begum and Islam, 2011 .(**

As a consequence, to overcome these dual problems, the impacts of biofertilizers are recently being gaining momentum and are successfully practical in in legumes in many countries.

The Significant contribution of blue green algae as an alternative source of nitrogen particularly in the legume and rice yields. The algalization technology has been reported to be successful to a great extent in many countries **(Kaushik, 2000)**. Therefore an attempt has been made to follow the impact of cyanobacteria inoculum on growth of *phaseolus vulgaris* plants grown in clay: sand soil (2:2 v/ v).

Materials and methods

- **Plants materials and growth conditions**

Phaseolus vulgaris (cv. baladi) seeds were surface sterilized by soaking in a 10^{-3} M HgCl solution for 3 min, then washed with sterile water and divided into two sections. Each section contains four groups and the seeds of each group were divided into a number of sets, with each set having 20 seeds. These sets are allowed to germinate in (30 X 28 X 26 cm) pots. The soil obtained from the Agriculture Research station of Mansoura, Dakahlia Governorate, was taken from the upper 30

cm arable layer. All pots contained equal amounts of soil (8 Kg). 12 pots were subdivided into 4 groups each of 3 pots, one group left to serve as control and the other 3 groups treated with cyanobacteria biofertilizer inoculum at 3 levels (10 %, 20 % and 30 %) incorporation with soil and planted the other 4 groups each of 3 pots, one of them left as control and the other 3 groups was planted with kidney bean seeds. Treatment of such seeds spray with different concentrations of cyanobacteria biofertilizers was carried out after 3 weeks from date of sowing. The biofertilizer was added to the plant by foliar spray to each pot.

All pots were irrigated with tap water every 3 days to maintain the field capacity throughout the experiment. Samples from each treatment were taken after 30 days from date of sowing respectively fully vegetative stage.

Sampling was made in a way so as to include all plants allotted for each treatment in the 3 pots. Samples were used for determination of growth parameters, electrolyte leakage as well as investigation of cyanobacteria colonies inside plant leaf tissues using Transmission electron microscopy (TEM).

- **Determination of electrolyte leakage**

Plant leaves were cut into discs (10 mm), 20 leaf discs were placed in a test tube, rinsed 3 times with 20 ml distilled water. Tubes were filled with 30 ml distilled water and stand in dark for 24 hours at room temperature. Electrical conductivity of the solution was measured at the end of incubation period using electrical conductivity (EC) meter. The tubes were then heated in water bath at 95^0C for 20 minutes and then cooled to room temperature. The final EC was measured as following: EC= (EC1⁄EC2) X100 **(Shi *et al.*, 2006)**.

- **Ultrastructural studies using transmission electron microscope (TEM)**

To verify the presence of the observed cyanobacterial inoculum biofertilizer inside kidney bean plants, TEM analysis of the plant leaves after applying cyanobacterial inoculum. Small parts (about 1 mm^3) of freshly harvested leaves were cut with a sharp razor blade under 2.5% (v⁄v) glutaraldehyde. Leaf tissues were transferred to vials of 2.5 % (v⁄v) glutaraldehyde in 1M phosphate buffer at pH 7.5 at 4^0C for 24 h. Following fixation, the specimens were embedded in gelatin capsules and left in an oven at 60 ^{0}C for 60 h. The gelatin capsules were dissolved in boiling water for 1-2 h.

Ultra-thin sections were cut on a Reichert ultra-microtome using glass knife. Silver or pale gold interference sections were picked up on the dull surface of form-coated 100 or 200 mesh copper grids (Juniper et al., 1970). The grids with sections were left on a clean filter paper to dry. Ultra-thin sections were stained by 2 % aqueous uranyl acetate **(Juniper *et al.*, 1970)**. A drop of stain was put in a clean plastic petri dish and the grids were gently floated, with the sections facing down, on a drop of the stain. The grids were washed by a stream of distilled water and then transferred to drops of lead citrate **(Reynolds, 1963)** which were placed on a wax plate in a petri dish. Pellets of sodium hydroxide were placed in the petri dish to remove carbon dioxide. The grids were left in lead citrate for 10 -20 min and then rinsed by distilled water, dried under a bench lamp and stored in a grid box. The stained sections were examined and photographed with a JEOL 1010 transmission electron microscope at 80 kv.

The data obtained from triplicate samples were remarkably close, thus only the mean value will be presented. Experimental data were subjected to one-way analysis of variance (ANOVA) with Post Hoc L.S.D. (least significant difference) test. * P value < 0.05 was accepted statistically significant. Statistical analysis was performed with statistical package for social science for windows (SPSS, version 13.0, 2004, Chicago, IL, USA)

Results and discussion

- **Changes in growth parameters**

The values of growth parameters (root length, shoot length, fresh weight, dry weight and leaf area) appeared to increase significantly with increase in cyanobacteria biofertilizer levels (Table 1). The magnitude of increase appeared to be progressive with the optimum levels of cyanobacteria biofertilizers (20 %).

Table 1: The effect of foliar application and soil incorporation of cyanobacterial inoculums biofertilizers on growth of kidney bean plants. Mean values are significantly different from control at $p \leq 0.05$.

Foliar spray										
Parameters Treatments	**Root Length (cm/ plant)**	**Change %**	**Shoot Length (cm/ plant)**	**Change %**	**Fresh matter (g/plant)**	**Change %**	**Dry matter (g/plant)**	**Change %**	**Leaf Area (cm/ plant)**	**Change %**
Control	**12.3**	**-**	**21.7**	**-**	**9.4**	**0.0**	**1.2**	**-**	**20.7**	**-**
Cyanobacteria (10%)	**13.4***	**10.89**	**26.1***	**20.27**	**10.0**	**6.38**	**1.3***	**8.33**	**22.6***	**9.71**
Cyanobacteria (20%)	**13.8***	**12.19**	**27.7***	**27.64**	**11.9**	**26.59**	**1.5***	**25.00**	**23.9***	**15.45**
Cyanobacteria (30%)	**13.6***	**10.56**	**26.9***	**23.96**	**10.11**	**7.50**	**1.25***	**4.00**	**22.0***	**6.28**
L.S.D at 5% level	**.61**	**-**	**.96**	**-**	**0.41**	**-**	**0.53**	**-**	**.44**	**-**
Soil incorporation										
Parameters Treatments	**Root Length (cm/ plant)**	**Change %**	**Shoot Length (cm/ plant)**	**Change %**	**Fresh matter (g/plant)**	**Change %**	**Dry matter (g/plant)**	**Change %**	**Leaf Area)cm/ plant (**	**Change %**
Control	**12.0**	**0.0**	**22.1**	**0.0**	**9.1**	**0.0**	**1.2**	**0.0**	**20.6**	**0.0**
Cyanobacteria	**13.3***	**10.83**	**24.8***	**12.21***	**11.8***	**29.67**	**1.6**	**33.33**	**25.3***	**22.81**

(10%)										
Cyanobacteria (20%)	**14.0***	**16.66**	**25.9***	**17.19***	**12.0***	**31.86**	**1.7**	**41.66**	**26.1***	**26.69**
Cyanobacteria (30%)	**12.8***	**6.67**	**23.7***	**7.23***	**11.0***	**20.87**	**1.5**	**25.00**	**22.6***	**9.70**
L.S.D at 5% level	**.60**	**-**	**.97**	**-**	**.42***	**-**	**0.52**	**-**	**0.93**	**-**

The percent increase in fresh weight in foliar spray treatments was as follows: 11.9% for 20% biofertilizer, 10.0% for 10% biofertilizer and 10.11% for 30% biofertilizer for plants treated with biofertilzer by soil incorporation. On the other hand, The percent increase was 26.59% for 20% biofertilizer, 6.38% for 10% biofertilizer and 7.50% for 30% biofertilizer from control plants treated with cyanobacteria by foliar spray.

In support of the present results, addition of biofertilizers resulted in significant increase in vegetative growth, plant dry weight and area of freshly expanded of a variety of crop plants **(Srivastava *et al*., 1998; Hasaneen *et al*., 2009)**. The promoting effect of cyanobacteria biofertilizer on growth of the variously treated kidney bean plants may be due to the active bacteria in biofertilizer which as stated by **Sherif *et al* (1997**), allow to convert insoluble phosphate to soluble forms, fix atmospheric nitrogen and secreting organic acids such as formic acids and lactic acids. Such acids lower the pH and bring about the dissolution of bounced forms of phosphate and render them available for growing plants **(Hasaneen *et al*., 2009)**.

The response of plants to bio-N- fertilizers was studied by **Fisinin *et al*. (1999), Rizk and shafeek (2000) and Adam (2002)** who reported that bio-N-fertilizers has a great number of bacteria which are responsible for nitrogen fixation by atmosphere. The stimulatory effects of cyanobacteria biofertilizer are attributed to the activation of the growth of microflora including production of many plant growth stimulators **(Hasaneen *et al*., 2009).**

The results suggest that application of cyanobacteria was effective in enhancing the growth attributes of kidney bean plants. This indicates the better efficiency of cyanobacteria in promoting the growth of kidney bean plants. Similar information in increasing the dry weight of kidney bean plants due to cyanobacteria has also been achieved by the other investigators **(Aiyer *et al*., 1972; Begum and Islam 2011; Hasaneen *et al*., 2012).**

- **Uptake and translocation**

a- Changes in electrolyte leakage

The observed changes in the electrolyte leakage from variously treated kidney bean plants throughout the entire period of the experiment are presented in table 2.

Electrolyte leakage of soil incorporation treatment plants appeared the decrease progressively from control values. The following sequence treatment 30% > 10% > 20% > control was displayed with respect to decrease in electrolyte leakage

(table 2). On the other hand, foliar application of cyanobacteria biofertilizers to kidney bean plants induce significant decrease in electrolyte leakage with the following order control> 10% biofertilizer > 30% biofertilizer > 20% biofertilizer.

Cyanobacteria biofertilizer appeared to reduce the amount of malonyldialdehyde (MDA) and ion leakage in treated kidney bean plants, as reported by **Oancea *et al*. (2009)** who hypothesized that controlled release of active plant growth stimulators. On the other hand, cyanobacteria biofertilizers appeared to increase the amount of MDA and ion leakage in kidney bean plants treated with cyanobacteria biofertilizer as soil incorporation as represented by **Adam (2002)** who reported that bio-N-fertilizer has a great number of bacteria which are responsible for nitrogen fixation by atmosphere. The stimulatory effects of cyanobacteria biofertilizer in the present study might be contributed to the activation of the growth of microflora including production of many plant growth stimulators **(Hasaneen *et al*., 2009)**.

Table 2: The effect of foliar application and soil incorporation of cyanobacterial inoculum biofertilizers on electrolyte leakage of kidney bean plants. Mean values are significantly different from control at $P \leq 0.05$

Soil incorporation		Foliar spray		Parameters
Change %	Electrolyte leakage	Change %	Electrolyte leakage	Treatments
-	1.15	-	1.16	Control
4.34	1.20*	-4.31	1.11*	Cyanobacteria (10%)
1.73	1.17*	-6.03	1.09*	Cyanobacteria (20%)
9.56	1.26*	-5.17	1.10*	Cyanobacteria (30%)
-	0.04	-	0.04	L.S.D at 5% level

b- Ultrastructure changes

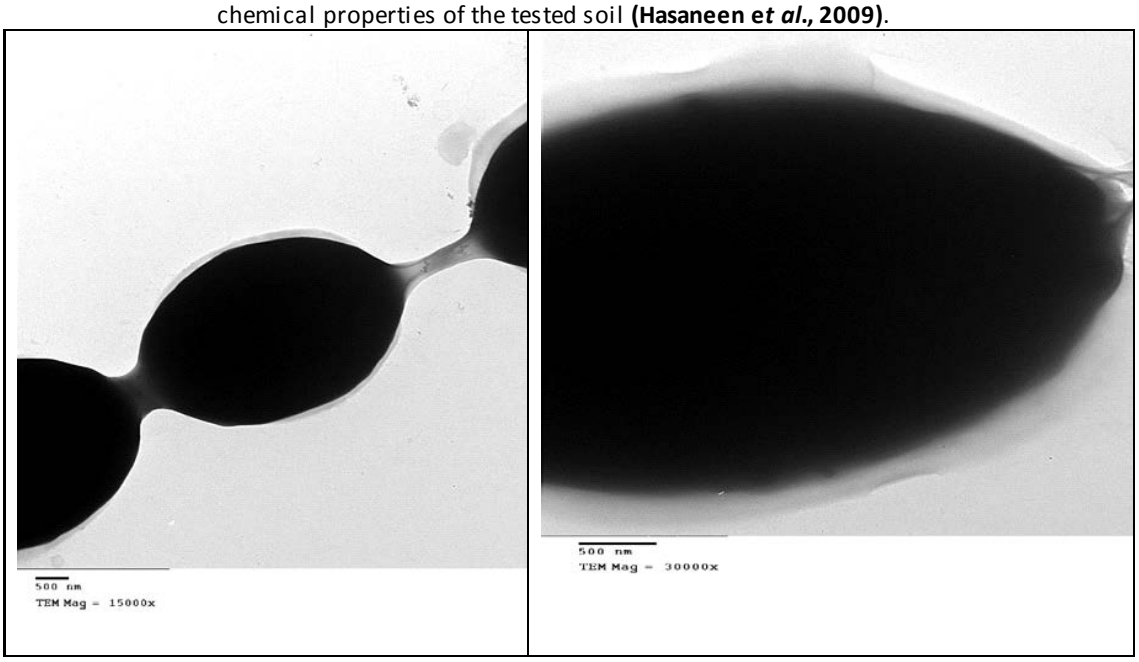
Examination of figures 1 and 2 revealed the following:

- In all cyanobacteria biofertilizers, treated kidney bean plants either by soil incorporation or foliar spray cyanobacteria were observed inside xylem vessels and in sieve tubes after entering the stomata, theses cyanobacteria were translocated by the xylem system and in consequence translocated into phloem system.
- The phloem consists of living vascular tissues that translocate photosynthetic products including sucrose and some mineral ions for plant growth.
- The observed results indicate that xylem and phloem tissues are the main and unique pathway for translocation of cyanobacteria biofertilizer and in consequence, confirm the penetration of either plant roots and leaves and lead to a strong support to the observed changes in growth of kidney bean plants affected by cyanobacteria biofertilizers.

Of interest, these results might indicate that cyanobacteria biofertilizers mitigated the increase in the plasma membrane permeability and cell mortality under cyanobacteria effects in kidney bean plants **(Hegazi *et al*., 2010)**.

It was observed that growth parameters in the present study were significantly increased compared with controls. These increases could be attributed to the nitrogenase as well as nitrate reductase activities of the alga penetration into kidney bean plants or the amino acids and peptides produced in the algal filtrate and/or other compounds that stimulate growth of crop plants **(Hegazi *et al*., 2010)**.

Moreover, **Jagannath *et al*. (2002)** studied the effect of blue green algae as potent biofertilizer on chickpea and they found that it enhanced all the morphological characters and biomass of the chickpea. Also **Nanjappan *et al*. (2007)** stated that cyanobacteria have growth promoting activity as inoculants of wheat. These stimulation effects of cyanobacteria on plant growth may be attributed to their influence on increasing the biological activity **(Hegazi *et al*., 2010)** and chemical properties of the tested soil **(Hasaneen e*t al*., 2009)**.



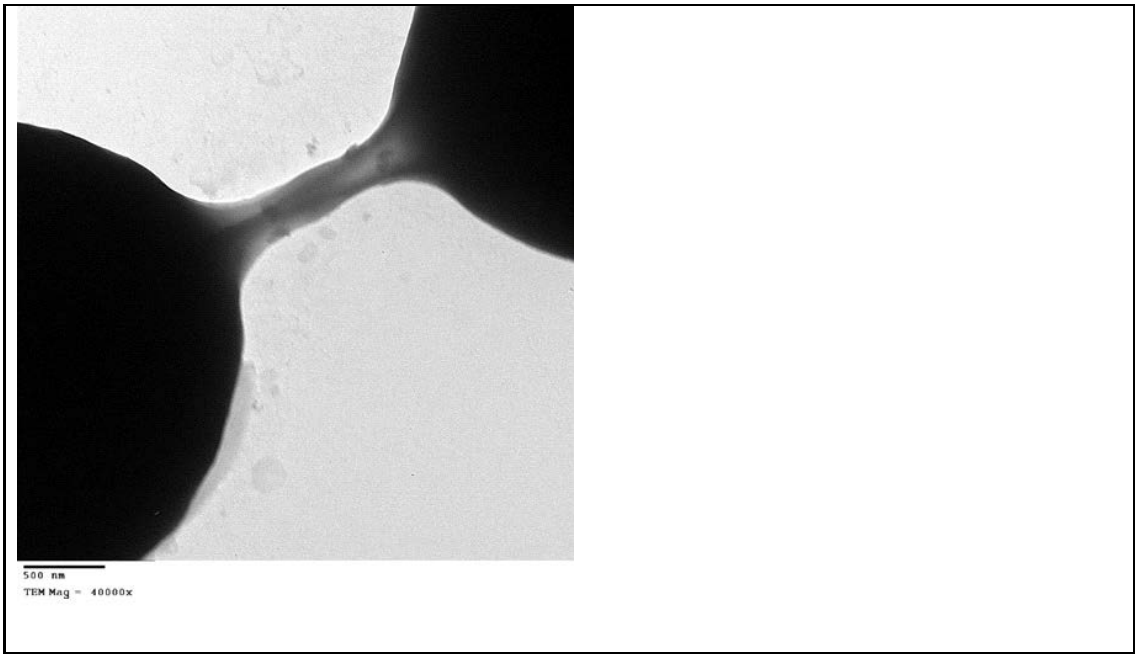
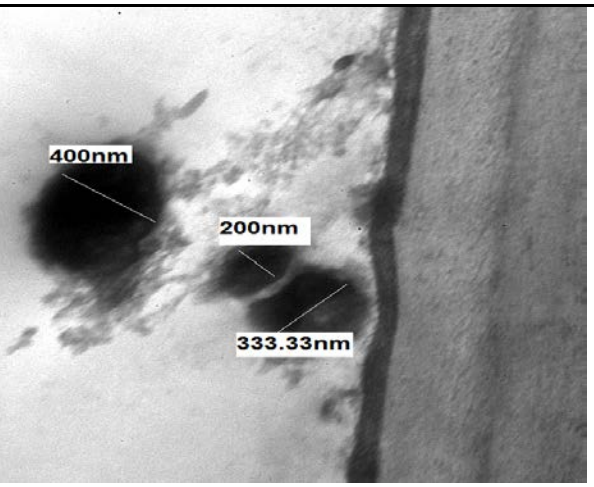


Figure 1: Transmission electron micrograph showing cyanobacterial inoculum culture medium.



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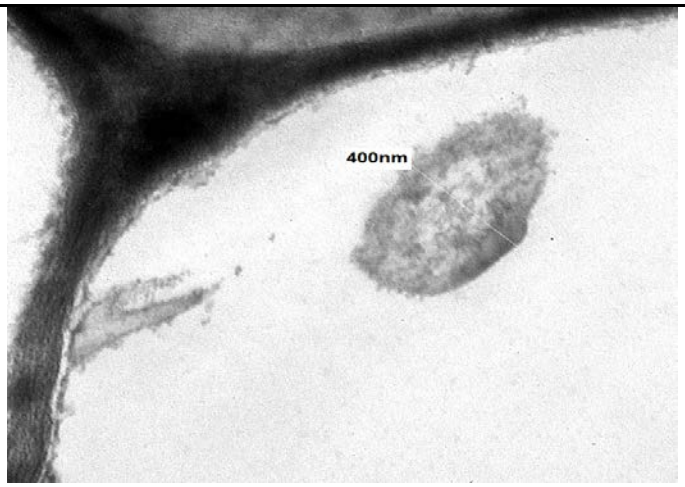

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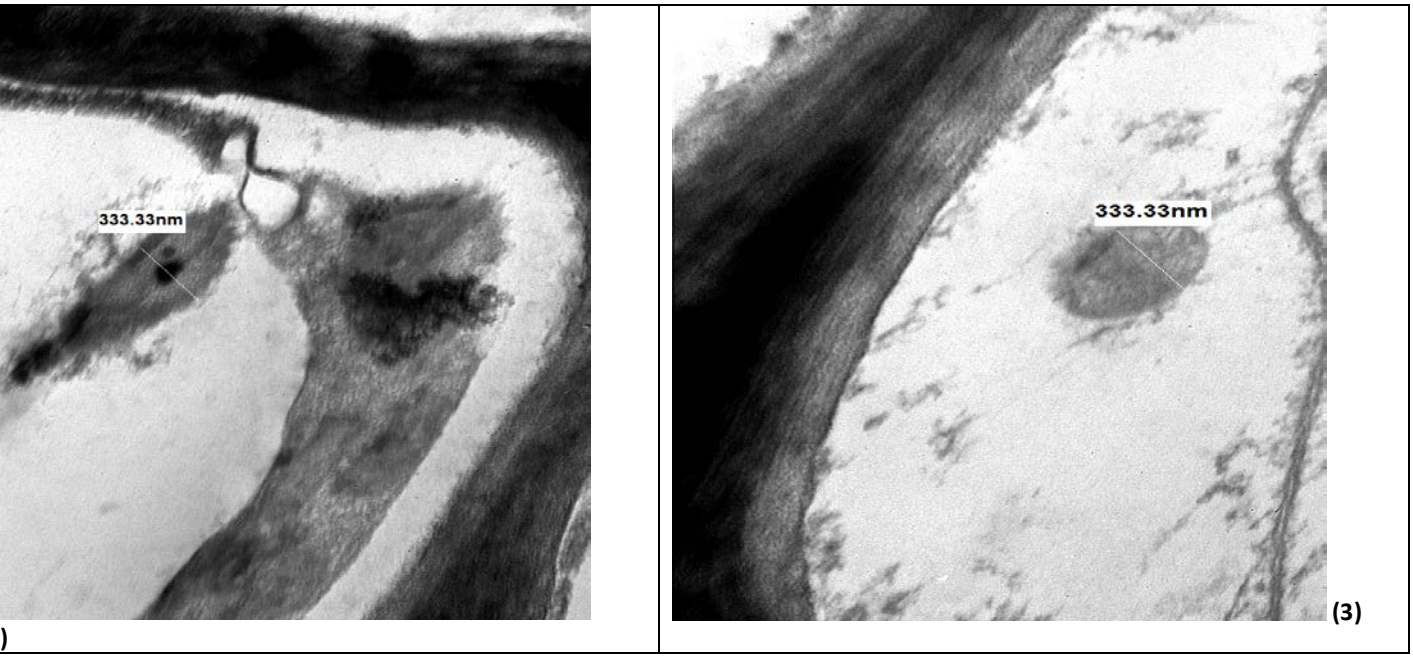
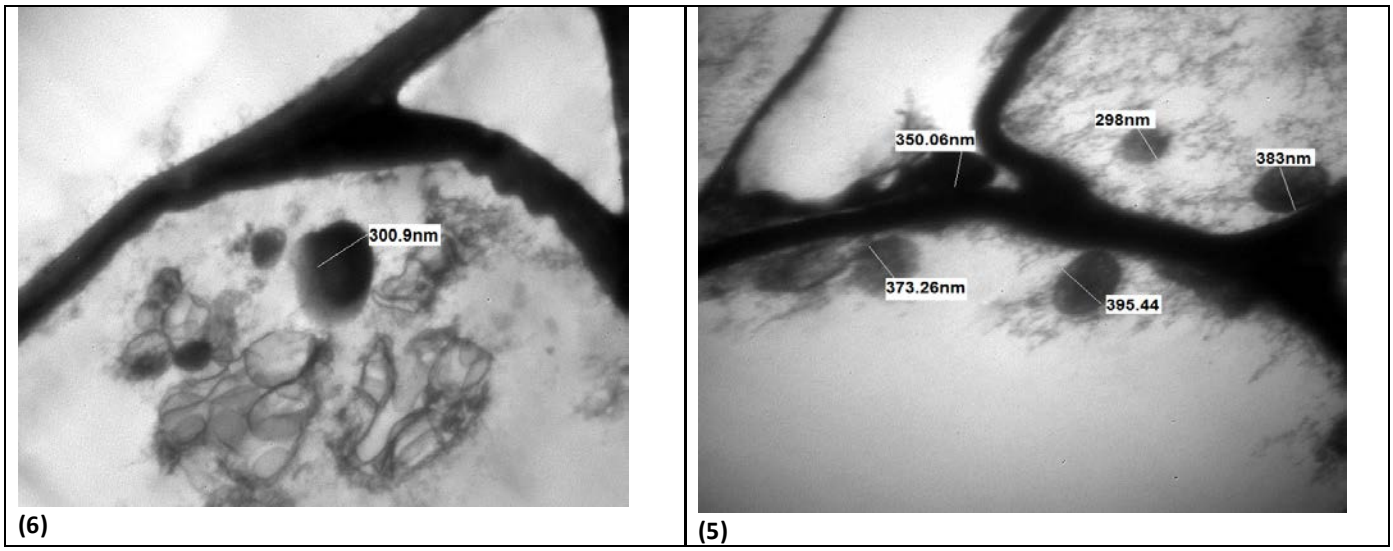


Figure 2: Transmission electron micrograph showing cyanobacterial inoculum inside phloem sieve tubes (1), (2) and xylem vessels (3), (4) of kidney bean plants treated with soil incorporation of cyanobacteria inoculum.



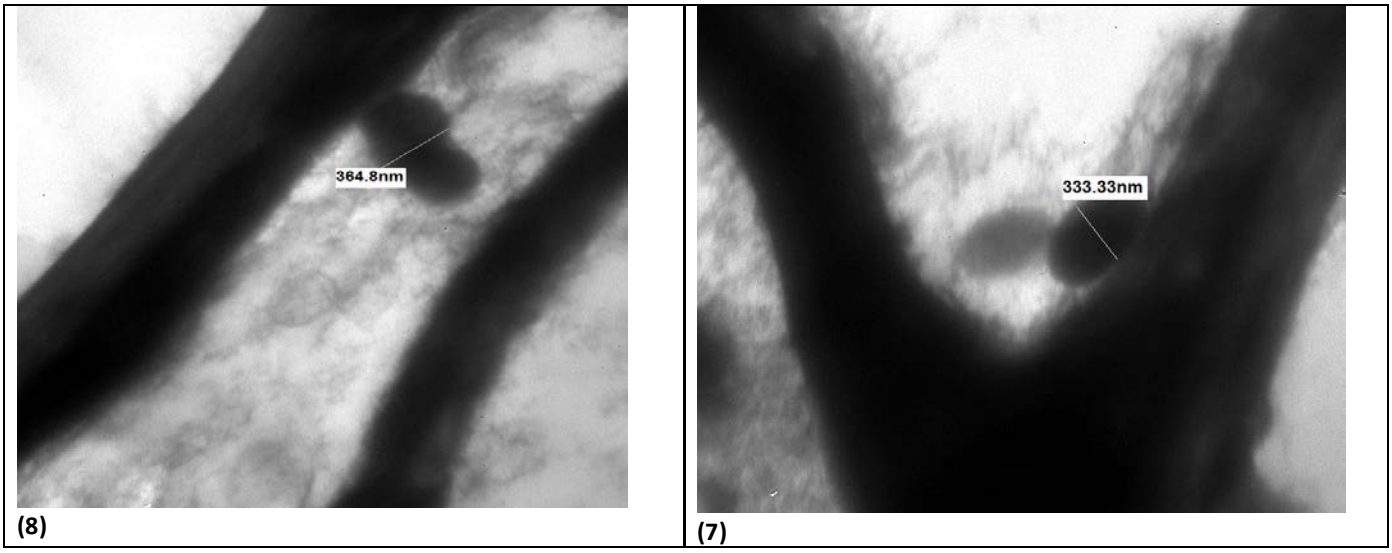


Figure 3: Transmission electron micrograph showing cyanobacterial inoculum inside phloem sieve tubes (5), (6) and xylem vessels (7), (8) of kidney bean plants treated with foliar application of cyanobacteria inoculum.

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Can cyanobacteria biofertilizer enhance the growth of *phaseolus Vulgaris* plants?

هل في إمكانية إستخدام طحلب السيانوبكتيريا كمخصب حيوي من زياده نمو نبات الفاصوليا ؟

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-يهدف هذا البحث إلي دراسة تأثير إستخدام الطحالب الخضراء المزرقة من النوع سيانوبكتيريا كمخصب حيوي علي نمو نبات الفاصوليا المزروع في أصص تحتوي علي تربه طينية رملية بنسبة حجمية 1:2 و لمدة 30 يوم.

- و لقد أظهرت النتائج أن المعاملة بالسيانوبكتيريا كمخصب حيوي بتركيزات 10 % و 20% و 30% سواء بالرش علي المجموع الخضري أو كإضافات أرضية أدت إلي زيادة ملحوظة في دلالات النمو و المتمثلة في (طول الجذر,طول الساق, الوزن الطازج, الوزن الجاف , مساحة الورقة) و معدل تسريب الإلكتروليتات ; و قد كان مقدار الإستجابة أكثر تأثيرا في المعاملات الأرضية بالمقارنة بالرش علي المجموع الخضري.

- و قد أظهر الفحص بإستخدام الميكروسكوب الإلكتروني لمقاطع من أوعية الخشب و اللحاء في الورقة إلي تواجد السيانوبكتيريا في كل من الخشب و الصفيحة الغربالية للحاء مما يدل علي نجاح إنتقال السيانو بكتيريا

داخل النبات سواء من خلال أوعية الخشب (من الجذر إلي الأوراق) في المعاملات الأرضية أو من خلال الصفائح الغربالية للحاء (من الأوراق إلي الجذر) في معاملات الرش.

- من خلال النتائج المتحصل عليها إستوضح لنا أن إستخدام السيانوبكتيريا كمخصب حيوي بتركيز 20 % و المعاملة به سواء بالرش علي المجموع الخضري أو الإضافة الأرضية قد أعطي نتائج ملموسة و ملحوظة بالنسبه لنمو نبات الفاصوليا.