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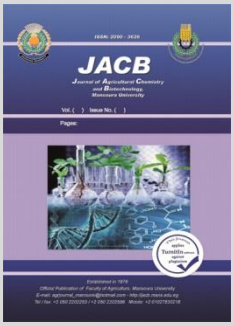
Cyanobacteria and Fungicide as Controlling Agents for Cotton Fungal Diseases

Aida H. Afify[1*] and A. Z. A. Ashour[2]

[1] Microbiol. Dept., Fac. Agric., Mansoura Univ., Mansoura, Egypt
[2] Plant Pathology Research Institute, Agricultural Research Center (ARC), Giza, Egypt



ABSTRACT

Biological control is a very important factor as sustainable alternative or complement to conventional pesticides for fungal plant disease management. This study was carried out on cotton plants to investigate the roles of cyanobacteria in controlling plant fungal diseases and pesticides biodegradation. Therefore, twelve cyanobacterial strains were isolated from soil samples that polluted with pesticides in Kafr El- Sheikh, Governorate and identified as four genera (*i.e.* Anabaena, Nostoc, Oscillatoria and Chroococcus). The *in vitro* study showed antagonistic activities of these cyanobacterial strains against *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotium rolfsii*. The superior cyanobacterial strains, which antagonised the phytopathogenic fungi were *Nostoc muscorum*, *Nostoc paludosum*, *Nostoc entophytum*, *Nostoc verrucosum*, *Anabaena oryzae*, *Anabaena variabilis* and *Oscillatoria brevis*. These cyanobacterial strains showed different levels of efficiency in increasing the surviving seedlings dry weight and plant height in greenhouse tests, depending on the fungus under consideration; however, the cyanobacterial strains significantly increased these parameters. Under field conditions, also the cyanobacterial strains were effective in increasing the surviving seedlings and yield in 2021 and 2022 seasons. In addition, the results of bioagents materials showed that all cyanobacterial strains produced ammonia and catalase enzyme. Whereas, only *Anabaena oryzae* was found to be positive for hydrogen cyanide and chitinase enzyme. Finally, the strong action of cyanobacteria is very important in development with fungicides against fungal pathogens.

Keywords: cyanobacteria; fungal plant diseases; antagonism, antifungal materials

INTRODUCTION

Cotton is the first large-scale crop to be treated with a biological control agent for suppression of seedling diseases and long-term chronic rhizosphere diseases (Brannen and Kenney 1997). Seedling diseases, which are ubiquitous in cotton-producing areas, are caused by one or more of pathogens acting single or in combination. Pre-emergence and post-emergence damping-off caused by these fungi must be controlled to obtain uniform stand and vigorous plants (Zaki *et al*., 1998). In some diseases, biological control was much more effective than that of chemicals (Manka and Fruzynska 1996). Antagonistic microorganisms have been used by many workers for controlling soil-borne plant pathogens (Afify and Ashour 1995; Safiyazov *et al*., 1995 and Perondi *et al*., 1996). Cyanobacterial isolates are among the widely used biocontrol agents. Biopesticides are living organisms or natural products that control agricultural pests including bacteria, fungi, weeds, viruses and insects (Lukmanul and Usman 2020). Micoorganisms present in biopesticides are responsible for the degradation of the synthetic pesticide residue found in the environment (Paulina and Ewa 2022).

Biological control agents are bacteria that can use many mechanisms to limit the development of plant disease and several bacterial products have been marketed as biopesticides (Bonaterra, *et al.*, 2022). New trends in crop protection have been oriented toward a reduction of reliance on conventional pesticides together with the compulsory implementation of integrated pest management (IPM), these are the program addressed in the regulations of different countries (Lamichhane *et al.*, 2016). Bacteria and fungi are the most important in integrated pest management (IPM) systems because their roles in pesticide degradation and the high number of commercial preparations containing these microorganisms available on the market (Paulina and Ewa 2022). Cyanobacteria, one of the least investigated microbes, may synthesize and generate a significant number of antimicrobial secondary metabolites. Many orders from phylum cyanobacteria are produced these metabolites such as: Chroococcales (16%), Pleurocapsales (6%), Stigonematatles (4%), Oscillatoriales (49%) and Nostocales (26%) (Yadav, *et al.*, 2022). Cyanobacteria are sources of several bioactive compounds and other fine chemicals (Hillary, *et al.*, 2022). The present study, aims to evaluate the possibilities of suppressing the incidence of cotton damping-off by bacterization with cyanobacterial strains. However, current research is improvement the action spectra, including mechanisms to reduce the use of chemical pesticides for protection cotton plants from soil born fungi.

MATERIALS AND METHODS

Source of cyanobacterial strains

Cyanobacterial strains were isolated from soil sample polluted with pesticides and identified according to Afify, *et al.* (2023).

Source of fungal isolates

The fungal isolates (*F. oxysorum* Schlech., *R. solani* Kuhn and *S. rolfsii* Sacc.) were obtained from Plant Pathology Research Institute, Agricultural Research Center (ARC), Giza, Egypt .

Plant used

Cotton seeds (*Gossypium barbadense* L.) cv. were obtained from the Agricultural Research Center (ARC), Giza, Egypt.

* **Corresponding author.**
E-mail address: aidaafify@yahoo.com
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Antagonism

In vitro study was carried out to investigate the antagonistic activities of the twelve cyanobacterial isolates towards damping – off fungi (*F. oxysorum, R. solani* and *S. rolfsii*) using plate assay. The plates were incubated at 28-30ºC and the inhibition of fungal growth was detected after incubation for 7 days (Sivamani and Gnanamanickam 1988). The cyanobacterial isolates which exhibited the highest antagonistic activities against pathogenic fungi were selected.

Preparation of fungal inoculum

Substrate for growth of each fungus was prepared in 500 ml glass bottles, each bottle contained 100g of sorghum grains and 80 ml of water. Contents of bottles were autoclaved for 30 min at 121ºC. Fungal inoculum taken from one-week-old culture on PDA, was allowed to colonize sorghum for 3 weeks on the bottles. In the greenhouse, the fungus-sorghum mixture was air-dried. In the present study batches of soil were placed on greenhouse and infested separately with inoculum of each fungus at the rates 5 g/kg soil of *F. oxysorum, R. solani* and *S. rolfsii*. Infested soils were conducted in 20 cm diamater clay pots and planted with 10 cotton seeds per pot (cultivar Giza 89). No fungi were added to soil of control pots.

Treatment of cotton seeds with cyanobacteria

Cotton seeds were surface sterilized by using 2.5% calcium hypochlorite solution for 3 min. After thorough washing in six changes of sterile distilled water, the seeds were aseptically air dried, placed in flasks containing 150 ml cyanobacterial suspension (10^9 cfu /ml) for 24hr and sown in greenhouse potted soil and/or field experiment (Mew and Rosales, 1986).

Seeds-dressing fungicide

Monceren combi (20% Pencycuran + 50% Captan) was applied at the recommended dose (3 g/kg seeds). Treated seeds were planted in greenhouse and field experiments.

Experimental conditions

The twelve strains of antagonistic cyanobacteria and the chemical fungicide Monceren combi were tested against *F. oxysorum, R. solani* and *S. rolfsii*. The greenhouse experiment was conducted by using clay pots of 20 cm in diameter. A field experiment was conducted at Plant Pathology Research Institute, Agricultural Research Center (ARC), Giza, Egypt , during summer season of 2022 to evaluate the biodegradation of Monceren combi in cotten field inoculated with liquid cultures of the most efficient cyanobacterial strains. In the field experiment, treatments were sown in 5.0 x 1.8 m plots having 3 (5.0 x 0.6 m) rows. The natural soil used in both experiments was a clayey soil (pH 7.5, clay 66.3%, E.C. 1.2 mmhos/cm). The design of layout of both trails was a randomized complete block design with four replicates.

The treatments in experiments were as follow:

Cod No.	Treatment
1	*Nostoc muscorum*
2	*Nostoc paludosum*
3	*Nostoc entophytum*
4	*Nostoc pruniforme*
5	*Nostoc viride*
6	*Nostoc verrucosum*
7	*Nostoc rivulare*
8	*Anabaena oryzae*
9	*Anabaena qelatinicola*
10	*Anabaena variabilis*
11	*Chroococcus minor*
12	*Oscillatoria brevis*
13	Nutrient broth
14	Monceren combi
15	Control

Variables of the tested plant

Cotton seeds treated with the cyanobacterial strains or monceren combi were planted one week after soil infestation with the fungal inoculum. Percentages of surviving seedlings, were recorded 40 days from sowing in greenhouse and field experiments. Dry weight (g/plant) and plant height (cm) in greenhouse were determined after 40 days from sowing. Seed cotton yield (kentar/fed.) was recorded at the end of growth season in the field trials.

Antagonistic materials analyses

Ammonia production

Ammonia was evaluated by Nessler's reagent according to the method of Dye (1962).

HCN production

Qualitative method was used for evaluated of HCN production Kermer and Souissi (2001).

Enzymes detection

Production of hydrolytic enzymes were detected on plate containing medium with enzyme substrate, according to Ngarajkumar, *et al.* (2004).

Statistical analysis

Data of greenhouse and field experiments were transformed into arc sine angles before carrying out analysis of variance (ANOVA) to produce approximately constant variance. Management and Analysis of Agronomic Research Experiments (MATAT- C, Michigan State Univ., USA).

RESULTS AND DISCUSSION

Twelve cyanobacterial isolates were obtained from a soil sample contaminated with insecticides in Kafr Elsheikh Governorate. These twelve isolates were found to be belonging to four genera (*Anabaena, Nostoc, Oscillatoria* and *Chroococcus*). Scientific names are presented in Afify, *et al.* (2023). These twelve isolates were chosen and they exhibited consistent *in vitro* antagonism against *F. oxysorum, R. solani* and *S. rolfsii* (Table 1).

Table 1. Antagonistic effect of cyanobacteria against damping-off fungi

Cod No.	Cyanobacterial isolates	Fungi tested		
		F. oxysorum	*R. solani*	*S. rolfsii*
1	*Nostoc muscorum*	++	++	++
2	*Nostoc paludosum*	++	++	++
3	*Nostoc entophytum*	++	++	++
4	*Nostoc pruniforme*	+	+	+
5	*Nostoc viride*	+	+	+
6	*Nostoc verrucosum*	++	++	++
7	*Nostoc rivulare*	+	+	++
8	*Anabaena oryzae*	++	+	++
9	*Anabaena qelatinicola*	+	+	+
10	*Anabaena variabilis*	++	++	++
11	*Chroococcus minor*	+	+	+
12	*Oscillatoria brevis*	++	++	++
13	Control (only fungus)	-	-	-

++ Inhibition of pathogen by over growth of cyanobacteria
+ Inhibition of pathogen
-No inhibition of pathogen (Full growth of pathogen)

Effects of cyanobacterial isolates under greenhouse and field conditions

The effect of interaction between biocontrol agents and the fungal pathogens were highly significant with variation in percentage of surviving seedlings, dry weight and plant height. To compare between the individual biocontrol agent means (arc-sine transformed values) within each fungus

a least significant difference (LSD) was used. The results in Table (2) indicated that the cyanobacterial strains showed different levels of efficiency in increasing the surviving seedlings depending on the fungus under consideration; however, most of the tested strains significantly increased the surviving seedlings compared to the control. For example, *Nostoc entophytum* which was ineffective in case of *S. rolfsii*, was found to be effective in all other cases. It is noteworthy that some of the cyanobacterial strains were as effective as the fungicide, or even superior in increasing the surviving seedlings. These findings are in conformity with the findings of Kulik (1995); Ashour and Afify (1999); Afify and Ashour (2023) Who reported that when cyanobacterial isolates added to the seeds plant were protected from soil-borne fungi. The same conclusions previously mentioned regarding the effects of biocontrol agents on the surviving seedlings held true in case of their effects on dry weight and plant height (Table 3). For example, the *Oscillatoria brevis* strain had no effect on the dry weight in case of *F.oxysporum* while it significantly increased in all other cases. *Nostoc rivulare* strain significantly increased the dry weight in case of *F. oxysporum*, while it had no effect on dry weight in case of *S.rolfsii*. *Anabaena oryzae* strain was ineffective in increasing plant height in case of *F. oxysporum*, while it significantly increased it in case of *R. solani* (Pierson and Weller 1994). Among *Nostocales*, the *Anabaena* species were active in controlling soil-borne pathogens under greenhouse conditions (Hillary, *et al.*, 2022).

Table 2. Effect of biocontrol agents on seedlings of cotton damping off in soil naturally and artificially infested with fungal pathogens under greenhouse conditions

Trearments	Fungal pathogen			Natural soil	Mean
	F. oxysporum	*R. solani*	*S. rolfsii*		
Nostoc muscorum	58.25[a](49.76)[b]	69.50 (56.49)	70.00 (56.86)	71.00 (57.48)	67.18 (55.14)
Nostoc paludosum	61.25 (51.58)	61.00 (51.41)	69.00 (56.22)	75.25 (60.21)	66.69 (54.85)
Nostoc entophytum	55.75 (48.31)	38.25 (38.18)	33.50 (35.34)	68.50 (55.86)	49.00 (44.42)
Nostoc pruniforme	57.00 (49.04)	47.00 (43.28)	47.00 (43.28)	63.50 (52.84)	53.63 (47.11)
Nostoc viride	62.00 (51.95)	67.50 (55.29)	43.75 (40.41)	63.75 (52.98)	59.25 (50.40)
Nostoc verrucosum	66.00 (54.35)	67.25 (55.11)	64.50 (53.46)	67.75 (55.42)	66.38 (54.58)
Nostoc rivulare	40.25 (39.37)	34.50 (35.92)	44.75 (41.98)	61.00 (51.36)	45.13 (42.16)
Anabaena oryzae	39.00 (38.58)	33.75 (35.51)	41.25 (39.95)	59.00 (50.19)	43.25 (41.06)
Anabaena qelatinicola	43.50 (41.25)	30.00 (33.17)	28.00 (31.94)	54.50 (47.59)	39.00 (38.49)
Anabaena variabilis	33.75 (35.48)	28.50 (32.20)	24.75 (29.79)	57.00 (49.03)	36.00 (36.62)
Chroococcus minor	59.00 (50.21)	61.75 (51.84)	69.75 (56.68)	65.75 (54.20)	32.81 (53.23)
Oscillatoria brevis	55.00 (47.88)	65.00 (53.77)	59.25 (50.34)	66.00 (54.34)	61.31 (51.58)
Nutrient broth	33.75 (35.51)	62.25 (30.78)	28.75 (32.40)	33.25 (35.19)	30.50 (33.47)
Monceren combi	46.50 (42.98)	62.00 (51.97)	62.75 (52.39)	62.75 (52.39)	58.50 (49.93)
Control	32.00 (34.31)	38.75 (32.31)	28.25 (32.06)	30.75 (33.67)	29.94 (33.09)
Mean	49.53 (44.70)	48.73 (43.81)	47.68 (43.60)	59.98 (50.85)	

L.S.D. for biocontrol (B)=1.82 (*P*=0.05)
L.S.D. for fungal pathogens (F)=0.94(*P*=0.05)
L.S.D. for B x F=3.64 (*P*=0.05)
[a]Percentage of surviving seedlings
[b]Arc sine – transformed data

Table 3. Effect of biocontrol agents on dry weight and plant height of cotton seedlings in soil naturally and artificially infested with fungal pathogens under greenhouse conditions.

Treatments	Dry weight (g plant^{-1})					Plant height (cm plant^{-1})				
	Fungal pathogens			Natural Soil	Mean	Fungal Pathogens			Natural Soil	Mean
	F. oxysporum	*R. solani*	*S. rolfsii*			*F. oxysporum*	*R. solani*	*S. rolfsii*		
Nostoc muscorum	1.93	1.80	1.89	1.88	1.88	30.5	27.4	28.5	30.9	29.3
Nostoc paludosum	2.03	2.01	1.76	2.00	1.95	32.4	31.1	29.5	33.5	31.6
Nostoc entophytum	1.99	1.94	1.81	1.98	1.93	30.8	29.9	32.2	31.4	31.0
Nostoc pruniforme	1.89	1.85	1.72	1.93	1.85	29.7	32.5	27.4	31.8	30.3
Nostoc viride	1.79	1.92	1.71	1.89	1.82	27.6	32.0	26.1	32.9	29.6
Nostoc verrucosum	1.87	1.76	1.66	1.89	1.79	29.4	28.3	28.3	30.2	29.0
Nostoc rivulare	2.00	1.80	1.78	1.93	1.88	32.0	27.9	29.6	31.4	30.2
Anabaena oryzae	1.94	1.77	2.00	1.94	1.91	28.6	30.1	32.9	31.4	30.7
Anabaena qelatinicola	1.83	1.84	2.07	1.92	1.91	30.9	39.2	32.2	28.7	30.2
Anabaena variabilis	1.85	1.84	1.94	1.96	1.90	29.6	29.4	31.9	31.4	30.6
Chroococcus minor	1.65	2.07	1.77	1.88	1.84	25.9	32.1	27.5	29.6	28.8
Oscillatoria brevis	1.65	1.79	1.88	1.83	1.78	27.5	29.1	30.9	29.5	29.2
Nutrient broth	1.75	1.70	1.70	1.79	1.74	27.3	29.8	28.4	29.0	28.6
Monceren combi	1.71	1.82	1.72	1.88	1.78	25.6	29.6	28.9	30.4	28.6
Control	1.68	1.66	1.73	1.71	1.69	26.9	24.8	26.7	28.8	26.8
Mean	1.84	1.84	1.81	1.89		29.0	29.5	29.4	30.7	

L.S.D. for biocontrol (B) =0.06 (*P*=0.05) ; 1.48 (*P*=0.05)
L.S.D. for fungal pathogens (F) =0.03(*P*=0.05) ; 0.76 (*P*=0.05)
L.S.D. for B x F =0. 12 (*P*=0.05) ; 2.96 (*P*=0.05)

Biocontrol agent should has two features. First, reduce disease development. Second, stable performance under different environmental conditions. In the present investigation (Table 4) among the 12 strains evaluated under field conditions, strains *Nostoc muscorum*, *Nostoc paludosum*, *Nostoc viride*, *Nostoc verrucosum Anabaena*

qelatinicola and *Oscillatoria brevis.* Data showed that the only strains which met the two features when increasing stand and yield in both years. While the other strains resulted in an increase in the surviving seedlings and seed cotton yield or only yield. The successful application of cyanobacterial strains for controlling cotton seedling damping-off under field conditions, is in agreement with the results obtained by Afify and Ashour (1995) and Safiyazov *et al*. (1995). Also, *Nostoc* strain introduced the development of growth in plants seedlings (Toribio, *et al.,* 2020).

Table 4. Effect of biocontrol agents on cotton seedling disease incidence and seed cotton yield under field conditions

Trearments	Seedling survival %		Seedcotton yield (ketar/fed.)	
	2021	2022	2021	2022
Nostoc muscorum	61.73[a](51.80)[b]	66.38 (54.57)	5.51	4.64
Nostoc paludosum	56.23 (48.60)	67.68 (55.36)	5.11	4.63
Nostoc entophytum	24.73 (29.70)	44.70 (41.96)	3.79	3.74
Nostoc pruniforme	24.25 (29.47)	29.03 (32.58)	3.98	3.08
Nostoc viride	55.08 (47.92)	49.33 (44.62)	5.44	4.38
Nostoc verrucosum	48.23 (43.98)	48.60 (44.14)	4.91	4.45
Nostoc rivulare	23.00 (28.55)	26.38 (30.87)	4.14	3.06
Anabaena oryzae	38.25 (38.19)	29.10 (32.64)	4.48	2.88
Anabaena qelatinicola	48.13 (43.92)	57.70 (49.43)	4.32	4.16
Anabaena variabilis	29.20 (32.70)	28.43 (32.22)	3.93	3.17
Chroococcus minor	33.83 (35.51)	66.95 (34.92)	4.97	4.33
Oscillatoria brevis	55.45 (48.14)	42.83 (40.87)	5.65	4.03
Nutrient broth	24.10 (29.36)	32.55 (34.75)	3.92	2.93
Monceren combi	61.88 (51.89)	68.70 (55.99)	5.87	4.78
Control	27.28 (31.47)	34.20 (35.70)	3.93	2.78
Mean	40.76 (39.41)	46.17 (41.38)	4.66	3.80
L.S.D. (*P*=0.05)	**3.64**	**2.79**	**0.25**	**0.27**

[a]Percentage of surviving seedlings
[b]Arc sine – transformed data

Detection of antagonistic substances

Cyanobacterial activities as antagonistic substances are presented in Table (5). The results showed that all strains were produced ammonia and catalase, but only *A. oryzae* produced HCN and chitinase. When detection enzyme of cellulase, all the tested cyanobacterial strains did not show any activity. The results were in agreement with the findings of Castenholz (2015). Cyanobacteria are a mother of wide categories of antagonistic substances with different biological metabolites, *i.e.*, antibacterial, antifungal properties (Yadav, *et al*., 2022).

Table 5. Detection of antagonistic substances produced by cyanobacteria.

Cyanobacterial strains	Ammonia	HCN	Cellulase	Chitinase	Catalase
Nostoc muscorum	+	-	-	-	+
Nostoc paludosum	+	-	-	-	+
Nostoc entophytum	+	-	-	-	+
Nostoc pruniforme	+	-	-	-	+
Nostoc viride	+	-	-	-	+
Nostoc verrucosum	+	-	-	-	+
Nostoc rivulare	+	-	-	-	+
Anabaena oryzae	+	+	-	+	+
Anabaena qelatinicola	+	-	-	-	+
Anabaena variabilis	+	-	-	-	+
Chroococcus minor	+	-	-	-	+
Oscillatoria brevis	+	-	-	-	+
Nutrient broth (control)	-	-	-	-	-

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السيانوبكتيريا والمبيد الفطرى كعوامل مقاومه لأمراض القطن الفطرية

عايده حافظ عفيفى[1] وعبد الودود زكى عاشور[2]

[1]قسم الميكروبيولوجى - كلية الزراعه – جامعة المنصوره – المنصوره – مصر
[2]معهد أمراض النباتات - مركز البحوث الزراعيه – الجيزه – مصر

الملخص

المقاومة الحيوية لها تأثير فعال فى وجود المبيدات الكيميائية عند مقاومة أمراض النبات الفطرية ، وتعتبر السيانوبكتيريا من عوامل المقاومة الحيوية وخاصة التي لها القدرة على تحليل تلك المبيدات الكيميائية. فى هذه الدراسة تم عزل وتعريف سلالات من السيانوبكتيريا لها القدرة على تحليل المبيدات الكيميائية وذلك من أراضى ملوثة بتلك المبيدات الكيميائيه من محافظة كفر الشيخ. وتم تعريف 12 سلالة من السيانوبكتيريا تنتمى لأربعة أجناس هى الأنابينا والنوستوك وأوسيلاتوريا والكروكوكس ، وأظهرت السلالات الإثنى عشر من السيانوبكتيريا تضاد عند إختبارها فى المعمل ضد ثلاث فطريات ممرضه لنباتات القطن وهى فيوزاريوم أوكسيسبورم وريزوكتونيا سولانى وأسكيرورشيم رولفسياى حيث سجلت سبعة سلالات من السيانوبكتيريا أعلى حالات تضاد مع الفطريات الممرضة وتشمل: أربعة أنواع تتبع جنس النوستوك ونوعين تتبعا جنس الأنابينا ثم نوع يتبع جنس الأوسيلاتوريا. وقد أظهرت سلالات السيانوبكتيريا مستويات مختلفة من الكفاءة فى القدرة على زيادة نسبة بادرات نبات القطن الباقية على قيد الحياة وزيادة كل من الطول والوزن الجاف لهذه البادرات من القطن عند إختبارها تحت ظروف الصوبة وذلك حسب الفطر المستخدم فى عدوى التجربة ولكن كانت معظم سلالات السيانوبكتيريا فعالة عند إختبارها تحت ظروف الحقل خلال موسمى زراعة القطن 2021/ 2022 حيث سجلت ستة سلالات من السيانوبكتيريا قدرة عالية فى زيادة نسبة الإنبات والمحصول. وعند تقدير قدرة سلالات السيانوبكتيريا على إنتاج المواد المضادة لنمو الفطريات الممرضة فى المعمل سجلت جميع السلالات قدرتها على إنتاج الأمونيا وإنزيم الكتاليز بينما سجلت سلالة واحدة من السيانوبكتيريا (أنابينا أوريزا) قدرتها على إنتاج سيانيد الهيدروجين وإنزيم الكيتينيز. من هنا نجد أن للسيانوبكتيريا دور هام فى تطور مقاومة أمراض النبات المتسببة عن الفطريات فى وجود المبيدات الكيميائية.