J. Agric. Sci. Mansoura Univ., 31 (8): 5385 - 5391, 2006

PRODUCTIVITY, CHEMICAL COMPOSITION AND ABUNDANCE OF CYANOBACTERIA IN CYANOBACTERIAL SOIL CARRIER BASED INOCULUM

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ABSTRACT

A greenhouse experiment was conducted to evaluate the productivity, chemical composition and the abundance of cyanobacteria in the soil based cyanobacteria inoculum (SBI) commonly used as biofertilizer in rice cultivation Four local cyanobacteria strains namely, *Anabaena oryzae, Nostoc calcicola, Microchaete tenera* and *Cylindrospermum muscicola* were used each separately in addition to the control treatment (soil only without cyanobacteria inoculation) in the production of SBI inoculum. Results showed a large variability of biomass production of SBI depending on the incubation period. The indigenous cyanobacteria (control) gave its highest SBI biomass of 71.04 g m^{-2} after one week then tended to decrease with increasing incubation time up to 4 weeks. Nitrogen percentages of the produced SBI ranged from 0.56 (control) to 2.00 (*C. muscicola).* Phosphorus Percentages ranged from 0.16 (*A.oryzae)* to 0.30 (, *M.tenera* or *C.muscicola*). Carbon percentage ranged from 1.33 (control) to 7.87 (*N.calcicola).* The highest C/N ratio (8.86) noticed with *A. oryzae* after 4 weeks incubation period, while the lowest one (2.25) was for *C.muscicola* after 3 weeks incubation. The highest mean N/P ratio of 9 72 was for *C.muscicola* against the lowest one (4.18) for *A.oryzae.* The cyanobacteria count decreased with increasing the incubation up to 4 weeks.

INTRODUCTION

Cyanobacteria constitute the largest, most diverse, and most widely distributed group of prokaryotes that performs oxygen photosynthesis. Several genera can fix atmospheric nitrogen (N_2), and thus contribute to maintaining the fertility of natural and cultivated ecosystems, especially wet land rice fields (Roger and Kulasooriya 1980).

Cyanobacteria are the only N_2-fixing microorganisms that generate their own photosynthate from CO_2 and water. This trophic independence has led many researchers to investigate their agronomic potential. Since De (1939) attributed the natural fertility of rice fields to cyanobacteria, hundreds of papers have been published on their use as bio fertilizer (Roger and Kulasooriya 1980 and Roger 1991).

Application of cyanobacteria inoculation into rice fields exerts a long lasting effect while mineral nitrogen like urea should be added more frequently. They are a promising alternative to avoid soil pollution caused by the use of agrochemicals, as well as recover the nutrient and structure lost after harvest (Mule *et al.*, 1999).

For a large scale application of cyanobacteria inoculation in rice cultivation, the production of the cyanobacterial inoculum has been adopted long- ago in India, China, Viet Nam, Japan, Philippines (De, 1939; Watanabe,

1966 and Venkataraman, 1972). In Egypt, Ghazal (1988) studied the ecology of the soil based cyanobacteria inocula production to be applied in rice cultivation. He concluded that the two main consideration in the utilization of these cyanobacteria inoculum in agriculture are (i) growing the cyanobacteria in abundance (mass culture), and (ii) preserving the produced cyanobacteria inoculum for seeding purposes. However, despite the abundance of literature on the role of N_2- fixing cyanobacteria inoculum and their possible use as a source of nitrogen for rice (Roger and Kulasooriya 1980), little is known about their chemical composition and the abundance of the cyanobacteria strains used in the production of these cyanobacteria inoculum. Roger *et al.*, (1987) find out that the relative abundance of N_2 fixing cyanobacteria in soil based inoculum (SBI) ranged from 2 to 32 % and averaged 13-18 % with density number of the $2.5 * 10^{6}$ CFU g dry weight SBI^{-1}. He also added that their chemical analyses ranged in average as 78-89 % ash, 2.1 %-4.7 % C, 0.2-0.8 % N, 640 – 1900 ppm P, N (0.5%), C (3.4%), and ash (80%) indicating that SBI inoculum contain high percentage of soil. Applying the recommended dose of 10 kg SBI ha-1 (Venkataraman, 1981) introduces $2.5 * 10^{11}$ CFU ha^{-1} or $2.5 * 10^{3}$ CFU cm^{-2}. This is 1/ 130 Th the average density of indigenous N_2 fixing cyanobacteria ($3.2 * 10^{5}$ CFU cm^{-2}) in the sols examined.

This paper is an attempt to evaluate (1) the cyanobacterial biomass of the produced soil based cyanobacteria inoculum (SBI), (2) its chemical composition, (3) the abundance of the cyanobacteria strains used in producing the (SBI) inoculum and (4) the suitable time for harvesting the produced cyanobacteria inoculum.

MATERIALS AND METHODS

Slant agar refrigerated (5^{0}C) cyanobacteria cultures were exposed to light (500 Lux) for 2 days then inoculated to liquid BG 11 (Rippika *et al.*, 1979) medium to reach the log phase growth of each alga strain. All cyanobacteria strains were occasionally purified using yeast extract agar medium. The development algal growth was homogenized and then introduced as inoculum of (1ml) to 500 ml conical flasks containing 100 ml sterilized BG11 medium (pH 7.2) and incubated on continuous rotary shaking incubator (100 rpm) equipped with continuous illumination (3000 Lux) at temperature of 28 – 32^{0} C . The developed cyanobacteria were then ready to use as inoculum starter for soil based cyanobacteria inoculum (SBI) production.

Shallow galvanized trays (65 cm x 100 cm x 20cm) containing 8-10 kg of slightly alkaline clay loamy soil with pH 8.1, EC 2 dSm^{-1}, organic matter 1.3 % (Allison 1965), total nitrogen 1.2 % (Jackson 1973) and available phosphorus 12.6 ppm Olsen (1972) were covered up to 10 cm height with tap water and supplied with 10g superphosphate (15 % P_2O_5), 12.5 ml sodium molybedate (in 1% solution, w/v) and 1.0 g carbofuran (ai 3 % granules) under the greenhouse condition to study the production of the soil based cyanobacteria inoculum (SBI) commonly used as biofertilizer in rice fields. The trays were inoculated with 50 ml inoculum starter from each of *Anabaena*

oryzae, *Nostoc calcicola*, *Microchaete tenera* and *Cylindrospermum muscicola* each separately along with control without inoculation but supplied with all chemical additives. The treatments were in six replicates in complete randomized design. At the end of each incubation periods of 1, 2, 3 and 4 weeks, the parameters of cyanobacterial biomass, total – N, phosphorus, total carbon, C/N, N/P ratio and cyanobacteria colonies formed per unit CFU g^{-1} cyanobacteria soil based inoculum were determined using the Plating technique of cyanobacteria enumerating (Allen and Stanier, 1968).

RESULTS AND DISCUSSION

In this work different strains of cyanobacteria were used in the production of the soil based inoculum to be utilized as nitrogen biofertilizer source in rice production commonly and recently in wheat (Abd EL-Rasoul et al., 2004 and Aref and AL-Kassas 2006). The phenomenon is currentely tried to produce cyanobacteria inocula using other carrier materials rather than soil. Such materials were neem powder, bel leaves, tobacco waste powder and paddy straw powder (Jaha and Prasad, 2003)

However, results showed a large variability of biomass production of SBI depending on the incubation period length. The indigenous cyanobacteria (control) gave its highest SBI biomass of 71.04 g m^{-2} after one week then tended to decrease with increasing incubation time up to 4 weeks. Same trend was achieved for the inoculated cyanobacteria strains treatments that gave their corresponding highest biomass of 131.75, 100.11, 87.38 and 59.07 g SBI m^{-2} after one week incubation period. Increasing time of incubation more than one week decreased significantly the biomass yield of SBI. The lowest SBI biomass of 37.39 g SBI m^{-2} was recorded by *C. muscicola*.

Nitrogen percentages of the produced SBI ranged from 0.56 to 2.00. The highest percentage ratio of nitrogen (2.00) had achieved by *C. muscicola* at third week of incubation. While, the least percentage nitrogen of 0.56 was for the control treatment after one week of incubation. However, the nitrogen percentage had averages of 0.81, 0.81, 1.25, 1.05 and 1.55 for control, *A.oryzae*, *N.calcicola*, *M.tenera* and *C.muscicola*, respectively.

In concern to phosphorus accumulation in the produced SBI inoculum, data in Table (1) and coincided that the incubation time elevation up to 4 weeks had decreased phosphorus percentages for both indigenous algae (control) and the inoculated ones. However, the respective mean threshold phosphorus percentages in harvested SBI inoculum were 0.16, 0.27, 0.30, 0.30 and 0.22 for control, *A.oryzae*, *N.calcicola*, *M.tenera* and *C.muscicola*, respectively. The highest phosphorus percentage (0.30) recorded by both *N. calcicola* and *M.tenera* against the lowest one (0.16) for the indigenous one (control).

Table (1): Biomass and chemical composition of produced cyanobacterial soil based inoculum

Treatment algae strains	control	*A. oryzae*	*N. calcicola*	*M. tenera*	*C. muscicola*
Incubation time week			Mass culture g / m^2		
1	71.04	131.75	100.11	87.38	59.07
2	67.08	74.47	57.77	53.98	57.61
3	56.12	60.04	44.61	45.67	54.02
4	45.20	43.24	40.00	39.32	37.39
			Nitrogen percentages		
1	0.56	0.57	0.79	0.71	0.78
2	0.90	0.93	1.26	0.93	1.83
3	0.86	0.89	1.35	1.38	2.33
4	0.90	0.85	1.58	1.19	1.60
			Phosphorus percentages		
1	0.20	0.40	0.52	0.42	0.26
2	0.19	0.40	0.37	0.31	0.20
3	0.15	0.17	0.16	0.26	0.23
4	0.10	0.11	0.15	0.12	0.17
			Carbon percentages		
1	1.33	2.77	3.27	3.48	4.17
2	4.97	4.83	5.03	5.37	7.57
3	4.26	5.05	7.87	6.50	5.24
4	4.10	7.53	6.29	7.98	5.00
			C / N ratio		
1	2.38	4.86	4.14	4.90	5.35
2	5.52	5.19	3.99	5.77	4.14
3	4.95	5.67	5.83	4.71	2.25
4	4.56	8.86	3.98	6.71	3.13
			N / P ratio		
1	2.80	1.43	1.52	1.69	3.00
2	4.74	2.33	3.41	3.00	9.15
3	5.73	5.24	8.44	5.31	10.13
4	9.00	7.73	10.53	7.44	10.00

L. S. D. values for the a forementioned parameters:

	Biomass		N	
	0.05	0.01	0.05	0.01
A	2.46	3.30	0.27	0.36
B	2.75	3.69	0.30	0.41
AB	1.23	1.65	0.14	0.18
	P		C	
	0.05	0.01	0.05	0.01
A	0.09	0.12	1.23	1.65
B	0.10	0.13	1.38	1.85
AB	0.04	0.06	0.62	0.83

A, Strain; B, period;

Carbon percentages ranged from 1.33 (control) to 7.87 (*N.calcicola*). The mean values of carbon percentage were found to be in ascending of 3.67 (control), 5.05 (*A. oryzae*), 5.50 (*C. muscicola*), 5.62 (*N. calcicola*) and 5.83 (*M. tenera*). Carbon / nitrogen ratio ranged from 2.25 to 8.86. The highest C/N ratio (8.86) noticed with *A. oryzae* after 4 weeks incubation period, while the lowest one (2.25) was for *C.muscicola* after 3 weeks incubation. Nevertheless, the C/N ratios could be arranged according to their mean values as 3.72 (*C.muscicola*), 5.90 (*A.oryzae*). It is also obvious that C/N ratio increased gradually with increasing incubation time up to the third week in parallel with produced algal biomass.

In contrast to the decrease of phospherus with increasing time of production, it was detected that N/P ratio increased with rising time of SBI inoculum production with average ratios of 5.57, 4.18, 5.98, 5.27 and 9.72 for control, *A.oryzae, N. calcicola, M. tenera* and *C. muscicola,* respectively. However, the highest mean N/P ratio of 9.72 was for *C.muscicola* against the lowest one (4.18) for *A.oryzae*. The nitrogen phosphorus ratio seemed to be in reverse relation with the produced algal biomass either for the control treatment or the other tested strains.

Table (2): Cyanobacteria count in the produced Cyanobacteria soil based inoculum (cfu g SBI^{-1})

Cyanobacteria strains	Incubation period (weeks)			
	1	2	3	4
Control	60730	62400	75060	22530
Anabaena oryzae	50100	24800	11100	9730
Nostoc cacicola	44060	41300	18300	6460
Microchaete tenera	32000	25400	18160	5630
Clyndrospermum muscicola	5400	4660	4260	3200

Total cyanobacteria count was evaluated in the produced SBI at intervals of 1, 2, 3 and 4 weeks (Table 2) by plating dilution technique (dry weight basis) on agarized BG 11 medium (Allen and Stanier 1968). The total cyanobacteria count in control treatment had increased with increasing the incubation period up to 3 weeks, then started to decrease at the fourth weeks. The corresponding total population counts were 60730, 62400, 75060 and 22530 CFU g SBI^{-1} in respective to 1, 2, 3 and 4 weeks. In the contrary with the control treatment, the population count for other cyanobacteria had been decreased with rising the incubation period up to 4 weeks. The corresponding total count in respective to incubation period of 1, 2, 3 and 4 weeks were 50100, 24800, 11100 and 9730 CFU g SBI^{-1} (*A. oryzae*), 44060, 41300, 18300 and 6460 CFU g SBI^{-1} (*N. calcicola*), 32000, 25400, 18160 and 5630 CFU g SBI^{-1} (*M. tenera*) and 5400, 4660, 4260 and 3200 CFU g SBI^{-1} (*C. muscicola*). Among the tested cyanobacteria strains, *A. oryzae* recorded the highest occurred population of 50100 CFU g SBI^{-1} at the first incubation week followed with respective population of 44060, 32000 and 5400 CFU g SBI^{-1} corresponding to *N.calcicola, M. tenera* and *C.muscicola.* The lowest population occurrence had achieved by *C.muscicola* (3200 CFU g SBI^{-1}) at the fourth incubation week.

However, it is obvious that to prepare the SBI biofertilizer, it is better to harvest the cyanobacteria mat produced after one week of cyanobacteria cultivation. This trend was to avoid the competition of the indigenous algae that inhabitant naturally the soil and which overcome the survival of the inculcated cyanobacteria with prolonged incubation time more than one week (Table 2).

The rice field ecosystem provides a favorable environment for the cyanobacteria with respect to their requirements for light, water, high temperature and nutrient availability. This may account for higher abundance of cyanobacteria in paddy soils than in other cultivated soils (Watanabe and Yamamoto, 1971). Therefore it was useful to save such conditions under the Egyptian condition to may produce high quality cyanobacterial inoculum to accelerate nitrogen saving for rice production.

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الانتاجية والتركيب الكيميائى وتواجد خلايا السيانو بكتريا فى لقاح السيانوبكتريا المنتج باستخدام التربة كمادة حاملة

السيدة على حسن

قسم بحوث الميكروبيولوجيا الزراعية ــ معهد بحوث الأراضى والمياة والبيئة- مركز البحوث الزراعية – الجيزة- مصر

فى هذه الدراسة أجريت تجربة في الصوبة باستخدام أربعة سلالات من السيانوبكتريا كل على حدة وكانت هذه السلالات هي:

Anabaena oryzae, Nostoc calcicola, Microshaete tenera, and Cylindrospermum muscicola

وذلك لتقييم لقاح السيانوبكتريا المنتج باستخدام التربة وذلك من حيث الكتلة المنتجة والتركيب الكيميائي له وكذلك أعداد خلايا السيانوبكتريا لكل جرام جاف منتج من هذا اللقاح.

ولقد أوضحت النتائج ما يلى:

١- لقد كان هناك اختلاف فى وزن الكتلة الحية من اللقاح الناتج وذلك باختلاف فترة التحضين.

٢- أعطت معاملة المقارنة(الكنترول) أعلى وزن جاف لها بعد اسبوع من النمو ثم انخفضت هذة الكمية بعد ذلك وحتى الأسبوع الرابع.

٣- تراوحت النسبة المئوية للنتروجين باللقاح الناتج بين ٥٦% و٢%.

٤- تراوحت النسبة المئوية للفوسفور باللقاح الناتج بين ١٦،% و٣٠،%.

٥- تراوحت النسبة المئوية للكربون باللقاح الناتج بين ١،٣٣% و٧،٨٧%.

٦- لقد تحققت أعلى نسبة من الكربون الى النتروجين(٨،٨٦) بواسطة سلالة *A . oryzae*

٧- لقد تحققت أعلى نسبة من النتروجين الى الفوسفور (٩، ٧٢) بواسطة سلالة *C. Muscicola*