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Degradation of Chlorpyrifos by the Cyanobacteria Strains in Rice Fields

Abou Elatta, A. E. A.[1]; H. A. H. El- Zawawy[2]; Aida H. Afify[1*] and F. I. A. Hauka[1]

[1]Microbiol. Dept., Fac. Agric., Mansoura Univ., Mansoura, Egypt.
[2]Botany Dept. (Microbiology), Fac. of Agric., Al-Azhar Univ., Cairo, Egypt.

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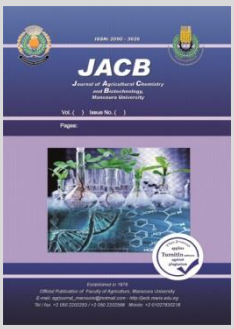

ABSTRACT

The important indiscriminate use of pesticides pollution of environment and leads a significant threat to beneficial microorganisms. The aims of this research is to perform the degradation of the pesticide by the cyanobacterial strains (*Anabaena oryzae* and *Nostoc muscorum*). So that the cyanobacteria are able to decrease insecticide contamination of rice fields by biodegradation of chlorpyrifos via measure nitrogen fixation rate, dry weight accumulation, total count of cyanobacteria, absorbance measurements by spectrophotometer and addition of gas chromatography–mass spectrometry (GC-MS) used to introduce the residuals components. The results refer to degrade all components of chlorpyrifos by cyanobacterial strains reach to 2.75 from 48 % after 12 days from incubation time. The mixture of *Nostoc* sp. and *Anabaena* sp. had increased in dry weight accumulation and raised the nitrogen fixation in environment. In addition to increasing in growth, yield and yield contributions for rice plant after inoculation with cyanobacterial strains particulars in mixture treatment compared to non-application by cyanobacteria.

***Keywords*:** cyanobacteria, insecticide, rice plant

INTRODUCTION

Recently, application of insecticides in crop fields for controlling of pests in agriculture practice led to dangerous environmental contamination, loss of crop productivity, growth and development of many beneficial micro-organisms, phytoplankton's (Chen *et al.*, 2016 and Singh *et al.*, a 2016). A great loss in rice crop yield when attacked by insects, pests, and fungi. Therefore, the farmers use many of these materials to develop the crop. However, the use of pesticides leads a high danger to cyanobacteria (Tariq *et al.*, 2007; Kumar *et al.*, 2008; Shen *et al.*, 2009; Galhano *et al.*, 2010). Nowadays, the farmers extensively used pesticides for the protection of crops from pests and insect (Parte *et al.*, 2017). Chlorpyrifos is a is wide spectrum organophosphate pesticide (OP), nonsystemic, used to control insects on field crops, fruits, vegetables (Low *et al.*, 2013). Chlorpyrifos is used as an act tool in rice fields by products bioactive in soil for long time and is compared with moderately persistent, with different from short periods (Getzin 1981; Lakshmi *et al.*, 2008). The different periods for degradation of chlorpyrifos in soil are affected by many factors such as initial concentration, soil moisture, temperature, and pH (Racke *et al.*, 1994; Awasthi and Prakash, 1997). The important factors of chlorpyrifos degradation are microbial degradation, and other chemical factors on soil (Getzin 1981; Racke *et al.*, 1988). Chlorpyrifos residues affect food chain (Aysal *et al.*, 2004; Gavrilescu *et al.*, 2005; Jha *et al.*, 2005; Mohapatra *et al.*, 2003). The toxicological and environmental risks associated with chlorpyrifos residues are the nature of insecticide therefore, there is a need to detoxify this effect (Mukherjee *et al.*, 2004). Development of resistance in the pests are affected by the stability of the ecosystem. In addition, this a big cause of environmental pollution due to accumulation of these pollutants in soil and water (Rani and Dhania, 2014; Verma *et al.*, 2014). Recently, cyanobacteria have been discovered to be a main source of biologically active compounds that can release soluble organic substances as secondary metabolites, which can be mineralized by the cyanobacteria, such as vitamins, enzymes, carbohydrates, peptides and amino acids, which have been found to enhance plant growth and productivity (Zulpa *et al.*, 2003; Singh *et al.*, b 2016 and Sammauria *et al.*, 2020). Lately, main attention to the beneficial effects of cyanobacteria in fields of rice are the amount of fixed nitrogen could supply rice plants with their needs of nitrogen. In addition, cyanobacteria are group of photosynthetic procaryotes. Adequate population dependent on N_2 fixation for cyanobacterial process (El-Saadny, 2013; Castenholz, *et al.*, 2015; El-Zawawy, 2016; Abou Elatta, 2018 and Abou Elatta, *et al.*, 2019). Little of reports are available for biodegradation of insecticide by cyanobacteria (Lee *et al.*, 2003; Barton *et al.*, 2004; El-Bestawy *et al.*, 2007; Cáceres *et al.*, 2008). The cyanobacteria strains were identified as *Anabaena oryzae* and *Nostoc muscorum* (Afify *et al.*, 2018). Many of OP compounds are degraded by microorganisms in the environment as a source of phosphorus or carbon or both (Karpouzas *et al.*, 2006). The treatment of wastewater by photoautotrophic microorganisms, such as cyanobacteria, lead to remove nitrogen and phosphorus (Ibrahim and Essa, 2003). And have potential to remove various pollutants, such as dyes (Rangabhashiyam, *et al.*, 2014). Heavy metals (Ibrahim, 2011) and pesticides (Ibrahim *et al.*, 2014). Cyanobacteria are a diverse group of oxygenic photosynthetic prokaryotes and growing in different ecological habitats. (Seckbach, 2007). Rice is the very important food of over half the world's population. It is the predominant dietary energy source for 17 countries in Asia and the pacific, 9 countries in America, and 8 countries in Africa (Yadav *et al.*, 2010). However, where population

* **Corresponding author.**
E-mail address: aidaafify@yahoo.com
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pressure is high, there is no option except to produce more food. It is necessary to increase productivity, but in ways that are environmentally safe, economically viable and socially sustainable, which has been christened an 'Evergreen Revolution' (Swaminathan, 2000). Thus, the aim of this study was to evaluate the survival of cyanobacterial strains *Anabaena oryzae* and *Nostoc muscorum* in several concentrations of chlorpyrifos as well as to evaluate the efficiency of these strains in biodegradation of chlorpyrifos. Moreover, effect of inoculation with cyanobacterial strains combined with chlorpyrifos on growth and yield of rice was also studied.

MATERIALS AND METHODS

Cyanobacterial strains:

Cyanobacterial strains (*Anabaena oryzae* and *Nostoc muscorum*) were isolated from soil sample obtained from Sakh, Kafer EL Sheikh Governorate, Egypt.

Growth rates of strains and dry weight of cyanobacteria were determined :

This experiment was carried out in glass bottles of 300 ml-capacity. Each bottle received 100 ml- of BG11 nitrogen free broth medium (El-Ayouty and Ayyad, 1972). The medium was inoculated with 1 ml-hormogonia growth of each of the different isolates. Three replicates were prepared for each isolate, and samples were collected after 7, 15, and 21 days incubation under (2500 lux) illumination. For each isolate, the content of one bottle (100 ml) was filtered using pre-oven dry folded filter paper; whatman No1 during each sampling period. The filter papers were kept till dryness under laboratory conditions, followed by oven drying at 105ºC for two hours. The differences in weights gave the dry weight of the cyanobacteria biomass according to Taha, (2000); El-Zawawy, (2016) and Abou Elatta, (2018). Total cyanobacterial strains were counted (*Anabaena oryzae* and *Nostoc muscorum*) according to Ellora Malakar *et al.* (2012) by Most probable Number (MPN) method.

Total nitrogen determination:

Total nitrogen in cyanobacterial strains was determined using micro-kjeldahl according to Jackson (1958).

Determination of cyanobacterial growth:

Absorbance measurements **s**pectroscopy was performed using a Cary 100 Bio UV-Vis spectrophotometer and a1 cm quartz cuvette. A spectral scan was performed at a wavelength of 720 nm.

Chromatographic determination of chlorpyrifos residues:

The Gas chromatography–mass spectrometry (GC-MS) system (Agilent Technologies) was equipped with gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, Cairo, Egypt. The GC was equipped with HP-5MS column (30 m x 0.25 mm internal diameter and 0.25 µm film thickness). Analyses were stored in Wiley and NIST Mass Spectral Library data (Rasekhi *et al.*,2014).

Rice grains:

Oryza sativa cv. Sakha 108 grains were kindly supplied by Rice Research Dept., Field Crops Res. Institute, Agricultural Research Center (ARC), Giza, Egypt.

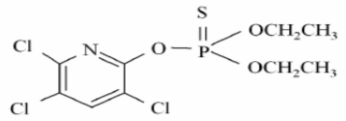
Nitrogen fertilizer:

Amount of nitrogen added is 75% of the recommended dose in the form of (46.5% N) was used in this investigation.

Insecticide used:

Chlorpyrifos (Bestban 48%) was used in this investigation. Chlorpyrifos is O,O-diethyl O-3,5,6- trichloro-2- pyridyl phosphorothi oate (TCP) obtained from Sigma - Co., USA. Some chemical and physical characteristics of chlorpyrifos are presented in Table (1) according to Venkta Mohan *et al.*, (2004).

Table 1. Physico-chemical characteristics of chlorpyrifos

Chemical name	Formula	Structure	Activity
O,O-diethyl O-3,5,6- trichloro-2- pyridyl phosphorothi oate	$C_9H_{11}Cl_3NO_3PS$		Insecticidal

Soil analysis properties:

Some chemical and physical properties of soil sample presented in Table (2) were determined according to Piper (1950) and Jackson (1958).

Table 2. Physical and chemical properties of soil sample

Characteristics	Sakha – KafrElSheikh Clay loam soil
Sand (%)	41.0
Silt (%)	32.0
Clay (%)	33.0
Ca CO3 (%)	1.1
(meq/ 100 g)	
Ca++	7.6
Mg++	5.1
Na+	9.1
K+	2.0
CO3--	0.0
HCO3-	4.6
Cl-	9.0
SO4--	8.2
E.C.(dS/m)	2.4
pH	7.7

The field experiment:

A field experiment was conducted during summer season of 2020 to evaluate the biodegradation of chlorpyrifos in rice field inoculated with liquid cultures of the most efficient cyanobacterial strains (*Anabaena oryzae* and *Nostoc muscorum).* The type of soil for experiment was presented in Table (2). Sakha 108 rice cultivar was used for this study and the seedlings were transplanted after month from planting in the field. The cyanobacterial inoculants were applied just after one week from transplanting. The insecticide (chlorpyrifos) was added 15 days after transplanting the rice at a rate of one liter /fed. and injected with irrigation water. Inorganic phosphorus fertilizer as recommended was added before planting and after irrigation.

The treatments in field experiment were as follow:

1- *Nostoc muscorum* + chlorpyrifos
2- *Anabaena oryzae* + chlorpyrifos
3- Mixture of *Nostoc muscorum* + *Anabaena oryzae* + chlorpyrifos

4- Chlorpyrifos
5- Control.

Yield of rice:

Rice yield and its components traits were collected and recorded as below:

Plant height (cm):

Plant height (cm) was estimated from the soil surface up to the panicle top.

Number of panicle in hill:

Panicles number was calculated as an average of five hills at maturity stage.

Fertility percentage:

Fertility percentage was determined by account full grains divide by all spikelet number for panicle.

Weight of 1000 -grain (g):

Sample was taken from threshed dry rice grains to measure the 1000-grain weight.

Rice yield (ton/ha):

Inner five square meters were identified in each plot, and harvested for grain and straw yields determination. The plants were left for air drying about five days, then threshed and the weight (kg) of grains of this area was recorded and calibrated to 14 percent moisture basis (the determined moisture % in rice grains), and then converted into tons/ha. The air-dried straw of the five square meters was weighed.

Harvest index in rice crop:

The harvest index in rice crop was determined according to the following equation of Yoshida, (1981)

$$\text{Harvest index in rice crop} = \frac{\text{Grain yield}}{\text{Grain yield + Straw yield}} \times 100$$

Statistical analysis:

The Randomize Complete Block Design were used for field treatments and LSD the least of significant differences was used to compare between means.

RESULTS AND DISCCUION

Results in Table (3) showed that in cultures of cyanobacterial strains the dry weight and fixed nitrogen increased with increasing the incubation period. At the same incubation period, there was an increase in dry weight and N_2 fixation with increasing chlorpyrifos concentration from 0 until 150 ppm, then there was a decline at the concentration of 200 ppm. The highest total nitrogen was noted with *Anabaena oryzae* (Sarnaik *et al.,* 2006; John *et al.,* 2015).

Table 3. Dry weight (mg/100ml-culture) and fixed nitrogen (mg N/100 ml - culture) in cyanobacterial strains cultures in presence of different concentrations of chlorpyrifos

Cyanobacteria strains + Concentration of chlorpyrifos	Dry weight (mg/100ml-culture) Incubation Period			Fixed nitrogen (mg N/100 ml-culture) Incubation Period		
	7	14	21	7	14	21
Anabaena oryzae	52	74	98	3.25	5.35	9.81
Anabaena oryzae +50 ppm Chlorpyrifos	55	80	99	3.31	5.45	9.92
Anabaena oryzae +100 ppm Chlorpyrifos	62	85	102	3.37	5.58	10.31
Anabaena oryzae +150 ppm Chlorpyrifos	65	89	108	4.28	5.85	10.81
Anabaena oryzae +200 ppm Chlorpyrifos	50	35	25	3.16	4.35	6.54
Nostoc muscorum	48	65	91	2.33	5.35	9.81
Nostoc muscorum +50 ppm Chlorpyrifos	55	80	95	2.46	5.54	9.89
Nostoc muscorum +100 ppm Chlorpyrifos	60	68	98	2.87	6.54	10.16
Nostoc muscorum +150 ppm Chlorpyrifos	64	70	105	4.97	7.34	10.46
Nostoc muscorum +200 ppm Chlorpyrifos	41	32	23	1.87	1.21	1.06
LSD 0.05	1.85	2.94	3.77	0.64	0.85	1.15

The ability of photoautotrophic nature to fix atmospheric nitrogen and to survive in polluted environments makes them more suitable for biodegradation (Sorkhoh *et al.,* 1995 and Kumar *et al*., 1998). The use of cyanobacteria to remove pollutants is inexpensive method since they have few requirements for growth (Chungjatupornchai and Fa-Aroonsawat, 2008). Therefore, they are an important microorganism in both terrestrial and aquatic ecosystems (Palanisami *et al.,* 2009). Nitrogen-fixing cyanobacteria are commonly seen in paddy fields where they introduced as bio-fertilizers (Kuritz, 2010). Cyanobacterial strains are able to accumulate high concentrations of pesticide (Chen *et al.,*2007 and Vijayakumar, 2012).

Results in Table 4 show that biodegradation of the pesticide (chlorpyrifos in culture) by cyanobacterial isolates was demonstrated by complete discoloration at 12 days of incubation, indicating that an acclimation period was necessary after 12 days. The cultured cells adhered to the culture wall, leaving the formation of a biofilm that absorbs some insecticides (chlorpyrifos) at concentrations of 50,100 and 150 ppm while at a concentration 200 ppm the color increased, which indicates that these isolates are tolerant until a concentration of 150 ppm only. These data were in accordance with Forlani *et al.,* (2008) and Nawaz *et al.,* (2011).

Table 4. Growth of cyanobacterial strains (determined as optical density) in presence of different concentrations of chlorpyrifos

Concentration of chlorpyrifos	Incubation Period	*Nostoc muscorum*	*Anabaena oryzae*
Control	8	0.27	0.26
	10	0.361	0.381
	12	0.471	0.486
50 ppm	8	1.65	1.95
	10	0.76	0.68
	12	0.67	0.58
100 ppm	8	3.66	3.86
	10	2.56	2.23
	12	1.64	1.58
150 ppm	8	4.56	4.76
	10	3.87	3.06
	12	2.21	2.02
200 ppm	8	6.76	6.82
	10	6.04	6.16
	12	6.79	6.36
LSD 0.05		1.03	1.02

Degradation of chlorpyrifos:

The data in Table (5) cleared that degradation of chlorpyrifos gave several types of compounds. The residual chlorpyrifos records 2.75% compared with the control

(chlorpyrifos at application time 48%). Ibrahim *et al.*, (2014) found that the *Nostoc* extract has different percentage of the bioactive compounds. The decrease of chlorpyrifos in *Nostoc* extract may be due to the ability of *Nostoc* to use chlorpyrifos as a source of phosphours (Anwar *et al.*, 2009) carbon and energy sources. And by intracellular degradation of chlorpyrifos (Yang *et al.*, 2005).

Table 5. Degradation compounds of chlorpyrifos insecticide by *Anabaena oryzae* culture through GC-Mass method.

Retention time	Name	Degradation component	Component Percentage
3.167	Decane, 2-methyl-	C11H24	5.45
3.201	Nonane, 4,5-dimethyl-	C11H24	4.22
3.356	Nonane, 4-ethyl-5-methyl-	C12H26	4.58
3.407	Decane, 2,3,5,8-tetramethyl-	C14H30	8.08
3.476	Octane, 4,5-diethyl-	C12H26	2.4
3.774	Tetradecane	C14H30	8.97
3.819	pentadecane	C15H32	3.3
3.848	Hexadecane	C16H34	2.65
3.957	Nonadecane	C19H40	1.42
3.991	Heptadecane, 2,6,10,14-tetramethyl-	C21H44	5.2
4.094	Dodecane, 2,6,10-trimethyl-	C15H32	3.61
4.123	Heptadecane, 2,6,10,15-tetramethyl-	C21H44	7.65
4.226	2,4-Di-tert-butylphenol	C14H22O	0.56
4.775	Methoxyacetic acid, 3-tetradecyl ester	C17H34O3	2.36
5.17	Eicosane	C20H42	3.43
5.221	Octadecane, 2-methyl-	C19H40	8.02
5.513	1-Decanol, 2-hexyl	C16H34O	3.16
6.217	Heneicosane	C21H44	2.37
6.852	Nonadecane, 2-methyl-	C20H42	8.01
7.235	Octacosane	C28H58	2.46
7.585	Chlorpyrifos	C9H11Cl3NO3PS	2.75
8.122	1-Ethynylcyclopentanol	C7H10O	1.7
8.54	Heptadecane, 2-methyl-	C18H38	2.61
8.872	Eicosane, 2-methyl-	C21H44	2.35
9.307	2-methyltetracosane	C25H52	2.68

Total count of cyanobacterial strains:

Data in Table (6) represent the changes in the total count of the two strains *Nostoc muscorum* and *Anabaena oryzae* at zero, 30 and 60 days after application to the soil. Results showed that the total count of cyanobacteria was increased with increase the days after transplanting. It was noted that the highest count was found after 60 days under all orders and reached to 0.212 x10^4 with mixture of *Nostoc* sp.+*Anabaena* sp. + Chlorpyrifos. The results are consistent with those observed by Shatta *et al*., (2014) who found that total count of cyanobacteria increased at all growth stages of rice.

Table 6. Total count of cyanobacteria (10^4cfu/g dry soil) in soil cultivated with rice plant.

Treatments	Days after transplanting		
	0	30	60
Mixture of *Nostoc* sp. +*Anabaena* sp. + Chlorpyrifos	0.083	0.188	0.212
Anabaena oryzae + Chlorpyrifos	0.076	0.175	0.198
Nostoc muscorum + Chlorpyrifos	0.045	0.135	0.188
Chlorpyrifos	0.025	0.096	0.124
Control	0.011	0.068	0.090
LSD 0.05	0.11	0.15	0.15

Determination of growth in rice plant:

The data in Table (7) indicated that the application of cyanobacteria combined with chlorpyrifos insecticide in rice fields led to a significant increase in plant height (cm), panicle length (cm), number of tillers/plant, number of panicles/plant and panicle weight (g). This increase in growth may be attributed to ability of cyanobacteria to degrade chlorpyrifos, which produce regulators that enhances the ability of crop to absorb nutrients from the soil. Ubiquities microorganisms in the environment may utilize the organic phosphors in chlorpyrifos as a source of phosphorus or carbon or both (Bohnert and Richard, 1996). Application of cyanobacteria combined chlorpyrifos insecticide resulted in increase in plant height as compared to control. In addition panicle length (cm) increased with application of cyanobacteria with chlorpyrifos than control values. Therefore, highest values number of tillers/plant were with application of mixture of *Nostoc* and *Anabaena* with chlorpyrifos and lowest value was recorded in control. Concerning number of panicles/plant the superior values was achieved with mixture while the lowest values were recorded in control. With application of cyanobacteria panicle weight (g) significantly increased as compared to control . The highest value of panicle was achieved with mixture treatment. These data were in accordance with Aziz *et al.*, (2014); Chittapun *et al*., (2018); El-Sheekh *et al.*, (2018).

Table 7. Effect of two strains of cyanobacteria and chlorpyrifos insecticide on rice growth.

Treatment	Plant height (cm)	Panicle length (cm)	No. tillers/plant	No. panicles/plant	Panicle weight (g)
Nostoc muscorum +Chlorpyrifos	99.8	24.4	25.45	25.41	4.72
Anabaena oryzae +Chlorpyrifos	98.3	23.6	24.5	22. 22	4.63
Mixture + Chlorpyrifos	101.6	25.3	26.6	25. 25	4.98
Chlorpyrifos	96.6	22.2	23.4	21.53	4.30
Control	96	22	18	17	4.18
LSD 0.05	0.98	0.77	1.18	1.27	0.37

Determination of rice yield and its components :

The data in Table (8) cleared that there are statistically significant differences in fertility percentage, 1000-Grain weight (g), grain yield t/ha and harvest index as affected by application of cyanobacteria combined with chlorpyrifos insecticide. The results illustrated that the highest values of fertility percentage, 1000-Grain weight, grain yield and harvest index were recorded with mixture of *Nostoc* and *Anabaena* combined with chlorpyrifos treatment. The *Nostoc muscorum* and *Anabaena oryzae* enhance plant biomass (Aziz *et al.*, 2014 and Yanni *et al.*, 2020). Also, the strains of *Nostoc muscorum* and *Anabaena oryzae* stimulate the secretion of growth substances which increase the uptake of nutrients N, P and K by continuous supply of nitrogen (Zebo *et al.*, 1998 and Nayak, 2004) .

Table 8. Effect of application two strains of cyanobacteria and chlorpyrifos insecticide on rice yield.

Treatment	Fertility Percentage	1000-Grain weight (g)	Grain yield t/ha	Harvest index
Nostoc muscorum+ Chlorpyrifos	0.95	28.08	10.95	51.69
Anabaena oryzae+ Chlorpyrifos	0.96	29.03	10.72	48.29
Mixture of *Nostoc* sp.+ *Anabaena* sp. +Chlorpyrifos	0.97	28.76	11.28	55.54
Chlorpyrifos	0.96	27.23	9.52	42.88
Control	92.00	26.04	9.22	41.24
LSD 0.05	0.09	0.71	0.62	1.58

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تكسير المبيد كلوروبيرفوس بواسطة سلالات السيانوبكتيريا فى حقول الأرز

أحمد السيد عبد الرحمن أبو العطا[1] ، حسن أحمد حسن الزواوى[2] ، عايده حافظ عفيفى[1] و فتحى إسماعيل على حوقه[1]

[1] قسم الميكروبيولوجي - كلية الزراعة - جامعة المنصورة - المنصورة – مصر

[2] قسم النبات (ميكروبيولوجى) - كلية الزراعة - جامعة الأزهر - القاهرة – مصر

الملخص

يؤدي الإستخدام العشوائي للمبيدات الحشرية إلى مشاكل بيئية ويشكل تهديدًا كبيرًا للكائنات الحية الدقيقة المفيدة. وقد أجريت هذه التجربه لدراسة تحلل المبيد الحشري كلوروبيرفوس حيويا بواسطة سلالات من السيانوبكتيريا *Nostoc muscorum* و *Anabaena oryzae* وذلك يكون من خلال قياس معدل تثبيت النيتروجين وكمية الوزن الجاف والعدد الكلى للسيانوبكتيريا وقياسات الإمتصاص بواسطة مقياس الكروماتوجرافيا (GC-MS) الذى يستخدم لتحديد المكونات المتبقية ، وقياسات الإمتصاص بواسطة مقياس الطيف الضوئي. وقد سجلت النتائج أن تحلل معظم مكونات الكلوروبيرفوس بواسطة سلالات السيانوبكتيريا إلى 2.75 ٪ بدلا من 48٪ بعد 12 يومًا من وقت الإضافة .كما أدى الخليط من السيانوبكتيريا المضاف إليه المبيد الحشرى الكلوروبيروفوس إلى زيادة كمية الوزن الجاف للحبوب وكذلك تثبيت النيتروجين فى البيئة بالإضافة إلى الزيادة في النمو والإنتاجية والمساهمة في زيادة محصول الأرز عند المعامله بسلالات من السيانوبكتيريا مقارنة بالكنترول (بدون التلقيح بالطحالب الخضراء المزرقة وعدم إضافة مبيد الكلوروبيروفوس).