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# Using Cyanobacteria (*Nostoc* spp.) in the Biological Control of Damping off Disease in Cotton

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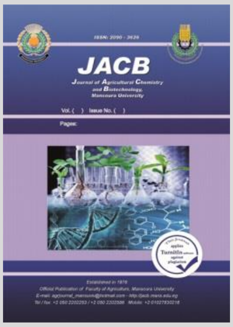

## ABSTRACT

Cyanobacteria are one from the main classes of bacteria that promote growth of plants and in biocontrol of soil-borne fungi. *In vitro* and *in vivo*, this study investigated the effect of application *Nostoc* spp. filtrates (*Nostoc lichenoides, Nostoc indistinguendun* and *Nostoc favosum*) on the *Fusarium oxysporum* and *Rhizoctonia solani* that infects cotton (*Gossypium barbadense* L.) plant. *Nostoc* spp. strains were tested to antagonize both pathogenic fungi. Total phenolic compounds, polysaccharides contents and dry weight were determined of *Nostoc* spp. strains. In a greenhouse test in soil infested with *F. oxysporum* and *R. solani* isolates the three filtrates from cyanobacterial strains and their mixture were effective in increasing the dry weight and the surviving seedlings percentage; however, the filtrates of strains had no effect on seedling height. The strains showed that the highest efficiency to inhibit the post-damping off of *F. oxysporum* and *R. solani* when they were applied in a mixture. Under field conditions the three strains were consistently effective in increasing stand and yield of cotton plant in 2021 and 2022 growing seasons; however, their efficiency was much higher when they were applied in a mixture. Finally, from the obtained data we concluded that using biotic factors for controlling damping off of cotton plant pathogens such as *F. oxysporum* and *R. solani* were effective.

***Keywords:*** *Nostoc* spp., biocontrol, cotton, pathogenic fungi

## INTRODUCTION

The cyanobacteria are the largest diverse and widely distributed groups of photosynthetic prokaryotes (Stanier and Cohen-bazire, 1977) and introduce big amounts of biomass (Cannell, 1993). Recently, cyanobacteria are very important source of biologically active compounds for pathogenic bacteria and fungi. It can release soluble organic substances as extracellular products by the cyanobacteria which to increase growth of plant (Zulpa *et al.* 2003; Singh *et al.* 2016 & Sammauria *et al.* 2020). Microalgae are different communities of photosynthetic prokaryotes they found in soil, water, and air ecosystems (Seckbach, 2007). Currently, the farmers extensively used pesticides for the protection of crops from pests and insects (Parte *et al.* 2017). The use of pesticides in agriculture has become very essential because of the reduced crop yield due to pests (Verma *et al.* 2014). A major cause of environmental pollution because their pollutants (pesticides) in soil and water (Rani and Dhania 2014). The important source of a vast assortment of biologically active products are the algae (Metting and Pyne, 1986; Cannell, 1993; Radmer and Parker, 1994). Cyanobacteria and microalgae could be applied for plants to protect against pathogenic fungi. However, it is a greater chance of success when using culture filtrates or cell extracts from cyanobacteria applied to seeds as protectants from damping-off fungi : *Fusarium* spp., and *Rhizoctonia solani* (Burris, 1994). A number of cyanobacteria, produce various biologically active compounds which inhibited bacteria and fungi that incite diseases of plants (Papavizas and Lewis 1981 & Martin, 1995). The aims of this study is to evaluate biological control with the cyanobacterial strains and their products for the control of phytopathogenic fungi in cotton plants.

## MATERIALS AND METHODS

**Cyanobacterial strains**

The three cyanobacterial strains (*Nostoc lichenoides, Nostoc indistinguendun* and *Nostoc favosum*) were isolated from clay loam soil which some physical and chemical characteristics and by culture, morphological and molecular according to Afify *et al.* (2023) were isolated and identified as Cyanobacteria phylum of the Bacteria domain. They form a monophyletic group with a high diversity in terms of morphology, physiology and molecular properties (Hillary *et al.* 2022).

**Pathogenic strains**

*Fusarium oxysporum* and *Rhizoctonia solani* isolates were isolated from diseased plants and identified by Plant Pathology Research Institute, Agricultural Research Center (ARC), Giza, Egypt .

**Plant used**

Cotton (*Gossypium barbadense* L.) cv. used as a host was obtained from the Agricultural Research Center (ARC), Giza, Egypt.

**Preparation of cyanobacterial culture filterate**

Culture at the exponential phase were centrifuged at 10,000 rpm for 3 min. and filtered through Wattman-4 filter paper (Starr *et al.* 1962).

**Antagonism**

*In vitro* plate assays were carried out for antagonism tests between cyanobacterial filtrate against damping-off fungi (*F. oxysporum* and *R. solani*). After incubated plates the inhibation zone were recorded when the control plate was full growth by tested fungus (Sivamani and Gnanamanickam, 1988).

**Estimation of phenols and polysaccharides content**

Phenols and Polysaccharides {Intracellular (IPS) and Extracellular Polysaccharides (EPS)} of tested cyanobacteria

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were estimated according to Jindal and Singh (1975) & Shi *et al.* (2007) respectively.

**Production of pathogenic fungi inoculum for soil infestation**

Substrate for growth of fungi isolates were prepared in 500-ml glass bottles. Each contained 50g of sorghum grains and 40 ml tap water and autoclaved for 30 minutes. Inocula were taken from one-week old PDA cultures and aseptically introduced into the bottles and allowed to colonize sorghum grains for three weeks.

**Prepartion of cyanobacteria inoculum for seed treatment**

Each cyanobacterial strain was grown for 48 h at 28ºC on broth medium. For single- strain inoculations 1.5 ml of a cyanobacterial filtrate and 5g of cotton seeds were mixed in a small plastic bag. For mixture strains inoculations, equal volumes of cell filtrate were mixed and 1.5 ml of this mixture was then applied to 5g of seeds. *In vivo*, cotton seeds treated with the cyanobacterial strains (individually or in mixture) were planted with one week after soil infestation. Percentage of surviving seedlings, dry weight (g/plant) and plant height (cm) were recorded 40 days from sowing (Afify and Ashour 2018).

**Statistical analysis**

ANOVA was performed by the software A Microcomputer Program for the Design Management (MSTATC Michigan State Univ., USA). Before carrying out analysis of variance (ANOVA) to produce approximately constant variance percentage data were transformed into arc sine angles.

## RESULTS AND DISCUSSION

**Antagonism**

Antagonism against the three *Nostoc* spp. filtrates and fungal isolates of *F. oxysporum* and *R. solani* (Table 1) *in vitro.* These cyanobacterial strains: *N. lichenoides, N. indistinguendun, N. favosum* and their mixture showed different of inhibition zone with the two tested pathogenic fungi. At the other side, cyanobacterial strain (*N. favosum*) was no effective or no antagonism, but the best antagonism showed with the mixture from strains (Table 1). Our data are in accordance with the reports *in vitro* , which reported that isolates of bacteria inhibited growth of pathogenic fungi (Ashour and Afify 1999 & Kumar and Kaur 2014) by various compounds as antifungal.

**Table 1. The antagonism between *Nostoc* spp. against two fungi ( *F. oxysporum* and *R. solani*) *in vitro***

| *Nostoc* spp. No. | Inhibition zone of fungal isolates (mm) *F. oxysporum* | *R. solani* |
|---|---|---|
| *Nostoc lichenoides* | 5.0[a] | 3.0 |
| *Nostoc indistinguendun* | 4.0 | 1.0 |
| *Nostoc favosum* | 0.0 | 0.0 |
| Mixture | 13.0 | 13.0 |
| Control (without cyanobacteria) | Full growth | Full growth |

**[a] Inhibation zone (mm); Values represent Means (n=5)**

All strains of *Nostoc* consistently showed no antagonism *in vitro* between their filtrates. Patterns of interaction among the three strains of *Nostoc* spp. filtrates on Nutrient Agar (NA) and Potato Dextrose Agar (PDA) media are shown in Table 2. All the three strains of *Nostoc* spp. filtrates were not inhibited by any strain with other. Fukui *et al.* (1994) reported that it is well-known that patterns of interacton among organisms *in vitro* do not necessarily occur *in vitro*, although the reasons are not clear.

**The antifungal agents as phenols and polysaccharides content**

Estimation of polysaccharides and phenol contents that produced from tested cyanobacteria strains and their antifungal activities. The results showed that as the phenol contents of the tested *Nostoc* species were increased (Table 3). Our data are in agreement with a some of authors e.g., De Cano *et al.* (1990) found that phenolic compounds from *Nostoc* sp. inhibited the growth of pathogenic yeast and bacteria. At the same line, to polysaccharides which significant role as bioagent act for cyanobacteria. Results showed that the content of polysaccharides of *N. lichenoides* was higher than *N. indistinguendun* and *N. favosum* as the antifungal activity. Marine algae were used to protect plants from infections of phytopathogenic fungi by production oligosaccharides (Potin *et al.* (1999).

**Table 2. Antibiosis between *Nostoc* spp. strains on agar media**

| *Nostoc* spp. filtrates inoculated second | *Nostoc* spp. inoculated first on nutrient agar (NA) *Nostoc lichenoides* | *Nostoc indistinguendun* | *Nostoc favosum* |
|---|---|---|---|
| *Nostoc lichenoides* | + | _+ | + |
| *Nostoc indistinguendun* | + | - | _+ |
| *Nostoc favosum* | + | _+ | _+ |
| | *Nostoc* spp. inoculated first on potato – dextrose agar (PDA) | | |
| *Nostoc lichenoides* | + | + | _+ |
| *Nostoc indistinguendun* | + | - | _+ |
| *Nostoc favosum* | + | +_ | _+ |

**Growth of strains inoculated second: + = normal growth equivalent to the growth in single inoculation; +: reduced growth compared to the growth in single inoculation; and - = no growth after 24h but slight growth observed after 72h.**

**Table 3. Total phenol and polysaccharides contents of *Nostoc* spp.**

| Biological agents Cyanobacteria | Total phenol contents (mg/ $g^{-1}$ DW) | Polysaccharides content (mg/ $g^{-1}$ DW) IPS (mg/$g^{-1}$ DW) | EPS (mg/$mL^{-1}$ ) |
|---|---|---|---|
| *Nostoc lichenoides* | 0.88 | 2.9 | 160.1 |
| *Nostoc indistinguendun* | 0.80 | 2.1 | 155.3 |
| *Nostoc favosum* | 0.44 | 1.9 | 151.7 |

**IPS: Intracellular Polysaccharides; EPS: Extracellular Polysaccharides; Values represent Means (n=5)**

All treatments showed significant variation in dry weight of seedlings. All *Nostoc* strains filtrats, whether they were applied singly or in a mixture effective in increasing dry weight of cotton seedlings; however, they showed the highest efficiency when applied in a mixture (Table 4). The cyanobacteria isolates are increased dry weight that described as indicator of cyanobacterial growth (Singh *et al*. 2016; Ramirez *et al*. 2019). *Nostoc* spp. had the highest biomass production, recording 348, 318 and 310 mg/100ml of liquid culture (Afify *et al.* 2023).

**Table 4. Effect of *Nostoc* spp. filtrates on dry weight in cotton seedlings under greenhouse conditions**

| Trearments | Fungi involved in cotton seedlings disease *F. oxysporum* | *R. solani* | Natural soil | Mean |
|---|---|---|---|---|
| *Nostoc lichenoides* | 2.00 | 2.02 | 1.88 | 1.96 |
| *Nostoc indistinguendun* | 2.04 | 2.99 | 2.06 | 2.36 |
| *Nostoc favosum* | 1.98 | 2.08 | 2.15 | 2.07 |
| Mixture | 2.16 | 2.09 | 1.86 | 2.03 |
| Nutreint broth | 1.86 | 1.78 | 2.18 | 1.94 |
| Control (without cyanobacteria) | 1.87 | 1.80 | 1.86 | 1.84 |
| Mean | 1.98 | 2.13 | 1.99 | |

**L.S.D. for Treatments (T) = 0.06 (P= 0.05)**

**Effects of tested cyanobacteria under greenhouse and field conditions**

Treatments were non significant source of variation in plant height that is *Nostoc* strains filtrates had no effect on plant height which was affected only by the fungal isolates (Table 5). In Table (6) mixture from *Nostoc* strains filtrates were significant effect on seedlings survival of cotton under greenhouse conditions. At the same time (Table 7), under field conditions *Nostoc* strains filtrates were consistently effective in increasing stand and yield in 2021 and 2022 growing seasons; however, their efficiency was mush higher when they were applied in a mixture. The current study demonstrates that, in greenhouse and field tests, certain *Nostoc* strains filtrates combinations have the potential for greater biocontrol activity, against soilborne fungi involved in cotton seedling disease, compared to the same strains applied individually. The data are agreement with those workers (Wolk and Sarkar 1993; Pierson and Weller 1994). Also, Kulik (1995) reported that when added cyanobacterial filtrates to seeds can protect plant from soil-borne fungi.

**Table 5. Effect of *Nostoc* spp. filtrates on plant height (cm) under greenhouse conditions**

| Trearments | Fungi involved in cotton seedlings disease | | Natural soil | Mean |
|---|---|---|---|---|
| | ***F. oxysporum*** | ***R. solani*** | | |
| *Nostoc lichenoides* | 31.1 | 30.2 | 35.3 | 32.2 |
| *Nostoc indistinguendun* | 31.0 | 30.9 | 33.7 | 31.8 |
| *Nostoc favosum* | 31.8 | 30.3 | 31.8 | 31.3 |
| Mixture | 33.53 | 35.0 | 34.5 | 34.3 |
| Nutreint broth | 29.08 | 29.1 | 30.6 | 29.5 |
| Control (without cyanobacteria) | 29.88 | 28.9 | 30.7 | 29.8 |
| Mean | 31.06 | 30.7 | 32.7 | |

**L.S.D. for Fungi (F) = 1.1 (P = 0.05)**

**Table 6. Under greenhouse conditions, *Nostoc* spp. filtrates effect of on seedlings survival of cotton**

| Trearments | Fungi involved in cotton seedlings disease | | Natural soil | Mean |
|---|---|---|---|---|
| | ***F. oxysporum*** | ***R. solani*** | | |
| *Nostoc lichenoides* | 37.5[a] (37.66)[b] | 45.0 (42.12) | 67.5 (58.61) | 50.0 (45.57) |
| *Nostoc indistinguendun* | 55.0 (47.89) | 47.5 (43.56) | 57.5 (49.33) | 53.3 (46.89) |
| *Nostoc favosum* | 55.0 (47.89) | 27.5 (31.39) | 57.5 (49.33) | 46.6 (42.77) |
| Mixture | 65.0 (53.78) | 67.5 (55.29) | 77.5 (61.78) | 70.0 (57.11) |
| Nutreint broth | 7.5 (13.83) | 7.5 (13.83) | 25.0 (29.89) | 13.3 (21.12) |
| Control (without cyanobacteria) | 7.5 (13.83) | 15.0 (22.50) | 22.5 (28.22) | 15.0 (17. 33) |
| Mean | 37.9 (37.73) | 35.0 (36.22) | 51.25 (45.89) | |

**[a]Percentage data** **[b]Arc sine – transformed data** **L.S.D. (transformed data) for TXF = 7.68(P= 0.05)**

**Table 7. Under field conditions, *Nostoc* spp. filtrates effect on the incidence of cotton seedlings disease and seed cotton yield**

| Trearments | Seedling survival % | | Seed cotton yield (kentar/ fed.) | |
|---|---|---|---|---|
| | **2021** | **2022** | **2021** | **2022** |
| *Nostoc lichenoides* | *57.25(49.18) | 54.00(47.31) | 4.57 | 3.76 |
| *Nostoc indistinguendun* | 45.50(42.42) | 37.75(37.75) | 4.03 | 3.58 |
| *Nostoc favosum* | 43.25(46.87) | 45.75(42.56) | 4.10 | 3.42 |
| Mixture | 67.75(55.43) | 67.25(55.14) | 5.14 | 4.40 |
| Nutreint broth | 32.00(34.33) | 33.00(35.05) | 3.21 | 3.23 |
| Control (without cyanobacteria) | 28.25(32.07) | 32.50(34.75) | 3.19 | 3.20 |
| Mean | 45.66(42.50) | 45.04(42.11) | 4.04 | 3.59 |
| **L.S.D. (P = 0.05)** | **5.09** | **4.67** | **0.38** | |
| | **0.41** | | | |

*** Before carrying out the analysis of variance percentage data were transformed into arc-sine angles.**

## CONCLUSION

This work is conducted for using cyanobacterial strains (*Nostoc* spp.) as bioagent against phytopathogenic fungi which the causal agent of cotton damping off. The bioagents factors contained phenols and polysaccharides used for their antifungal activity. Using filtrats of the *N. lichenoides*, *N. indistinguendun*, *N. favosum* and their mixture expressed potency in the improvement of cotton plant. Finally, from the obtained data we recommended use biotic factors for controlling damping off of cotton.

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## إستخدام السيانوبكتيريا ( أنواع من النوستوك ) فى المقاومه الحيويه لأمراض موت البادرات فى القطن

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### الملخص

السيانوبكتيريا هى أحد مجموعات البروكاريوتات (بدائيات النواه) الهامه لما لها من تأثير على تطور نموالنبات بالإضافه لدورها الكبير فى المقاومه الحيويه لفطريات موت البادرات فى نبات القطن . فى هذه الدراسه أستخدمت راشح لكلا من ثلاثة أنواع من جنس النوستوك معمليا لتقدير قدرتها على تضاد الفطريات الممرضه وكذلك تقدير التضاد فيما بينها ثم تم تقدير منتجات التضاد مثل المواد الفينوليه والمحتوى من السكريات وكذلك الوزن الجاف . وقد أظهرت نتائج التضاد المعمليه قدره عاليه فى مقاومة الفطريات الممرضه بينما لم يوجد أى تضاد بين سلالات النوستوك وبعضها وعند تقدير المواد المنتجه وجد أن النوع *Nostoc lichenoides* أظهر أعلى قيمه من إنتاج المواد الفينوليه والسكريه والوزن الجاف . وعندما أختبر راشح السلالات الثلاث ومخلوطها تحت ظروف الصوبه فى تربه ملوثه بعزلات الفطرين كانت كلها فعاله فى زيادة النسبه المئويه للبادرات الباقيه على قيد الحياه وفى زيادة الوزن الجاف لهذه البادرات . إلا أن جميع السلالات لم يكن لها تأثير على طول البادرات وقد أظهرت السلالات أعلى درجات الفاعليه عند إستعمالها على شكل مخلوط . ولكن أدى إستعمال نفس المعاملات تحت ظروف الحقل فى موسمى 2021/ 2022 إلى زياده مئويه فى نسبة الإنبات ومحصول القطن الزهر حيث كانت أعلى درجات الفاعليه عند إستعمال السلالات فى شكل مخلوط . من هذه النتائج يمكن إستخدام العوامل الحيويه مثل السيانوبكتيريا كعوامل فعاله فى مقاومة مرض موت البادرات فى القطن وذلك بوقف نمو هذه الفطريات الممرضه *F. oxysporum* and *R. solani*