THE POTENTIAL FOR USING CULTURE FILTRATE OF *CHROOCOCCUS MINUTUS* AS FUNGICIDAL AGENT AGAINST PHYTOPATHOGENIC *PYTHIUM* SP.

***Ibraheem, I. B. M.; Abdel-Raouf, N.; Hammouda O. and Abdel-Wahab, N.**

Botany Department , Faculty of Science, Beni-Suef University, Beni-Suef, Egypt.
E-mail Ibraheemborie@yahoo.com

Abstract

Culture filtrate of the blue-green alga *Chroococcus minutus* isolated from Wadi-Sannur, Egypt, was screened for its antimicrobial activity against 5 Gram positive and 3 Gram negative bacterial species, unicellular fungus *Candida albicans* and 9 filamentous fungal species. The filtrate exhibited an inhibitory activity against all bacterial species under investigation except *Staphylococcus aureus*, has a selective inhibitory effect against the fungal tested organisms. Further studies were conducted to optimize the growth conditions and to enhance the productivity of *C. minutus*. The data revealed that pH 8, light/dark period of 16/8 h and light intensity of 2000 Lux were the optimum growth conditions for enhancing the cyanobacterium productivity. Moreover, the cyanobacterial culture filtrate was tested as a biocontrol agent against the phytopathogen *Pythium* sp. which parasite on the tomato plant. Significant stimulating effects were produced on the tomato seeds which previously soaked in *C. minutus* culture filtrate, in retarding the *Pythium* sp. symptoms. This was obvious such as increasing seed germination percentages, survival percentages and growth parameters such as increasing fresh weight shoot and root length and chlorophyll contents.

***Key words*:** Cyanobacteria, *Chroococcus minutus*; antimicrobial activity*; Pythium* sp.; biological control.

Introduction

Cyanobacteria produce a large number and variety of bioactive allelochemical substances with a diverse range of chemical structures and biological activities. Such chemicals are likely to be involved in regulating natural populations. They are potentially useful as biochemical tools as herbicidal or biocontrol agents (Smith and Doan, 1999). There are number of reports by many authors on antibiotic and other pharmacological effects from cyanobacteria. The most investigated gensu of cyanobacteria which generally reported for the antimicrobial activities are *Nostoc* sp. and *Anabaena* sp. (De Mulé *et al.*, 1991 and Ibraheem and Abdel-Raouf, 2007), *Scytonema* sp. (Chetsumon *et al.*, 1995 and 1998), *Microcystis aeruginosa.* (Carmichael *et al*., 1988 and Ishida *et al.*, 1997), *Oscillatoria* sp. (Bagchi *et al.*, 1990) and *Phormidium* sp. (Fish and Codd, 1994), but little attention was paid to *Chroococcus* sp. (Safonova and Reisser, 2005). Microalgae like cyanobacteria were found to be rich source of various

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products of commercial, pharmaceutical or toxicological interest, some of them are primary metabolites such as proteins, fatty acids, vitamins or pigments (Borowitzka, 1988 a, b and 1995) and other are secondary metabolites which synthesized by the organism in the cultures at the end of primary growth phase and have different bioactivities (antibacterial, antifungal, antiviral and others (Falch, 1996 and Skulberg, 2000).

Several workers reported that cell-free extracts of some blue-green algae such as *Oscillatoria* sp., *Nostoc* sp., *Synechoccus* sp. *Scytonema* sp. *and Schizothrix* sp. exhibited antibacterial activities against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* (Chetsumon *et al.*, 1998 and Soltani *et al.*, 2005). Furthermore, the blue-green alga *Lyngbya majuscule* has been found to produce antifungal compounds, as extracellular metabolites, pahayokolide A, which inhibits the growth of *Saccharomyces cerevisiae* (John *et al.*, 2004). Also, the extract and extracellular products from *Nostoc muscorum* were found to inhibit the growth of *Sclerotinia scleroliorum, Candida albicans* and secondary fungal infection in AIDS patients as well curing tomato against damping-off that caused by *Pythium* sp. (Potts, 2000 and Rainer and Franz, 2006).

Recently, different researchers aimed to replace the chemical pesticides by natural components of different plant and microalgal sources as insecticide agents (Nassar *et al.*, 1999) and acricide agents (Ibraheem and Abdel-aziz, 2002 and Duke, 2002). Furthermore, Ibraheem and Mohammed (2002) demonstrated that different concentrations of extracellular metabolites of the blue-green algae *Phormedium* sp., *Anabaena* sp. and *Aphanocapsa* sp. were efficient in controlling damping-off in cotton plants caused by *Rhizoctonia solani* and *Fusarium oxysporium.* In the same manner, Abdel-Raouf (2004) observed that extrametabolites of green algae *Chlorella vulgaris* were efficient in controlling rot root disease in tomato plants caused by *Rhizoctonia solani* and *Fusarium oxysporium.*

The natural materials and their unlethal activities are friendly to the environment, because they didn′t affect the natural balance of funa and keep the beneficial animals such as predators or parasites. Therefore, the present study aimed to use the cyanobacterium *Chroococcus minutus* as antimicrobial agent against some pathogenic bacteria and fungi, through optimizing its growth conditions and studying the biocontrol activities of its filtrate against the damping-off disease caused by *Pythium* species on tomato plants (pots study).

Material and Methods

Alga isolate:

Local blue-green alga *Chroococcus minutus* (kütz.) Näg, was isolated from Wadi-Sannur on different media: Z-medium (Staub, 1961), Allen's medium (Allen, 1968), Bold's basal medium (Bischoff and Bold 1963) and BG11 (Rippka *et al.*1979) and identified according to Desikachary, (1959). To obtain bacteria free cultures (axenic culture) of microalga, antibiotic treatment (according to

Felfoldy and Zsuzsa 1959) and washing with chlorine water (according to Fogg, 1942) techniques were applied.

Culturing and optimization of growth conditions:

In this respect, different pH values (6, 7 and 8), different light / dark periods (12 / 12, 16 / 8 and 24 / 0.0) and different light intensities (1000, 2000 and 3000 Lux) were used with the optimum growth medium (at constant temperature 34 ± 1ºC) to optimize the growth conditions of *C. minutus* for maintanence the productivity against only the sensetive tested microorganisms.

Test organisms:

Gram positive: *Bacillus subtilis,* NCTC 1040, *Bacillus pumulis,* NCTC 8214, *Micrococcus kristinae,* ATCC 27570, *Sarcina maxima,* ATCC 33910, and *Staphylococcus aureus,* NCTC 7447

Gram negative: *Escherichia coli*, NCTC 10416, *Klebsiella pneumonia,* NCIMB 911 and *Pseudomonas aeruginosa*, ATCC 10145.

Unicellular fungi : *Candida albicans,* IMRU 3669.

Filamentous fungi: *Alternaria alternata, Aspergillus flavus,* IMI 111023, *Fusarium solani*, *Pythium* sp., *Macrophomina phaseolina, Penicillium chrysogenum, Rhizoctonia solani, Verticillium dahliae,* and *Curvularia hunata*.

All tested organisms were kindly supplied from Biotechnological Research Center, Al-Azhar University (For Boys), Cairo, Egypt. These organisms were subcultured on specific culture media, nutrient agar medium for bacteria (Case, 1984) and modified Czapek-Dox′s medium for fungi (Davet and Rouxel, 2000) until needed in the antimicrobial experiment.

Determination of antagonistic activity

***In vitro* studies:**

Culture filtrate of *C. minutus* at exponentially growth phase was investigated as antimicrobial agent against the 18 tested microorganisms as follows:

Preparation of culture filtrate: 1 liter culture filtrate of *C. minutus* at day 14 was centrifuged at 3000 rpm for 5 minutes then the supernatant was concentrated under reduced pressure by using rotary evaporator to 1 ml and kept in refrigerator until used.

Preparation of tested organisms: The media of the tested organisms were dispensed in 15 ml aliquots in the cotton-plugged test tubes and then autoclaved. The contents of one test tube were poured in a pre-oven sterilized 9-cm diameter Petri-dish. After solidification of agar medium the plates were seeded with 0.1 ml of a test bacterial or yeast suspension containing approximately 6×10^7 bacterial cell / ml or 6×10^6 fungal cells / ml. Bacterial suspensions were prepared from 24

h- old cultures and fungal suspension from 48 h-old cultures. Suspensions were spread on the surfaces of the bioassay media using alcohol- sterilized hock-shaped glass rods (Case, 1984).

Anitimicrobial test: According to Eugene and Carol (1988 a and b), disk diffusion method was used for detection of any antimicrobial activity as follows: oven-sterilized filter-paper discs Whatman number 3 (five millimeters in diameter) were moistened each with 20 µl (these were found experimentally to be quite enough for the test) of the concentrated *C. minutus* culture filtrate and placed on the surface of an agar medium pre-inoculated by the desired tested organisms. Plates were placed in a refrigerator at 4ºC for 2 hours to allow the expected antimicrobial agents in the disk to diffuse in the agar medium then inoculated at 37ºC for 18 h for bacterial test organism and at 24-36h for fungi. Zones of no growth (inhibition zone) rounding disk indicate the presence of an active agent in the sample used to moisten the disk.

***In vivo* studies (Biocontrol study of *C. minutus*):**

Culture filtrate of alga *C. minutus* was tested to determined its efficiency on controlling damping-off disease of tomato plant caused by *Pythium* sp.

Plant material: A vital seeds of pure variety of tomato plant (*Lycopersicum esculentum* mill CV. Marmand), was kindly provided by Agricultural Research Center, Ministry of Agriculture, Giza, Egypt.

Plant cultivation and inoculation: Cultivation and inoculation of tomato plants were performed by applying the method of Fuchs and Sacristan (1996). Tomato seeds were sterilized (before treatment) with mercuric chloride solution 0.1% for two minutes then rinsed several times with sterile distilled water. The sterilized tomato seeds were immersed in the concentrated culture filtrate of *C. minutus* for 24 h before planted (in control samples, the seeds were immersed in culture medium free of cyanobacterium). Pots containing two kg of sterilized sandy and clay soils (1:1 v/v) were inoculated separately by the pathogen. It is achieved by mixing 2 ml of arresting spore suspension at standard concentration of 106 spore ml^{-1} with the upper 10 cm of the soil. The treated tomato seeds (5 seeds) were put in each pot. Each treatment was replicated three times. The potes were irrigated each 5 dayes and placed in opend air throughout the experimental period. Percentages of seed germination and survival were recorded after 10 and 30 dayes of planting, respectively.

Four groups of pots were established:

C = Healthy seeds + sterilized soil (control).

T_1 =Healthy seeds previously soaked in cyanobacterial metabolite for 24 h + sterilized soil.

T_2 =Healthy seeds + infested soil with *Pythium* sp.
T_3 =Healthy seeds previously soaked in cyanobacterial metabolite for 24h + infested soil with *Pythium* sp.

At the end of experimental period (40 dayes), fresh weight (g), shoot length (cm) and root length (cm) were determined. Chlorophyll contents (μg gm^{-1} fresh weight) were estimated using the spectrophotometric method as described by Harborne (1984).

Statistical analyses. Data were analyzed and treatments compared using the one way ANOVA with 95 % and 99 % confidence limits ($P < 0.05$ and 0.01).

Results and discussion

Data recorded in Table 1, showed the antimicrobial screening of culture filtrate of *C. minutus* against some pathogenic Gram positive and Gram negative bacteria as well as one unicellular and some filamentous fungi. The culture filtrate showed different degrees of activity against the tested microorganisms. It was achieved by production of a clear zone around the discs loaded by this filtrate. The higher activity was shown against *Bacillus subtilis, B. pumilus, Pseudomonas aeruginosa, Pythium* sp. and *Macrophomina phaseolina*. Also it was recorded a weak activity against *Micrococcus kristinae, Sarcina maxina, Escherichia coli, Klebsiella pneumonia, Candida albicans, Alternaria alternata, Aspergillus flavus* and *Fusarium solani*., Whilst the culture filtrate exhibited no activity against *Staphylococcus aureus, Macrophomina phaseolina, Penicillium chrysogenum, Rhizoctonia solani, Verticillium dahliae,* and *Curvularia hunata*.

In this respect, some investigators reported that extracellular culture filtrate of cyanobacteria was suppressive to some microorganisms (Kulik, 1995; Ishida *et al*., 1997; Abdel-Aziz and Abdel-Raouf, 2002; Ibraheem and Mohammed, 2002; Mian *et al*., 2003; Abdel-Rahman *et al*., 2004; Soltani *et al*., 2005; Safonova and Reisser, 2005 and Hikmet *et al.* ,2006). These beneficial effects could be attributed to the nature of *C. minutus* exudates which has inhibitor properties (secondary metabolites). The clear inhibition zones indicating that the excudate retard the growth of other microorganisms and antagonize the infection mechanisms of these organisms. Campbell (1984) found that algal excudate may have peptides, alkaloids and phenols compound and other staining mono- and di-valent cations (Abdel-Rahman *et al*., 2004). The current hypothesis was in agreement with those of Kobbia and El-Sayed (1978), who demonstrated that cyanobacterial filtrates excreted certain inhibitory effects on growth and physiological activities of other microorganisms (*Aspergillus niger* and *A. candidus)* and retarding effects increased with doubling the concentration of the cyanobacterial filtrates. In this regard, Mousa and Shanab (2001) reported that the extent of inhibition in radial growth of *Fusarium* sp. increased with cyanobacterial metabolites and/or incubation period.

Table 1. Antimicrobial screening of *Chroococcus minutus* culture filtrate against some pathogenic bacteria and fungi on the agar plate by diffusion assay method.

Tested organisms	Activity
Bacillus subtilis, NCTC 1040	++
Bacillus pumilus, NCTC 8214	++
Micrococcus kristinae, ATCC 27570	+
Sarcina maxina, ATCC 33910	+
Staphylococcus aureus, NCTC 7447	-
Escherichia coli, NCTC 10416	+
Klebsiella pneumonia, NCIMB 911	+
Pseudomonas aeurginosa, ATCC 10145	++
Candida albicans, IMRU 3669	+
Alternaria alternata	+
Aspergillus flavus, IMI 111023	+
Fusarium solani	+
Pythium sp.	++
Macrophomina phaseolina	-
Penicillium chrysogenum	-
Rhizoctonia solani	-
Verticillium dahliae	-
Curvularia hunata	-

(-) No activity, (+) Weak activity and (++) considerably higher activity.

The ineffective behavior of the cyanobacterial filtrate against some of the tested organisms may be attributed to insufficiency of the concentrations of antimicrobial compounds. In this regard, Zulpa *et al.*, (2003) reported that the same cyanobacterial strain produced opposite effects (promotion or inhibition) depending on the target organism's interactions with the biomass extract or the extracellular products of cyanobacteria. The ability of *C. minutus* to produce antibiotics (antibacterial and / or antifungal) may be an advantage for their survival in desert habitat (Teuscher *et al.*, 1992). In this respect, it was reported that *in situ* soil microalgae might be able to promote or inhibit the growth of heterotrophic bacteria which might either be competitors or may deliver substances supporting heterotrophic growth of algae (Safonova and Reisser, 2005).

Our study also illustrated the optimization sequence of growth conditions for enhancement of the productivity of *C. minutus* (Table 2). It was revealed that pH 8, light intensity of 2000 Lux and 16/8 light/dark cycle were the most favorable conditions to the experimental organism for more antibiotic productivity. It may be concluded that these parameters affect the biosynthesis of antimicrobial agent products as secondary metabolites. Several studies have

shown that the production of the active compounds depends on the growth phase and / or culture conditions (Noaman *et al.*, 2004). In this respect, Hikmet *et al.*, (2006) showed that cultivation of *Chroococcus* sp. on BG11 medium under light intensity less than 3000 lux for 16 / 8 light / dark cycle were the favorable conditions for more antimicrobial production.

Table 2. Effect of different pH values, light duration and light intensity of the productivity of *Chroococcus minutus* against only the sensitive tested organisms.

Test organisms	pH values			Light duration Light / dark period (h)			Light intensity (Lux)		
	6	**7**	**8**	**12 / 12**	**16 / 8**	**24 / 0.0**	**1000**	**2000**	**3000**
Bacillus subtilis, NCTC 1040	-	+	++	+	++	+	+	++	+
Bacillus pumilus, NCTC 8214	-	+	++	+	++	+	+	++	+
Micrococcus kristinae, ATCC 27570	-	+	++	+	++	+	+	++	+
Sarcina maxina, ATCC 33910	-	+	++	+	++	+	+	++	+
Escherichia coli, NCTC 10416	-	+	++	+	+	+	+	+	+
Klebsiella pneumonia, NCIMB 911	-	+	++	+	++	+	+	++	+
Pseudomonas aeurginosa, ATCC 10145	-	+	++	+	++	+	+	++	+
Candida albicans, IMRU 3669	-	+	++	+	++	+	+	++	+
Alternaria alternata	-	+	++	+	++	+	+	++	+
Aspergillus flavus, IMI 111023	-	+	++	+	++	+	+	++	+
Fusarium solani	-	+	++	+	++	+	+	++	+
Pythium sp.	-	+	++	+	++	+	+	++	+

(-) no activity, (+) weak activity and (++) considerably higher activity.

In the pots experiment (Tables 3, 4 and 5), it was found that the soil infested with *Pythium* sp. significantly decreased seed germination and survival percentages of tomato seedlings (damping-off), in addition to the reduction in growth parameters (fresh weight, shoot length and root length). It may be attributed to the destructive or harmful effect of the pathogen. The current results were in harmony agreement with those of Schwarz and Grosch (2003) and Roberts *et al.*, (2005) who reported that *Pythium* sp. reduced both fresh and dry weight of tomata plant and significant reduced the emergence, wet and dry weight of shoot and root and shoot length.

Due to the highly inhibiting effects of culture filtrate of *C. minutus* against the phytopathogenic *Pythium* sp., this alga tested as a biocontrol agent against this pathogen. In this respect the data presented in Table 3, shows the effect of cyanobacterial treatment on seed germination and survival percentage of tomato planting in soil infested with *Pythium* sp. Generally, the treatment of tomato seeds with *C. minutus* filtrate induced seed germination and survival percentage. Whereas, soaking tomato seeds in culture filtrate of *C. minutus* (T1) for 24 h before seeding in non-infested soil, significantly increased seed

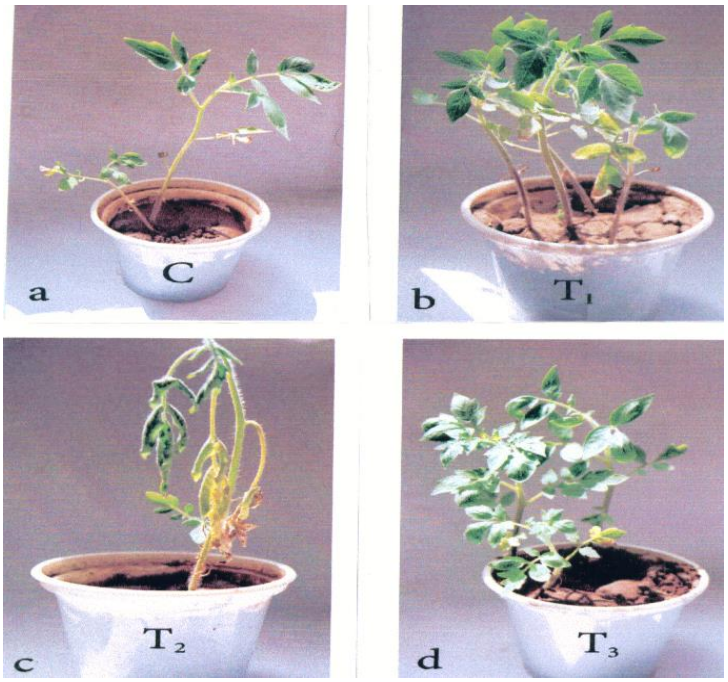
germination and survival percentage 80 and 80 % respectively as compared to 60 and 40 % in unsoaked seeds (C), respectively.

On the other hand, seeding the seeds of tomato which previously pre-soaked in algal filtrate for 24 h in infested soil with *Pythium* sp.(T_3) increased seed germination and survival percentage to 80 and 60 % respectively compared to 40 and 20 % for unsoaked seeds (T_2), respectively. In confermation of thses results (Plate1), it was notable that *Pythium* sp. caused a wilt to the stem and leaves in addition to yellowish color to the leaves (T_2) while the treated plants (T_3) did not exhibit any diseased symptoms on the leaves or stems. These effects may be attributed to the ability of cyanobacterial treatment to induce the resistance mechanism in tomato plant and also to avoid the pathogenic weapons and/or the ability of *C. minutus* to produce exudates which produced retarding effects on growth of pathogenic fungi. These findings were in agreement with those of Haroun and Hussein, (2003) who demonstrated that the "fast death factor" present in some microalgae and in the culture fluid (extracellular products) and the treatment of some plants by some microalgal culture filtrate resulted in increase in seed germination and dry weight in addition to increasing their growth characters.

Table 3. Effect of *Chroococcus minutus* culture filtrate on seed germination and survival percentage of tomato planting in soil infested by *Pythium* sp. The values are means of three replicates ± standard deviation. The LSD values at 5% and 1% are calculated.

	Treatment	Seed germination %	Survival %
Control (C)	Healthy seeds + non-infested soil (control).	60 ±2.0	40±3.0
Treatment 1 (T_1)	Healthy seeds previously soaked in algal metabolite for 24 h + non-infected soil.	80 ±3.0	80±2.0
Treatment 2 (T_2)	Healthy seeds + infested soil with *Pythium* sp.	40 ±1.0	20 ±1.0
Treatment 3 (T_3)	Healthy seeds previously soaked in algal metabolite for 24 h + infested soil with *Pythium* sp.	80 ±3.0	60±2.0
LSD at 5 %		4.5	3.99
LSD at 1 %		6.6	5.81

Plate I



a= Control, b= Treatment 1, c= Treatment 2 and d= Treatment 3

Table 4, show some growth characteristics (fresh weight, shoot and root length) of survival tomato plants whish previously treated by culture filtrate of *C. minutus* and planting in soil infected by *Pythium* sp. It was revealed that, the treated plants grown in non-infested soil (T_1) significantly increased in fresh weight, shoot and root length by 9.2 g, 29.6 and 10.6 cm, respectively compared to 8.3 g, 27 and 10.1cm for untreated plants (C) respectively.

The recorded data further demonstrated that the survival plant grown in infected soil with *Pythium* sp. (T_2) significantly decreased in fresh weight, shoot and root length of 3.2 g, 19.8 and 6.4 cm respectively compared to 8.3 g, 27 and 10.1cm for the survival ones in non-infected soil (C), respectively. Finally, it was observed that, the treated survival plants planted in infested soil (T_3) exhibited significantly increased in fresh weight, shoot and root length of 5.6 g, 26.3 and 9.5 cm respectively compared to 3.2 g, 19.8 and 6.4 cm of untreated ones (T_2), respectively (Table 4)

The enhancement of germination of seeds and development of tomato seedlings by the cyanobacterial filtrate may be attributed to a liberation of a very large portion of the bioactive substances from their assimilating nitrogen outside their cells or the amino acids and peptides produced in algal filtrate and/or other compounds that stimulate growth of crop plants as plant growth regulators and

hormones including auxins like substances, cytokinins like substances (Ghallab and Salem, 2001 and Haroun and Hussein, 2003).

Table 4. Some growth characters of the survival tomato plants which previously treated with Chroococcus minutus Culture filtrate and planting in soil infested by Pythium sp. The values are means of three replicates ± standard deviation. The LSD values at 5% and 1% are calculated.

	Treatment	Fresh weight (g/individual)	Shoot length (cm)	Root length (cm)
Control (C)	Healthy seeds + non-infested soil (control).	8.3 ± 0.2	27 ± 0.5	10.1 ± 0.2
Treatment 1 (T_1)	Healthy seeds previously soaked in algal metabolite for 24 h + non-infested soil.	9.2 ± 0.5	29.6 ± 0.6	10.6 ± 0.4
Treatment 2 (T_2)	Healthy seeds + infested soil with *Pythium* sp.	3.2 ± 0.1	19.8 ± 0.4	6.4 ± 0.2
Treatment 3 (T_3)	Healthy seeds previously soaked in algal metabolite for 24 h + infested soil with *Pythium* sp.	5.6 ± 0.3	26.3 ± 0.3	9.5 ± 0.08
LSD at 5 %		0.58	0.87	0.47
LSD at 1 %		0.85	1.26	0.68

Table 5 recorded the chlorophyll contents of survival tomato plants (treated and untreated ones). Data revealed that, the treatment with *C. minutus* culture filtrate (in seed stages) represented by T_1 and T_3 was increased the chlorophyll contents (chl. a, and b) compared to control (C) and treatment 2 (T_2). It was clearly that, the treated plants seeded in non-infested soil (T_1) exhibited significantly higher contents of chl. a, and chl. b which reached 9.5, and 19.3 μg g^{-1} respectively compared to 7.2, and 17 μg g^{-1} for controlled plants (C) respectively. However, seeding the tomato plants in infected soil (T_3) had significantly higher contents of chl. a, and chl.b reach to 8.1, and 12.6 μg g^{-1} respectively compared to 4.0, and 5.4 μg g^{-1} for untreated tomato plants grown in infested soil with *Pythium* sp. (T_2) respectively.

These results were similar to those obtained by Haroun and Hussein, (2003) and Abdel-Raouf (2004) who revealed that the presoaking of *Lupinus* and

tomato seeds in some microalgal filtrate resulted significant increase in chl. a, and chl. b of these plants. It was reported that cyanobacterial extract enhanced the chlorophyll formation (Wake *et al.*, 1992), this stimulative effect of cyanobacterial filtrates may be attributed to the elevated level of GA3 of the filtrates which is known to inhibit chlorophyllase activity (Drazkiewicz, 1994). In this regard, Ordog (1999) documented that the suspension of extract of some cyanobacteria and microalgae contain a special set of biologically active compounds including plant growth regulators which can be used to decrease senescence, transpiration as well as to increase leaf chlorophyll.

The present study further revealed that seeding the tomato plants in soil infested with *Pythium* sp. resulted in significant decrease in chl. a, and chl. b of the survival tomato plants. The results obtained are in partial agreement with that of Mahdy, (1981) who reported that *Fusarium oxysporum* induced a significant reduction in chlorophyll and total pigments content of susceptible cotton cultivars.

Table 5. Effect of the *Chroococcus minutus* culture filtrate treatment on the chlorophyll contents (µg g^{-1} fresh weight) of tomato leaves seeded in infested soil with *Pythium* sp. The values are means of three replicates ± standard deviation. The LSD values at 5% and 1% are calculated.

	Treatment	Chlorophyll- *a*	Chlorophyll-*b*
Control (C)	Healthy seeds + non-infested soil (control).	7.2 ± 0.3	17 ± 0.7
Treatment 1 (T_1)	Healthy seeds previously soaked in algal metabolite for 24 h + non-infested soil.	9.5 ± 0.2	19.3 ± 0.3
Treatment 2 (T_2)	Healthy seeds + infested soil with *Pythium* sp.	4.0 ± 0.1	5.4 ± 0.1
Treatment 3 (T_3)	Healthy seeds previously soaked in algal metabolite for 24 h + infested soil with *Pythium* sp.	8.1 ± 0.5	12.6 ± 0.2
LSD at 5 %		0.58	0.74
LSD at 1 %		0.85	1.08

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أمكانية أستخدام راشح مزرعة السيانوبكتيريا كروؤكوكس منيتس كمبيد حيوي لتثبيط نمو فطر بثيم الممرض

إبراهيم برعي محمد إبراهيم ، نيفين عبدالرؤف محمد ، علا حمودة إبراهيم ، نهي عبدالوهاب محمد
قسم علم النبات – كلية العلوم – جامعة بني سويف

أُجري في هذه الدراسة أختبار أمكانية أستخدام راشح مزرعة السيانوبكتيريا كروؤكوكس مينيتس كمبيد حيوي ضد نمو فطر بثيم الممرض لنبات الطماطم والتي تسبب خسارة للأنتاج المحلي منه. أُجريت دراسة أولية لتقييم مدي كفاءة الراشح ضد 5 كائنات بكتيرية موجبة لصبغ جرام ، 3 سالبة لصبغ جرام ، بالأضافة إلي كانديدا البيكانس و 9 أنواع من الفطريات الخيطية. أثبتت نتائج التجربة فاعلية الراشح لتثبيط نمو بكتيريا الأختبار عدا ستافيلوكوككس أريس ، بينما أظهر الراشح كفاءات متفاوتة ضد الفطريات المُختبرة ، كان أقصي ما أحدثه من تثبيط لنمو فطرة بثيم. تم عمل دراسة معملية من تهيئة أفضل ظروف نمو من درجة الحموضة وطول وشدة الضوء للحصول علي أعلي كفاءة ممكنة للراشح ضد الكائنات التي أظهرت نتائج إيجابية وعددها 12 كائن . أظهرت الدراسة أن أفضل ظروف نمو يستطيع الطحلب أن يعطي فيها أفضل نشاط ضد ميكروبي له هي 8 رقم حمضي , 16- 8 ساعات للضوء و الظلام بالإضافة إ لي 2000لاكس شدة إضاءه. تم أخذ الراشح الناتج من المزرعة التي نمي فيها كروؤكوككس منيتس تحت الظروف المثلي وتم تركيزة بأستخدام جهاز التبخير الدّوار ، وعوملت به بذور الطماطم لمدة 24 ساعة والتي تم زراعتها مباشرة في أصيصات تم حقن بعضها بفطر بثيم الممرض وترك الآخر كتجربة ضابطة. أظهرت النتائج أن بذور الطماطم التى سبق ونقعت فى راشح الطحلب قد زادت زيادة ملحوظة فى نسبة الإنبات ونسبة البقاء و التغلب على الإصابة سواء قد زرعت فى تربه غير محقونة بفطرة بثيم (T_1) (80،80 %) مقارنه بالتجربة الضابطة (60،40 %) على التوالى أو فى تربه محقونة بالفطر (T_3) (80،60%) مقارنه بـ T_2(40،20%) على التوالى. أثبتت النتائج أيضاً أن المعالجة براشح الطحلب قد أظهرت زيادة ملحوظة فى وزن وطول الساق والجذرومحتوى الكلوروفيل للنباتات الحية سواء كانت مزروعة فى تربة مصابة بفطر بثيم او غير مصابة (T_1،T_3). إن ما أثبتتة نتائج التجربة يوجه أنظارنا إلي أهمية مايكمن في مثل هذه الكائنات السيانوبكتيرية من فوائد مازالت مجهولة ، ويحثنا أيضا إلي تدعيم فرق عمل بحثية ترتكز دراساتها علي محاولة أختراق هذا العالم الملئ بالمقومات المفيدة للبشرية.