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Use of Cyanobacteria for Controlling Flax Seedling Blight

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ABSTRACT

Cyanobacteria are one of important groups of prokaryotes due to their effects on growth and development of plants and its role in biological control of phytopathogenic fungi such as : *Fusarium oxysporum* and *Rhizoctonia solani* on flax by producing various biologically active substances . In the present study , fourteen cyanobacterial strains were isolated from rice rhizosphere in kafr El–sheikh (k) (North Delta Region) and El- Dakahliya (D) (East Delta Region) . Only five cyanobacterial strains showed antagonistic effects against the both pathogenic fungi and were indentified as *Nostoc muscorum* k , *Anabaena oryzae* D , *Anabaena oryzae* k , *Nostoc pruniforme* D and *Oscillatoria brevis* D. The strains were analysed for phosphate solubilization , production of IAA , ammonia , HCN , production of some enzymes , and effect of their filterates on seed germination of flax seeds . Flax seeds treated with cyanobacterial filtrates germinated faster and produced higher seedlings compared with the nontreated ones .

Keywords: Prokaryotes , Blue – green algae , Flax , Phosphate solubilization , IAA , Biological control substances , Cellulase , Chitnase , Catalase productions .

INTRODUCTION

The cyanobacteria are often the dominant microalgae in soils (Zimmerman , 1992) . The use of cyanobacteria as Plant Growth Promoting Rhizobacteria (PGPR) can fulfill these criteria by carry out photosynthesis and can fix nitrogen , thus add the mass growth (algal blooms) and increase nitrogen to soils (Metting , 1981) . In addition , although the cyanobacteria (blue – green algae) , which constitute the largest , most diverse , and most widely distributed group of photosynthetic prokaryotes (Stanier and Cohen – Bazire 1977) make up most of the world's biomass (Cannel , 1993) they have received little attention as potential biocontrol agents of plant diseases . In the present study , *F.oxysporum* and *R.solani* which are important pathogens on flax , were inhibited *in vitro* by substances produced by various cyanobacteria . Many cyanobacteria produce a large number of antifungal materials , thus they are suitable candidtes for exploitation as biocontrol agents of plant pathogenic fungi (Martin , 1995).The cyanobacteria also produce auxins the most important and diverse group of plant hormone used by plant as in the regulation of diverse biological processes including cell division differentiation , root elongation . Sergeeva *et al.,* 2002 reported that cyanobacteria have the capability to accumulate IAA , production of lytic enzymes , hydrogen cyanide and catalase to improve plant health (khan , 2006) .

The objective of this study were to evaluate the the five cyanobacterial strains *N. muscorum* k , *A. oryzae* D, *A. oryzae* k , *N. pruniforme* D and *O. bervis* D for controlling flax seedling blight caused by *F.oxysporum* and *R.solani* and study the effects of their filtrates on seedling behavior of flax.

MATERIALS AND METHODS

Isolation and identification of cyanobacteria :

The five cyanobacterial strains " *Nostoc muscorum* k , *Anabaena oryzae* D, *Anabaena oryzae* k , *Nostoc pruniforme* D , and *Oscillatoria brevis* D " were isolated from rhizosphere of rice *(Oryzae sativum* L.) plant , cyanobacterial strains were identified morphologically , biochemically , and by cultural characters as described previously (Afify *et al.* , 2018) .These five cyanobacterial strains are chosen from fourteen strains because they were the most antagonistic ones against the flax pathogenic fungi *F.oxysporum* and *R. solani* . All cyanobacterial strains were isolated from rhizosphere of rice plant in the governorates of El-Dakahliya (D) and kafr El - Sheikh (k).

Source of pathogenic fungi isolates:

Fusarium oxysporum and *Rhizoctonia solani* isolates used in this study were obtained from the Fungal Collection of Cotton and Fiber Crops Diseases Reseach Section , Agric . Res.Center (ARC) Giza , Egypt .

Host plant :

Flax (*Linum usitatissimum* L.) Sakha1 cv. was supplied by Field crop Research Institute , ARC, Giza, Egypt.

Antagonism:

In vitro tests for antagonism of cyanobacterial filtrates against damping – off fungi *F. oxysporum* and *R.solani* were carried out by plate assays . The plates were incubated at 28 – 30 °C and observations were made up to 7 days on the inhibition of fungal growth (Sivamani and Gnanamanickam , 1988).

Phosphate solubilization :

For assay of phosphate solubilization , cyanobaterial strains were inoculated with 5% inoculums of P – straved , 15 day old actively growing culture of the algae for 35 days . The P-starved cultures were grown in presence of TCP at concentrations 20mg by replaing K2HPO4 in the usual BG11 medium . The method was described as Watanabe and Olsen (1965) and Jackson (1966) .

IAA estimation :

Five cyanobacterial strains were inoculated in 250 ml conical flask containing 100 ml BG11 medium with different concentrations (10 to 100 µg/ml) of tryptophan and incubated at 28 -30° C . IAA estimation was conducted in triplicate and OD was taken at 330 nm after 10 , 20 , 30 days according to Patten and Glick (2002).

Ammonia production :

Ammonia was evaluated by Dye (1962) the cynobacterial strains were grown in peptone water in 30 ml tubes and incubated at 28° C for 4 days . After that 1 ml of Nessler's reagent was added to each tube. Development of afaint yellow colour was indicative of weak reaction and deep yellow to brownish colour was indicative of strong reaction .

HCN production :

HCN production was evaluated by the qualitative method of kremer and Souissi (2001) .

Detection of enzymes by antagonistic cyanobacteria :

Production of hydrolytic enzymes were detected on plate by adding cyanobaterial filterate individually on the medium containing enzyme substrate , Ngarajkumar *et al* ., (2004) .

Germination test :

Late phase (21days) of the cyanobacterial strains culture were centrifuged and the cell free filterate were used for seed germination studies . The healthy seeds of flax were surface sterilized using 0.01% $HgCl_2$ solution for 5 min followed by several washing with distilled water for about one h. selected number of seeds (20) was then distributed on water agarized Petri plates (0.5% agar) and 10 ml of the following different treatments were added , N_1 , A_2 , A_3 , N_4 , O_5 and control .

N_1: *Nostoc muscorum* K
A_2: *Anabaena oryzae* D
A_3: *Anabaena oryzae K*
N_4: *Nostoc pruniforme* D
O_5: *Oscillatoria brevis* D

In control Petri dishes cyanobacterial filrate were replaced by water for evaluation . Perentage radical emergence and seed germination speed was recorded at 25°C after every 24 h time interval .Time for initial signs of radical emergence and maximum emergence was recorded up to three days .

Germination Velocity Index :

The germination speed index (CVI) was calculated as described in the Association of Official Seed Analysis (1983) by following formula :

$$G = \frac{N1}{1} + \frac{N2}{2} + \frac{N3}{3} + \text{..... so on}$$

Here N1 N2 N3 etc. are the no. of new germinate on day 1 , 2 , 3 etc . following the start of germination test . Since the no . of new germinate on a particular days divided by the serial number of that days the GVI is higher if more seeds geriminate in the fewest number of days .

Vigor Index :

Seedling vigor index was calculated following modified method of Abdul- Baki & Anderson (1973) :

VI = Seedling length (mm) x Germination %

Statistical Analysis :

The data were subjected to one way analysis of variance (ANOVA) . The treatment means were compared by LSD at a significance level of 0.05 .

RESULTS AND DISCUSSION

Antagonism:

Of the fourteen cyanobacterial strains tested for *in vitro* antagonism against pathogenic fungal isolates of *F .oxysporum* and *R. solani* ; these cyanobacterial strains : *N. muscorum* K , *A.* oryzae D , *A. oryzae* k , *N. pruniforme* D, and *O. brevis* D. consistently showed levels of inhibition *in vitro* antagonism against the two tested fungi . Other cyanobacterial strains were no effective or showed no antagonism (Table 1) , These results are in agreement with the previous reports , which indicated that bacterial isolates suppressed fungal growth *in vitro* Ashour and Afify (1999) and kumar and kaur (2014) by production of antifungal antibiotics .

Table 1. Antagonistic activity for fourteen cyanobacterial strains against two phytopathogenic fungi on flax .

Cyanobacterial strains	Fungi tested	
	F . oxysporum	*R. solani*
Nostoc paludosum D	-	-
Anabaena oryzae D	++	++
Anabaena oryzae K	++	+
Nostoc muscorum k	++	++
Nostoc pruniforme k	-	-
Nostoc pruniforme D	+	+
Nostoc verrucosum k	-	-
Nostoc verrucosum D	-	-
Nostoc entophytum k	-	-
Nostoc rivulari k	-	-
Nostoc viride k	-	-
Chroococcus minor k	-	-
Oscillatoria brevis k	-	-
Oscillatoria brevis D	-	+
Control (only funges)	-	-

Cyanobacterial strains isolated from two locations of : D= Dakahlia governorate ; K = Kafr El- Sheikh governorate
++ Inhibition of pathogen : by over growth
– no inhibition of pathogen + Inhibition of pathogen

Plant Growth promoting and protect substance:

In Table (2) when the determination of P-solubilization by the five cyanobacterial strains showed positive results for phosphate solubilization , IAA and all strains showed growth in nitrogen free media, All of the products improved plant growth by mounting up the availiability of phosphate and produce growth regulators such as auxin – like substances IAA (Hameeda *et al*., 2008) . Moreover , cyanobacterial strains may protect plants from phytopathogens due to ammonia , hydrogen cyanide and lytic enzymes production (kremer and Souissi ,2001) . The results showed high amount of P-soluliblization and IAA production with the strain *N . muscorum* (4.20 and 6.50 µg/L) respectively . In case of producing substances for protect plants from phytopathogens , all strains produced ammonia and catalase , while only *A.oryzae* D produced HCN and chitinase . Production of cellulase was not shown by all the tested cyanobacteria . These results were in agreement with Castenholz (2005) .

Table 2. Detection of different plant promotion and antagonistic properties by filterate of cyanobacterial strains

Cyanobacterial strains	P- solubilization (µ g/ml)	IAA (µg/ml)	Ammonia	HCN	Cellulase	Chitinase	Catalase
N.muscorum K	4.03	6.5	+	-	-	-	+
A.oryzae D	4.20	6.1	+	+	-	+	+
A.oryzae K	4.02	6.0	+	-	-	+	+
N. pruniforme D	3.85	4.5	+	-	-	-	+
O.brevis D	3.70	3.5	+	-	-	-	+
Control	3.58	3.5	+	-	-	-	+

Effect on seed Germination behavior of flax :

Culture filtrates of the tested cyanobacterial strains stimulated germination of flax seeds . A highly significant increase in percentage germination was 78.33 % with the *N.muscorum* K and *A.oryzae* D, respectively (Table 3) . These results are agreement with Martin (1995) who reported that culture filterate or cell extracts from cyanobacteria and algae applied to seeds protected them against damping –off fungi .

Data in Table 4&5 indicated that seeds treated with cyanobacterial filtrate faster , while in control seeds germinate slowly , so cyanobacterial filrate led to significantly higher value of the germination velocity index compared to seeds in under controlled treatment . Treatment

of *N. muscorum* K shows higher value of mean difference in germination velocity index . Further the seedlings arised from cyanobacterial filtrate treated seeds also had significantly higher values of the viger index . Martin(1995) also showed that the seedlings arised from cyanobacterial filterate treated seeds had significantly higher values of the vigor index . The germ – ination percentage , germination velocity index , vigor index were notably enhanced .

Table 3. Effect of cyanobacterial filtrate on germination percentage

Treatments	Germination % of total seeds
N.muscorum K	78.33 ± 7.26
A.oryzae D	78.33 ± 1.67
A.oryzae K	78.00 ± 1.67
N. pruniforme D	70.00 ± 2.88
O.brevis D	70.00 ± 0.00
Control	58.33 ± 3.33
LSD ($p \leq 0.05$)	9.50

Table 4. Effect of cyanobacterial filtrate on Germination Velocity Index (GVI)

Treatments	GVI
N.muscorum K	9.50
A.oryzae D	9.25
A.oryzae K	8.00
N. pruniforme D	7.90
O.brevis D	6.50
Control	4.00

Table 5. Effect of cyanobacterial filtrate on Velocity Index (VI)

Treatments	VI
N.muscorum K	8000
A.oryzae D	7950
A.oryzae K	6800
N. pruniforme D	6000
O.brevis D	6000
Control	3000

The result showed that cyanobacteria were effective in growth promotion and protection of flax from infection by soil born fungi.

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استخدام السيانوبكتيريا لمقاومة لفحة بادرات الكتان

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تعتبر الطحالب الخضراء المزرقه (السيانوبكتيريا) من اهم مجموعات البروكاريوتات (بدائيات النواة) التي لها تأثير علي تطور ونمو النباتات وذلك من خلال دورها كمشجعة لنمو النباتات وفي المقاومة الحيوية لعديد من الفطريات الممرضة للنباتات عن طريق إنتاج مواد نشطة حيويا . فى خلال هذه الدراسة تم احتيار خمسة سلالات سيانوبكتيريا من ضمن مجموعة سلالات السيانوبكتيريا المعرفه و المعزولة من محافظتي كفر الشيخ (k) والدقهلية (D) وهي : نوستوك مسكورم (K) ، أنابينا أوريزا (D) وانابينا أوريزا (k) ونوستوك بيرنيفورم (D) واوسلاوتوريا بريفس (D) هذه السلالات أظهرت معمليا قدرة عالية علي تضاد إثنان من فطريات التربة التي تسبب أمراض بادرات الكتان هما فيوزاريوم أكسوسبورم وريزوكتونيا سولاني وذلك بتقدير قدرتها علي إنتاج بعض المواد المشجعة لنمو النبات مثل إذابتها لعنصرالفوسفور وإنتاج إندول حمض الخليك بالإضافة للمواد المضادة لنمو الفطريات الممرضة مثل إنتاج الأمونيا وسيانيد الهيدروجين وبعض الإنزيمات حيث اظهرت جميع السلالات قدرتها علي إذابة الفوسفور حيث سجلت السلالة أنابينا أوريزا (D) أعلي إنتاج (4.20, µg/ml) بينما سجلت السلالة نوستوك مسكورم (k) أعلي قيمة في إنتاج إندول حمض الخليك (6.5 µg/ml) ولكن جميع السلالات كان لها القدرة علي إنتاج الأمونيا وإنزيم الكتاليز بينما فشلت في إنتاج إنزيم السليلوليز وبالنسبة لإنتاج سيانيد الهيدروجين كان مع السلالة أنابينا اوريزا (D). أما بالنسبة لإنزيم الكتينيز سجلت السلالتين انابينا أوريزا (D&K) نتيجة موجبة بينما فشلت باقي السلالات في إنتاجه وباستخدام راشح سلالات السيانوبكتيريا مع بذور الكتان كانت هناك سرعة في إنبات بذور الكتان المعاملة براشح السيانوبكتيريا عن البذور الغير معاملة (الكنترول) بالإضافة إلى أن البادرات المعاملة براشح السلالات كانت أطول من البادرات الغير معاملة وكذلك زيادة نسبة وسرعة الإنبات في بذور الكتان كانت مع البذور المعاملة أعلي من البذور الغير معاملة (الكنترول) .