Using of Cyanobacteria in controlling potato brown rot disease

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Abstract-- The aim of the present study was to evaluate the antibacterial activity of culture filtrates of three Cyanobacterial species namely: *Nostoc muscorum*, *Spirulina platensis* and *Anabaena flos-aquae* on the growth of *Ralstonia solanacearum*, the causal agent of potato brown rot disease, *in vitro.* The efficiency of the Cyanobacterial cultures in disease suppression was also evaluated under greenhouse conditions. Six virulent isolates of the pathogen isolated from infected potato tubers were used in this study. The three tested Cyanobacterial species exhibited inhibitory effect against *R. solanacearum* growth; *A. flos-aquae* showed the highest inhibition zone of 13.7 mm on Kings'B medium followed by 11 and 7.25 mm for *S. platensis* and *N. muscorum*, respectively. Also, the three tested species of Cyanobacteria significantly reduced the bacterial wilt incidence. *A. flos-aquae* was the most effective treatment that completely suppressed the disease. Both of *N. muscorum* and *S. platensis* treatments effectively reduced wilt severity up to 5 and 3.33%, respectively as compared to positive control. In addition, the application of the tested Cyanobacterial species improved the estimated plant growth parameters and increased plant content of the photosynthetic pigments (chlorophyll a, b, total chlorophyll and carotenoids), ascorbic acid, total phenols and proline as well as NPK and Na contents in the treated plants as compared with the positive control plant.

Key words : Potato , *Ralstonia solanacearum*, bacterial wilt, blue green algae, growth parameters , photosynthetic pigments, ascorbic acid, total phenols , proline , NPK and Na contents.

1 INTRODUCTION

Potato (*Solanum tuberosum* L.) is an important vegetable crop in Egypt for local consumption and exportation. It ranks the fourth most important staple food after wheat, rice and maize. Egypt is ranked the first African potato producer. The United Nations FAO reported that the world total cultivation of potatoes reached about 439.847 feddan, which produced about 4,800,000 ton of tubers (Anonymous, 2014).

Potato brown rot disease caused by *Ralstonia* (*Pseudomonas*) *solanacearum* was reported for the first time in Egypt at El-Gemmeiza farm, Gharbia governorate based on symptomtology only by Briton-Jones (1925). *R. solanacearum*, is a soil borne phytopathogenic bacterium in the *ß*-subdivision of the Proteobacteria with a global distribution (Yabuuchi *et al.*, 1995). *R. solanacearum* is subdivided into five races on the basis of host range and five biovars on the basis of biochemical properties (Schaad, 1988 and Hayward ,1991).Recently, the dominant race in Egypt and Europe is race 3 biovar 2, classified on the basis of genetic sequencing as Phylotype II, sequevar 1 (Fegan and Prior, 2005). As a diverse species complex, *R. solanacearum* has developed an extremely broad host range throughout the world, including over 450 host species representing 54 plant families (Wicker *et al.*, 2007).

The wide host range and broad geographical distribution have made *R. solanacearum* an economically significant pathogen. It considered to be a severe obstacle to the production of Solanaceous plants in tropical, subtropical and temperate regions .European and Mediterranean Plant Protection Organization (EPPO) has listed *R. solanacearum* as an A_2 quarantine pest (Lee *et al*., 2012). Integration of different management practices such as using disease-free planting materials, selecting less susceptible crop varieties, rotating with non-host crops and amending soil with biological or non-biological agents have been employed to manage bacterial wilt (Akiew and Trevorrow, 1994 and Saddler, 2005).

Biocontrol agents (BCAs) employ several mechanisms such as antagonism and competition, in addition to suppressing plant diseases. Bioagents are able to induce plant growth via several mechanisms, including production of phytohormones and other secondary metabolites, changing host physiology (Kim *et al*., 2006).Also, induction the host resistance to different plant pathogens (Mc Spadden Gardener, 2004; Pal and Mc Spadden Gardener, 2006 and Pieterse and Wees, 2015). Cyanobacteria, also known as blue-green algae (BGA), Cyanoprokaryotes and Cyanophytes, are oxygenic photosynthetic prokaryotes that capable of carrying out photosynthesis and nitrogen fixation simultaneously; a morphologically diverse group of gram negative eubacteria. Recently many researchers have been focused on the biotechnological potentials of Cyanobacteria for obtaining pharmacologically active secondary metabolites and that led to identification a wide range of compounds possessing antimicrobial, antiviral, and toxic properties (Namikoshi *et al.*, 1996). The blue green algae possess a diverse structure and have a wide distribution throughout the globe. They are considered to be a rich source of products having pharmaceutical and toxicological potential, which primarily include metabolites such as carbohydrates, proteins, vitamins, pigments, fatty acids and antioxidants (Archana and Shivani, 2012) and /or various secondary metabolites such as phenolic compounds (De Cano *et al.*, 1990; Pedersen and Da Silva, 1973 and Volk, 2005), peptides, alkaloids or terpenoids and glycosides which showed some different bioactivities as antifungal, antiviral. (Ramamurthy, 1970 and Bonjouklian *et al.*, 1991).

Several blue green algal species contain natural bioactive compounds that seemed to be potent antimicrobial agents, for example *Spirulina* species, have some valuable antiviral, antibacterial and antioxidant compounds (Ozdemiret *et al*, 2004 and Khan *et al.*, 2006). Also, *Anabaena* s pp. (Frankmolle *et*

al., 1992 and Moore *et al.*, 1986) and *Nostoc* spp. (Bloor and England, 1989) produce biologically active compounds that have antifungal and antibacterial activity against several plant pathogens (Kulik, 1995 and Schlegel *et al*, 1998).

The present study was carried out to evaluate the antibacterial activity of three species of Cyanobacteria named as: *Nostoc muscorum*, *Spirulina platensis* and *Anabaena flos-aquae* against *R. solanacearum in vitro* and their efficacy on the disease suppression as well as the plant growth under greenhouse conditions .Also, the changes in some chemical constituents of the treated plants was estimated.

2 MATERIALS AND METHODS

Isolation and identification of the pathogenic bacteria

Infected potato tubers were collected from Ismailia and El - Minufiya governorates. Isolation procedures were carried out using a semi selective medium of South Africa SMSA (Elphinstone *et al*., 1996) under incubation conditions according to European and Mediterranean Plant Protection Organization (EPPO) (Anonymous, 1990). Pathogenicity test was conducted in 3 leaves tomato plants. Inoculation was made by stem puncture technique according to Janse (1988). Morphological, cultural, physiological and biochemical characteristics were determined according to Hayward (1964) and Palleroni (1984). The immunofluorescence antibody staining (IFAS) described by Janse (1988) as a serological method for rapid detection and presumptive identification of bacteria was also conducted. In addition, Conventional PCR amplifying the internally transcribed spacer region between 16S and 23S (Pastrik *et al.*, 2002) was carried out.

Preparation of Cyanobacterial culture filtrates:

Both of *Nostoc muscorum* and *Anabaena flos-aquae* were grown on media described by Watanabe (1951) at the Lab. of Phycol. , Dept. of Botany, Fac. of Sci., Cairo Univ., Giza, Egypt , under continuous fluorescent white light , intensity was kept at 200 LUX and temperature 28 ºC, while *Spirulina platensis* was grown on Zarrouk media (Zarrouk 1966) under temperature 30^{o}C and continuous fluorescent light . After 21 days the biomass was separated from the cultural medium by centrifuging (40 min, 800 g , 10 c) , the supernatant was sterilized using 0.25 µm syringe filter.

Effect of Cyanobacterial culture filtrate on *R. solanacearum* growth *in vitro*

Testing the antibacterial activity of the three species of Cyanobacteria was carried out using filter paper disc diffusion assay (Bajpai *et al.*, 2009). 1 ml of bacterial suspension (10^{7}cfu/ml) prepared from 24-48 h. virulent culture of *R. solanacearum*, evenly spread onto the surface of king's B medium. Whatman filter paper discs (0.5 mm in diameter) were sterilized by autoclaving at 121°C for 15 min. The sterilized paper discs were aseptically dipped in the culture filtrate of each Cyanobacteria, then were placed on the surface of the plates. Four discs per plate and five replicates for each treatment were used, discs dipped in sterilized water served as negative control. The plates were incubated upside down at 28 °C for 48 hours. Antibacterial activity was evaluated by measuring the diameter of the cleared inhibition zone

Effect of Cyanobacteria on the disease incidence *in vivo*

Sandy soil from Ismalia governorate, collected from specific area with no disease history, were used. Pots of 25 cm diameter were filled with 3.5 kg/pot with unsterilized sandy soil. Soil inoculation was made using collection of six virulent isolates of *R. solanacearum*. The isolates were previously propagated on King's B medium for 72 hour at 28 °C. The inoculum density was adjusted to give final concentration of 10^{7}cfu/g dry soil.

This experiment was performed under greenhouse conditions at PBRP (potato brown rot project), where temperature was adjusted to 25±2°C during the day and 20±2°C during the night, with a RH of 75 to 80% and a 14 h light d^{-1}. Experimental blocks design was completely randomized. One tuber of susceptible Bellini potato cv. was planted per pot. The tubers were soaked in one of the three tested Cyanobacterial culture filtrate for 10 min before planting in the pots. Six replicates for each treatment were made. Six pots un-inoculated with the pathogen were served as positive controls, where three pots were treated only with water and the three others were treated only with Cyanobacteria served as negative control.

Watering of plants was made regularly throughout the growth period. Soil samples were collected at the end of the experiment for bacterial count. The disease progress was determined according to the key proposed by Winstead and Kelman (1952). Disease incidence was estimated by AUDPC (area under disease progressive curve) and the progress of the wilt severity was recorded daily till the end of the experiment. CFU (colony forming unit) count in soil / g dry soil, rhizosphere and in crown area (lower stem area) was detected on SMSA medium (Elphinston *et al.*,1996). Random typical and suspected colonies were confirmed by IFAS (Janse, 1988) and conventional PCR (Pastrik *et al.*, 2002).

Morphological and physiological characteristics

I-Plant growth parameters

At the end of the experiment, 69 days after planting, vegetative growth characteristics was determined by random sampling of 3 plants. Vegetative growth was determined as indicated by plant height (cm), shoot fresh and dry weight (g), leaf weight (g), leaf area using disc method (cm^{2}), pathogen inoculated and non-inoculated plants were determined.

II-Chemical constituents

II.1-Photosynthetic pigments

Fresh leaf samples from the third terminal leaf (0.5 gm) 69 days after planting were extracted by methanol for 24 h. at laboratory temperature after adding trace of sodium carbonate, then chlorophylls a, b, total and carotenoids were determined by spectrophotometer (Jenway 6300) absorbance at wave lengths of 650, 665, 452 and calculated by equations of Mackinney,(1941).

Chlorophyll a (mg/l) =16.5 OD665 -8.3 OD650

Chlorophyll b (mg/l) = 33.8 OD650 -12.5 OD665

Carotenoids = (4.2×OD452)-(0.0264×Chl a) + (0.496×Chl b) =mg/l

II.2- Estimation of non-enzymatic antioxidants

a. Total ascorbic acid

Content of ascorbic acid was determined by using the dye 2, 6 dichlorophenol indophenols for mg/100g fresh weight. Method was described by Ranganna (1979).

b. Total phenols

Sample of 1 g was homogenize in 80% volume ethanol 10 times, then centrifuge at 10,000 rpm for 20 min save the supernatant, re-extracted the residue with 5 times in 80% ethanol, centrifuge and pool the supernatant evaporate the supernatant to dryness then add 0.5ml of folin ciocalteu

reagent, after 3 min 2 ml of 20% Na_2CO_3, was added and mixed thoroughly. Place the tube in boiling water for exactly one min followed by cooling and measure at 650nm absorbance against a reagent blank (Singleton, 1999).

c. Proline

Half gram of fresh leaf tissues were homogenized in 10 ml of 3 % (w/v) aqueous salphosalicylic acid and filtered. 2 ml of the filtrate, 2 ml of ninhydrin were added, followed by the addition of 2 ml of glacial acetic acid and boiling for 60 min. the mixture was extracted with toluene and free proline was quantified spectrophotometrically at 520 nm (Bates *et al.*, 1973).

NPK and Na content

NPK content was estimated in dried plant material. Nitrogen was determined using micro Kjedahl method according to Black *et al.*, (1965), phosphorus content was determined spectrophotometrically according to Olsen and Sammers (1982) and total potassium according to the method described by Jackson (1958).Na was determined by flame photometric method according to Cottenie *et al.* (1982)

Statistical analysis

All analysis was conducted using SPSS v23. Data of different physiological characteristics were normally distributed so they were subjected to ANOVA (post-hoc, LSD) .The data of disease incidence were not normally distributed and were subjected to Kruskal-Wallis, non-parametric analysis.

3 RESULTS

Isolation and identification of the pathogenic bacteria

Fluidal, irregular, white and/or white with pink centers colonies, typical for *R. solanacearum* virulent form, were picked. Six isolates from infected potato tubers showed an agreement with *R. solanacearum* race 3 biovar 2 characteristics according to Bergey's Manual of systematic Bacteriology (Palleroni, 1984). Serological testing by means of Immuno-fluorescence antibody staining (IFAS) and a single band at 718 pb from all isolates confirmed the identification of the pathogen.

Effect of Cyanobacterial culture filtrate on *R. solanacearum* growth *in vitro*

Antibacterial activity of three species of Cyanobacteria namely: *Nostoc muscorum, Spirulina platensis* and *Anabaena flos-aquae* against the bacterial pathogen *R. solanacearum* were evaluated using paper disc diffusion method. Data in Table (1) and Fig. (1) show that the Cyanobacterial culture filtrate of the three tested species were suppressive to the growth of *R. solanacearum* on the King's B medium compared with the control as indicated by a free clear zone free from the pathogen.

Table 1. Effect of the tested Cyanobacterial culture filtrates on the growth of *R. solanacearum in vitro*

Cyanobacteria	Inhibition zone (mm)(mean±SE[4])
N. muscorum	7.25±0.4[1, 2]
S. platensis	11±1.3[2, 3]
A. flos-aquae	13.7±0.7[1, 3]

[1] *P* value < 0.001, [2] *P* value = 0.008, [3]*P* value = 0.046, 4=Standard error.

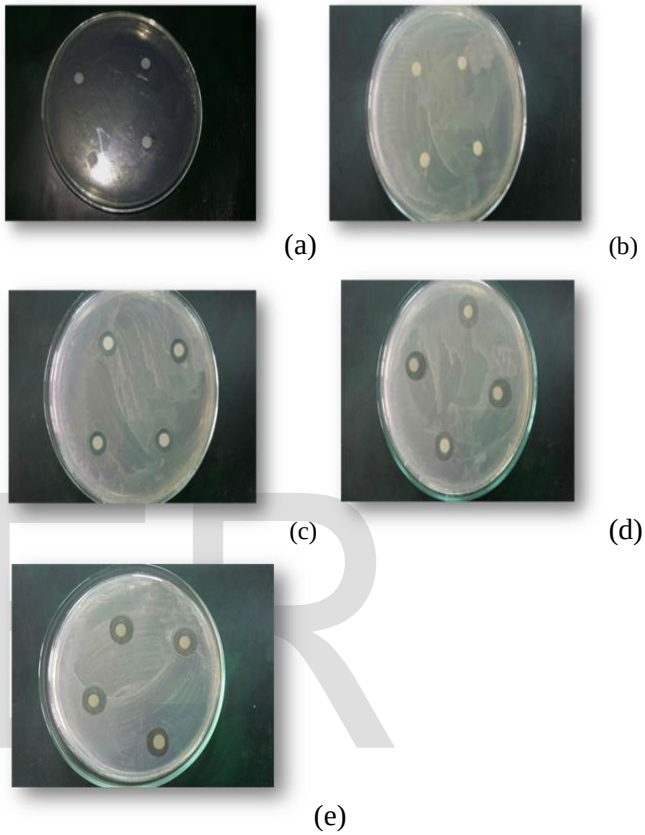

Figure 1. Effect of cultural filtrates of three Cyanobacteria on the growth of *R. solanacearum in vitro* (a) Negative control,(b) Positive control(c) *N. muscorum* , (d) *S. platensis* (e) *A. flos-aquae.*

Effect of Cyanobacteria on the disease incidence under greenhouse conditions

Effect of the three tested species of Cyanobacteria on potato brown rot disease was estimated by the count of *R. solanacearum* per gram dry soil, rhizosphere, plant crown area as well as AUDPC (area under disease progressive curve) and wilt severity as shown in (Table, 2). A Significant difference was observed in the counting of the pathogen in soil, rhizosphere and crown area in each treatment as compared with the positive control at the end of the experiment (69 days).

The three tested species of Cyanobacteria were very effective in reducing the wilt severity to 5.0 and 3.33 %with the treatments of *N. muscorum* and *S. platensis*, respectively. *A. flos-aquae* was the most effective treatment was which resulted in completely inhibition of the disease as expressed by the count of the pathogen (cfu/g) in the crown area, AUDPC and disease severity 0.0%.

Effect of the tested Cyanobacteria on the growth of infected and non-infected potato plants:

The effect of the tested Cyanobacteria on the growth of potato plants was indicated by plant height, leaf fresh weight, leaf area, shoot fresh weight and shoot dry weight is shown in table (3). Results (Table,3) show significant increasing in all vegetative growth parameters of the treated plants as compared to the positive control infected with the pathogen.

Also, it was observed that the growth of non-infected potato plants treated with the tested Cyanobacteria ,which served as negative controls showed significant increasing in plant height values compared with the non-treated plants. Most of the vegetative growth parameters were nearly similar to the non-treated negative control as shown in Table (4).

Table 2. Effect of the tested Cyanobacteria species on potato bacterial wilt disease as expressed by cfu[1] of *R. solanacearum* per g dry soil and plant crown area as well as wilt severity and AUDPC.

Treatments	Zero time cfu[1]/g dry soil	Final cfu/g dry soil	Rhizosphere area cfu/g dry soil	Crown area,cfu/g	Wilt severity %[c]	AUDPC[4,c]
P. C[2]	3.91E+05	2.77E+05	1.20E+08	4.21E+07	70.00	9.49E+02
N.C[3]	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00	0.00E+00
N. muscorum	3.93E+05	3.86E+03[a]	3.40E+05[a]	3.54E+03[a]	5.00	3.00E+01
S. platensis	3.92E+05	3.02E+03[a]	2.77E+05[b]	0.00E+00[a]	3.33	2.83E+01
A. flos-aquae	3.92E+05	1.76E+03[a]	2.14E+04[a]	0.00E+00[a]	0.00	0.00E+00

1=Colony forming unit; 2= Positive control (pathogen only), 3=N.C: Negative control, 4=Area under disease progressive curve;
[a]*P* value <0.001, [b] *P* value <0.001 as compared to negative control, [b] *P* value <0.001 as compared to positive control.

Table 3. Effect of the tested Cyanobacteria on the growth of potato plants infected by *R. solanacearum*

Positive treatments	Plant height(cm)	Leaf fresh weight (g)	Leaf area(cm)[2]	Shoot fresh weight(g)	Shoot dry weight(g)	% shoot dry wt.
P.C	34.00[1, a]	1.10[1, b, c]	55.00[1, b, c]	10.67[1, d, e, 6]	0.73[a, d, 7]	6.8
N.C	49.33[a]	2.57[1, 3, 4]	128.33[4, 1, 3]	20.33[1, 5, a]	1.63[1, a, e]	8.0
N. muscorum	44.00[2]	1.83[4, c]	91.67[4, c]	18.00[e]	1.10[1, 7]	6.1
S. platensis	52.33	1.60[3]	80.00[3]	13.50[a, 6]	1.47[e]	10.9
A. flos-aquae	52.00[1, 2]	2.07[b]	103.33[b]	17.67[5, d]	1.70[d]	9.6

[1, a , d, e] *P* value < 0.001, [2]*P* value = 0.008, [3,b]*P* value = 0.003, [4, c] *P* value = 0.017, [5]*P* value = 0.047, [6]*P* value = 0.036 and [7]*P* value = 0.006; P.C=Positive control; N.C=Negative control.

Table 4. Effect of the tested Canobacteria on the growth of non-infected plants

Negative treatments	Plant height(cm)	Leaf fresh weight (g)	Leaf area(cm)[2]	Shoot fresh weight(g)	Shoot dry weight(g)	% of shoot dry wt.
N.C[1]	49.33	2.57	128.33	20.33	1.63	8.0
N.muscorum	65.33	2.37	118.33	22.00	1.83	8.3
S. platensis	60.67	1.93	96.67	22.33	1.50	6.7
A. flos-aquae	67.33	2.23	111.67	23.00	1.57	6.8

1=Negative control. No significant difference as compared to negative control.

Effect of the tested Cyanobacteria on photosynthetic pigments in infected and non-infected plants:

Data presented in Table (5) show an increase in the total chlorophyll contents in infected potato leaves treated with the three tested species of Cyanobacteria compared with the positive control but still less than the negative control. Also, the infected plants content of chlorophyll b and carotenoids were increased after Cyanobacteria treatment compared with the positive control.

Photosynthetic pigments content including chlorophyll a, chlorophyll b, total chlorophyll and carotenoids of non-infected potato plants treated with Cyanobacteria were nearly similar to those of negative untreated control as presented in Table (6)

Table 5. Effect of the tested Cyanobacteria on photosynthetic pigments in infected plants:

Photosynthetic pigments (mg/g1.F.Wt.)				
Positive treatments	Chloro. a	Chloro. b	Carotenoids	Total chlorophyll
P.C[a]	0.40	0.89	0.62	1.29
N.C[b]	0.42	1.14	0.77	1.56
N. muscorum	0.40	1.01	0.68	1.41
S. platensis	0.40	1.01	0.67	1.41
A. flos-aquae	0.42	1.08	0.74	1.51

a =Positive control; b= Negative control. No significant difference as compared to negative and positive control.

Table 6. Effect of the tested Cyanobacteria on photosynthetic pigments in non- infected plants:

Photosynthetic pigments (mg/g1.F.Wt.)				
Negative treatments	Chloro. a	Chloro. b	Carotenoids	Total chlorophyll
N.C[1]	0.42	1.14	0.77	1.56
N.muscorum	0.42	1.12	0.76	1.54
S. platensis	0.42	1.12	0.77	1.54
A. flos-aquae	0.42	1.12	0.76	1.54

1= Negative control. No significant difference as compared to negative control.

Treating potato plants with the three species of Cyanobacteria resulted in increasing the endogenous ascorbic acid and proline content compared with both positive and negative control. The endogenous phenol content significantly increased in infected plants treated with the three species compared with the negative control but, it was less than positive control in both treatments of *N. muscorum* and *S. platensis*, while the treatment of *A. flos-aquae* increased this content as shown in Table (7).

Table 7. Effect of tested Cyanobacteria on some chemical constituents of infected potato plants

Endogenous Non-enzymatic antioxidants			
Positive treatments	Ascorbic acid (µg g-1 F.W)	Proline (mg /g-1.F.Wt.)	Phenol (mg /g -1.F.Wt.)
P. C[a]	10.00[1,2, 3,4]	0.53[1]	0.19[5, 1]
N.C[b]	7.33[2]	0.24[1]	0.04[1]
N. muscorum	14.00[3]	0.60	0.17
S. platensis	13.33[4]	0.62	0.18
A. flos-aquae	14.67[1]	0.74	0.23[5]

a=Positive control; b=Negative control
[1] *P* value =0.001, [2]*P* value =0.04,[3]*P* value =0.003,[4]*P* value =0.012,[5]*P* value =0.02.

Effect of the tested Cyanobacteria on some chemical constituents in infected and non-infected plants

Data shown in Table (8) show that treating potato plants with the tested Cyanobacteria in the absence of the pathogen caused increasing in the endogenous phenol content and decreased in proline content compared with the negative control while the contents of endogenous ascorbic acid were almost similar to those of negative control plant.

Table 8. Effect of tested Cyanobacteria on some chemical constituents of potato plants

Endogenous Non-enzymatic antioxidants			
Negative treatments	Ascorbic acid (µg g-1 F.W)	Proline (mg /g-1.F.Wt.)	Phenol (mg /g-1.F.Wt.)
N.C[1]	7.33	0.24	0.04
N. muscorum	7.33	0.17	0.06[c]
S. platensis	7.67	0.15	0.07[a]
A. flos-aquae	6.33	0.15	0.06[b]

1= Negative control; [a, b and c] *P* value <0.001, = 0.001 and = 0.004 respectively as compared to negative control.

Effect of the tested Cyanobacteria on Na and NPK content in plants

Data present in Table (9) indicate that Na and NPK contents of the non-infected plants treated with Cyanobacteria increased compared with the untreated plant. The treated plants with the three Cyanobacterial species showed marked increasing in potassium (K) content compared with the control, the highest one was with *A. flos-aquae* application. Also, The N content was most increasing in plants treated with *A. flos-aquae* followed by those treated with *N. muscorum*. On the other hand, plants treated with *S. platensis* had the highest Na and P contents.

Table 9. Effect of the tested Cyanobacteria on the percentages of Na and NPK content in non- infected plants

Treatments	N	P	K	Na
N.C	6.25	0.69	2.69	0.31
N.muscorum	7.07	0.71	4.16	0.44
S. platensis	6.85	0.88	5.03	0.50
A. flos-aquae	8.39	0.87	6.71	0.49

N.C= Negative control.

4 DISCUSSION

Potato brown rot disease, caused by *R. solanacearum* (Yabuuchi *et al.*, 1995) is difficult to manage due to the genetic diversity; aggressiveness of the pathogen; wide host range; the large number of weed hosts; long survival in soil and wide biological variation (Martin and French, 1985). Recently, there has been an increasing demand for searching of effective alternative strategies to control this disease. The objective of this study was to investigate the efficacy of the three cyanobacteria species namely: *Nostoc muscorum, Spirulina platensis* and *Anabaena flos-aquae* in suppression of the bacterial wilt disease.

In the present study, the three tested Cyanobacterial species exhibited inhibitory effect on the growth of *R. solanacearum in vitro*, observed as clear inhibition zone, these results are in harmony with those obtained by Archana and Sharma (2013) who found that the Cyanobacterial extracts of *Anabaena* sp. had strong antibacterial activity towards several pathogenic bacteria such as *E. coli*, *Enterococcus* sp. and *Klebsiella*, and with those of Fujii *et al*,(2002) who indicated that *Anabaena* sp. produces lipopeptidase that has an antibacterial effect. Also, Ozdemir *et al* (2004) and Abedin and Taha (2008) reported that the *Spirulina platensis* extract exhibited different degrees of antimicrobial activity against both gram-positive and gram negative organisms, as it contains a wide variety of bioactive compounds such as heptadecane and tetradecane.

El-Sheekh *et al*, (2006) noted that the phenolic compounds in the culture filtrate of *Nostoc muscorum* shows antimicrobial activiy towards several pathogenic bacteria. Also, Bloor and England (1989) reported that the antibiotic produced by *N. muscorum* inhibited the growth of multiple-resistant bacteria *Staphylococcus aurens* .

In the present study, the three tested Cyanobacteria have significantly reduced the disease incidence under artificial inoculation in the greenhouse. Complete suppression was observed in case of *A. flos-aquae* with an improvement of growth parameters of the plant. These data are in accordance with those of Prasanna *et al.*(2013a and 2013b) who proved that, the existence of Cyanobacteria in the crops rhizosphere shows positive influence on plant growth and availability of nutrients. Cyanobacteria play an important role as natural bio fertilizer, build-up soil fertility, consequently increasing the growth and yield of different crops (Song *et al.*, 2005). They have potential to produce many metabolites which includes the phytohormones (IAAs, cytokinin and gibberillin-like

compounds) and iron chelators (schizokinen, anachelin and synechobactins) that exhibit a great influence on the productivity of the ecosystem (Yadav, 2011).

Also, the results of this study in accordance with those of Adam (1999) and Wang *et al*.(1991) who found that the pre-soaking of several plants in Cyanobacterial cultures enhanced the germination rate. Also, Ordog (1999) noted that the blue-green algal extract excretes a great number of substances that influence plant growth by producing growth promoting regulators, vitamins, amino acids, polypeptides, antibacterial ,antifungal substances against phytopathogens and polymers, especially exopolysaccharides, that improve plant growth and productivity (Zaccaro,1999).

The present data also revealed that the disease suppression was accompanied by increasing in the photosynthetic pigments, proline, ascorbic acid and the total phenol contents in the treated plants. The role of phenolic compounds in the disease resistance was postulated by Mittelstrass *et al.*, (2006) who reported that, its role may be attributed to the toxic effect of phenols on the pathogen. In addition to that phenols are essential for the biosynthesis of lignin, which is considered to be an important structure component of plant cell walls (Hahlborck and Sheel, 1989).

In this regard, Sakr *et al.* (2010) reported that accumulation of the proline content is one of the most frequently reported compound that involved in the plant resistance mechanisms of stress. Also, Fabro *et al.* (2004) and Verslues and Sharma (2010) demonstrated that the proline content had been increased during plant defense against several pathogens in *Arabidopsis thaliana*. In addition, it has been suggested that the ascorbic acid may play an important role in protection of plants against several environmental stresses such as heavy metal action, salinity, pesticides, and pathogenesis (Shalata and Neumann, 2001; Vwioko *et al*., 2008). Mehlhorn, *et al*. (1996) and Vanacker *et al*. (1998) noted that the ascorbate is a substrate for cell wall peroxidases, and play an important role in the regulation of the cell wall lignification, especially during the HR, through its capacity to inhibit the oxidation of phenolic compounds by peroxidases.

It has been found that the obtained results showed an increase in the NPK content in the treated plants, these results are in agreement with those of Rana *et al.* (2015) who reported that the application of Cyanobacteria significantly increased nitrogen, phosphorus, and potassium (NPK) content and improved rice yield by 21.2%, as compared to the application of recommended dose of NPK fertilizers. They illustrated that the Cyanobacteria enhanced the yield of rice and micronutrient concentrations, consequently the nutrients mobilization in the soil facilitates uptake by plants, enhancing root growth, plant biomass and yield. Also, Xiao *et al.,* (2006) found that, potassium nutrition status do not only affects plant growth and development, but also plays an important role in plant resistance to diseases by regulating various plant physiological metabolism pathways.

Based on the obtained results, it could be concluded that the three selected Cyanobacterial species were able to reduce the disease severity and promoting the plant growth under the greenhouse condition. Application of Cyanobacteria (blue-green algae) is a promising environment-friendly approach for controlling potato bacterial wilt disease.

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