Biological Controls of *Fusarium* Tomato-wilt Disease by the Blue-green Alga *Nostoc* Spp.

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Abstract

This study investigates the effect of extract and culture of certain cyanobacteria on pathogenic *Fusarium oxysporum* f. sp. *lycopersici* (FOL) that infects tomato (*Solanum lycopersicum*) plant *in vitro* and *in vivo*. Some cyanobacteria isolates were isolated from saline soils of El-Hamoul and Seidy Salem location and identified. Bioactive compounds of isolates were analyzed by GC–MC. Dry weight, carotene, chlorophyll content, and total phenolic compounds of isolates were measured. Plant height (cm), dry weight (g), fruit number, and fruit weight (g) of tomato were estimated. Isolates were defined as *Nostoc calcicola* and *Nostoc linckia*. GC/MS analysis showed 49 and 35 bioactive compounds from *N. calcicola* and *N. linckia*, respectively. *N. calcicola* possess a higher amount of dry weight, chlorophyll a, carotenoid, and total phenol that measured 670.43 mg.L^{-1} , 1.91 mg/g fresh wt., $466.67 \text{ }\mu\text{g/g}$ fresh wt., and $47.00 \text{ mg (GAE) g}^{-1}$, respectively, compared to *N. linckia*. After 100 days, the results showed highest yield values of tomato fruits with *Nostoc* sp. compared with untreated plants and plants infected with *Fusarium*.

Introduction

Agriculture requires extensive use of chemical pesticides to protect crops against pests and diseases. Despite the protective effects of chemical pesticides, they can be harmful to crops, in whole or in part. Several chemicals that may be phytotoxic to plants also pollute soil, groundwater, drinking water, and food (Harman et al. 2004). Excessive use of chemical pesticides to combat pests pollutes the environment. Furthermore, international markets demand that food be clean and devoid of toxins and chemicals. As a result, several governments have made the decision to reduce chemical inputs. As a result, developing alternative pest management methods that lessen our reliance on chemical pesticides is a public concern. Biological control agents, which refers to the use of introduced or resident living organisms such as cyanobacteria, are one of these options. Biological control is sometimes defined as the suppression of one organism's harmful activity by one or more other species (Prasanna et al. 2012). Implementing a biological control agent reduces the effects of pesticide use in the long term and strikes a balance between harmful plant pathogens and their natural enemies. In this regard, antagonistic bacteria and fungi are widely used to control plant diseases (Shahzad et al. 2018). *Fusarium oxysporum* f. sp. *lycopersici* (FOL) is a highly destructive pathogen that infests greenhouse and field-grown tomatoes in warm vegetable production areas. FOL can be found soilborne, airborne, or as plant residue and is transmitted through any part of the plant (Summerral et al. 2003; Manikandan et al. 2018). Wilt caused by *F. oxysporum* has appeared as wilted plants with yellowed leaves, which significantly decreases the quantity and quality of the crop (Ajigbola and Babalola 2013; Akram et al. 2013). There may be a 30–40% yield loss (Kirankumar et al. 2008). Cyanobacteria (blue-green microalgae) are ubiquitous, gram-negative photoautotrophic prokaryotes. They are considered one of the most efficient sources of bioactive secondary metabolites. Known as the spearhead organism in major habitats and possessing diversity in structure, cyanobacteria produce natural products that increase their ability to survive in a variety of environmental conditions. Natural products have been used in disease control for decades. Cyanobacteria have been utilized to develop new drugs that were proven effective in treating incurable

diseases (Bethan and Carole 2018). They are considered one of the most efficient sources of bioactive secondary metabolites, such as pigments, vitamins, and enzymes (Seddek et al. 2019; Qamar et al. 2021). The presence of various bioactive compounds within the plant and cyanobacteria (secondary or primary metabolites) were recommended for phytopharmaceutical importance (Prakash et al. 2011; Shelk and Bhot 2019). Cyanobacteria are used as antioxidant, anticancer, and antiviral properties that have applications in agriculture, industry, medicine, and biotechnology (Patra et al. 2008). Chemically diverse natural compounds induce cytotoxicity and potentially kill various cells by inducing apoptosis or altering the activation of cell signaling, thus influencing protein kinase-C family members, cell cycle arrest, mitochondrial dysfunctions, and oxidative damage (Qamar et al. 2021). The mode of action of cyanobacteria antimicrobial compounds is damaging the structure or function of the cytoplasmic membrane, destruction of enzymes, and suppression of protein synthesis (Swain et al. 2017). The antimicrobial activity depends on algal species and the type of solvents used (Radhika et al. 2012). Modern advances in biotechnology are geared toward increasing the production of desired products in cyanobacteria for various industrial applications (Abed et al. 2011; Rama et al. 2012; Lau et al. 2015). Cyanobacteria produce a number of biocidal metabolites, including antitumor activity (Yadav et al. 2016; Camila et al. 2018), toxins (Agrawal et al. 2006), and enzyme inhibitors (Skulberg 2000). The present work aimed to explore the inhibitory effect of *Nostoc* spp. extracts against the tomato-wilt pathogen *Fusarium oxysporum* f. sp. *lycopersici* (FOL) *in vitro* (laboratory) and *in vivo* (pot experiment). In addition, it aimed to detect the phytochemical components of *N. spp.* extracts by GC/MS analysis.

Materials And Methods

Study area

The soil samples were collected from the upper layer (0–15 cm) of saline clay soil from El-Hamoul and Seidy Salem at Kafr El-Sheikh Governorate, Egypt, to sort and isolate some of the most common cyanobacteria that inhabited these soils (Fig. 1).

Cyanobacteria isolation

Free- nitrogen BG₁₁ medium was used to isolate N₂-fixing cyanobacteria after serial dilutions of the soil samples in an agar medium with an alcohol-sterilized triangle glass rod. Any colored growth that appeared on the agar surface of the Petri dishes was picked out, subcultured, and streaked several times in a new plate. The previous procedure was repeated several times to obtain unialgal cultures with a light microscope (Pringsheim 1949).

Cyanobacteria identification

For characterization of cyanobacterial isolates, 500 ml Erlenmeyer flasks, each containing 250 mL of BG11₀ medium and/or plates containing 1% agarized BG11₀ medium, were inoculated with a loopful of 10 days old culture of each isolate and incubated at 28 ± 2 °C under continuous illumination (3000 Lux) for 10 days. The developed colonies were examined for a cultural appearance on solid and liquid media and characteristics of trichomes, sheath vegetative cells, and heterocyst produced by each isolate (Venkataraman 1981) and (Roger and Ardales 1991). The length and the width of the vegetative cells also the width of the sheath, type of spores, presence or absence of hormogonia, presence or absence of spores and its position, number of heterocysts and its repetition, presence of akinte and its type, the nature of cell wall, presence or absence gas vacuoles, as well as pigment color was taken in consideration according to Desikachary (1959).

Cyanobacteria Harvesting

After the cultivation of cyanobacterial species, the biomass was harvested after the incubation period by centrifugation for 15 min at 6,000 rpm (Bettina and Gerd 2013).

Estimation of total dry weight

A definite volume of cyanobacterial culture (100 ml) was centrifuged at 6,000 rpm for 10 min. The precipitated cells were washed twice with distilled water to eliminate the salts, dried overnight in an oven at 50°C until constant weight, and weighted (Rafiquel et al. 2005).

Estimation of the chlorophyll a content

Chlorophyll a content was calculated by Mackinney (1941).

Estimation of carotenoid contents

A similar procedure for chlorophyll extraction was followed. After that, the pigment extracted was measured at 470 nm, and the amount of carotenoid was estimated by using the extinction coefficient given by Davis (1976).

Estimation of total phenolic compounds

Total phenolic (TP) contents were determined by the spectrophotometric method of Slinkard and Singleton (1977). The TP was expressed as milligrams of gallic acid equivalents (GAE) per g of dried sample.

Preparation of cyanobacterial methanol extracts

The cells from the exponential phase were centrifuged at 10,000 rpm for 3 min, then filtered through filter paper (Wattman-4) and air-dried (Starr et al. 1962). The dried cell mass of cyanobacteria was extracted by dissolving it in methanol (1 g/10 ml). The supernatant was separated by a filter with a 5 µm pore size diameter. The dry residue was re-dissolved in dimethyl-sulfoxide, which is called crude extract (100%). Then, they were kept in fresh glass vials in the dark at 4°C until they were using for phytochemical screening by GC/MS (Lefort et al. 1988).

Gas chromatography-mass spectrophotometry (GC/MS) analysis

The GC/MS is a direct and fast analytical method used for the identification of cyanobacterial extracts. Extracts of *Nostoc calcicola* and *Nostoc linckia* were obtained using Trace GC-TSQ Quantum mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG-5MS (30 m × 0.25 mm × 0.25 µm film thickness). The column oven's temperature was initially held at 50°C and then increased by 5°C/min to 200°C and held for 2 min. Then, it was further increased to the final temperature of 290°C by 30°C/min and held for 2 min. The injector and MS transfer line temperatures were kept at 270°C and 260°C, respectively. The carrier gas was helium at a constant flow rate of 1 ml/min.

Fungus cultivation

Potato dextrose agar (PDA) medium was used to cultivate the plant pathogenic fungus *Fusarium oxysporum f. sp. lycopersici* (FOL), then incubated at 28°C ± 2°C for 3–5 days and stored on PDA slants at 4°C until use.

Antifungal activity using cyanobacterial isolates (the disk diffusion test).

The antifungal activities of cyanobacterial extracts and cultures were evaluated by using an agar plate diffusion test. Plates were incubated at 37°C, and the inhibition zones were measured (Lefort et al. 1988).

Pot Experiment (50 days, 100 days)

Fungal inoculum preparation

The selected phytopathogenic fungus *Fusarium oxysporum* was multiplied using sorghum seeds (*Sorghum bicolor*) moistened with water in equal proportions and autoclaved thrice for 90 min on three consecutive days (Paulitz and Schroeder, 2005). Flasks were inoculated within one-week-old fungal mycelium grown on PDA media and incubated at room temperature ($27^{\circ}\text{C} \pm 2^{\circ}\text{C}$) for four weeks, shaking at weekly intervals. Colonized sorghum seeds were used as fungal inoculum at a rate of 50 g in 500 g of potting mix. Sterilized soil was inoculated with the FOL spores (1.0×10^3 spores/g of soil mixture). Pots were filled with 5 Kg of soil; two seedlings of tomato grown in soil were transplanted into each pot (24 pots $\times$ 3). Three times were carried out.

Preparation of seedlings

Tomato (*Lycopersicon esculentum* L.) seeds were surface sterilized (0.1% sodium hypochlorite), then washed three times. The seeds of the two cyanobacterial species (*N. calcicola* and *N. linckia*) were soaked in cyanobacterial methanol extract and cyanobacterial culture at room temperature ($27^{\circ}\text{C} \pm 2^{\circ}\text{C}$) for 24 hours. Then, the seeds were grown in a greenhouse and were transferred gently after growing for one month in the previously prepared pots. Seedlings without any inoculation served as the control.

Pots were arranged in a glass house on a rack and watered as required. Then, 5 ml of extract and 10 ml of culture were added to each treatment as an inoculum 3 times every two weeks. Plants were observed daily to record symptoms and growth. After 50 days, plant height, fresh weight, dry weight, and chlorophyll were recorded. Ripe fruits were collected daily and their numbers and weight were recorded. After 100 days, the plants were collected. Then, the plant length and dry weight were recorded, following a method described by Perveen et al. (2007), with slight modifications.

Estimation of photosynthetic pigments for tomato plant

The photosynthetic pigments (chlorophyll a, b, and carotenoids) were determined as mg/ml. They were estimated from the apex of the third leaf after 50 and 60 days using the spectrophotometric method of Metzner et al. (1965), and they were applied to higher plants by Ahmed et al. (1977).

Chlorophyll a (mg/ml) = $10.3 A(663) - 0.918 A(644)$.

Chlorophyll b (mg/ml) = $19.7 A(644) - 3.87 A(663)$.

Carotene (mg/ml) = $4.2 \times A(452 \text{ nm}) - (0.0246 \times \text{Chl a} + 0.426 \times \text{chl b})$

The results obtained from these equations were expressed as mg/g of fresh matter from all extraction using different treatments. Measurements were carried out in light within a maximum of six hours to avoid the decomposition of pigments.

Statistical analysis

The collected data were subjected to statistical analysis using SPSS version 22 and (Steel and Torrie, 1980).

Results And Discussion

The physical and chemical characteristics of the collected soil samples are presented in Table 1. Soils were classified as saline due to the large variation between those samples in electric conductivity, which ranged from 6.32 to 15.93 dS.m⁻¹. The cyanobacterial species were *N. caldicola*, which were located in sites 6, 7, and 8, in Seidy Salem; and *N. linckia*, which was located in the Seidy Salem site (13) and El-Hamoul site (10), as shown in Fig. 1. This relative frequency distribution reflects the fact that cyanobacteria can live in all environmental conditions, which agreed with findings by Muruga et al. (2014).

Cyanobacterial isolates

The cyanobacterial isolates *N. caldicola* and *N. linckia* were described by EL-Gamal et al. (2008) in Fig. 2, respectively. The pictures were taken by an OPTICA microscope (Italy) fitted with a Canon Powershot G12 digital camera.

Total dry weight and pigment contents of cyanobacteria

The total dry weight and pigments were among the most important factors for de cyanobacterial growth, especially chlorophyll a. Their biomass is one of the most beneficial bio-fertilizers, which improves soil characteristics such as water-holding capacity and improvements of mineral nutrients (Singh et al. 2016; Ramírez et al. 2019). As shown in Fig. 3, *N. caldicola* recorded 670.43 mg dry wt. /L followed by *N. linckia* 577.38 mg dry wt. /L. These results were in agreement with Hegazi et al. (2010). Cyanobacterial growth also belongs to their chlorophyll and carotenoids at harvesting time (exponential phase). The highest chlorophyll a and carotenoid contents were of *N. caldicola*, followed by *N. linckia*, representing 1.91, 466.67 and 1.64, 350.67 mg per g fresh wt., respectively. Statistical analysis revealed a highly significant difference in the content of chlorophyll of tested cyanobacteria. Photosynthetic pigments like chlorophyll and carotenoids were essential for photosynthesis in cyanobacteria as a primary electron donor. These results agreed with those obtained by Zavřel et al. (2015); Park et al. (2018). In addition, photoprotection was simultaneously shared. (Kim 2015).

Estimation of total phenolic compounds

There were highly significant variations between cyanobacterial species in their total phenol contents. Figure 4 revealed that the total phenols were evaluated to investigate compounds on the antioxidant activities of these extracts. The results showed that as long as the mean value of total phenol content was in the range of 38.0–57.3 mg GAE/g. These agreed with Park et al. (2018).

Gas chromatography/mass spectroscopy (GC/MS) analysis of methanol extract

Profiling of metabolite has been developed as a new technology platform for investigating biological samples, as it describes complex chemical matrices and identifies various compounds. In particular, GC/MS is a fast, precision tool that is commonly used in diagnostics, functional genomics, and screening (Rohloff 2015). The bioactive compounds in *N. caldicola* and *N. linckia* are summarized in Tables 2 and 3. *N. caldicola* and *N. linckia* contained numerous bioactive compounds that belong to different classes as fatty acids, phenolics, antioxidants, alkaloids, flavonoids, and steroids, which agrees with Michalak et al. (2016); Guiheneuf et al. (2016).

The major constituents, retention time, concentration (area %), the chemical structure of bioactive components, molecular formulas, and molecular weight are presented in Table 2 and Fig. 5. *N. caldicola* has forty-nine bioactive compounds, and 9-octadecenoic acid and (Z) methyl ester (Oleic acid) (19.48%) was the most common, which is used as antimicrobial, antibacterial, antioxidant, anti-arthritic, hypocholesterolemic, and anti-cancer (Lee et al. 2007; Mishra and Shree 2007; Wu et al. 2011). In addition, 12, 15-octadecadienoic acid methyl ester; 9.04%, p-xylene benzene, 1, 4-dimethyl; 8.25%, and hexadecenoic acid methyl ester (palmitic acid methyl ester) represent as 6.92% and must have the antimicrobial activity against different plant pathogen (Liu and Huang 2012; Johannes et al. 2016). Each extract also contains cyclooctasiloxane hexadeca methyl; 3.58%, and Di-n-octyl phthalate 1,2-benzenedicarboxylic acid, dioctyl ester; 3.51%.

N. linckia has thirty-five bioactive compounds in the methanolic extract. The most dominant percentage was 10-octadecenoic acid methyl ester (27.67%), followed by 9-hexadecenoic acid methyl ester, (Z); 24.10%, 12,15-octadecadienoic acid methyl ester; 10.16%, octacosane (Al3-52615); 4.89%, thieno (3,4-C) pyridine,1,3,4,7 tetraphenyl; 4.86%, and 10,13-octadecadienoic acid methyl ester [(methyl (10E,13E)10,13octadecadienoate)]; 2.98% as represented in Table 3 and Fig. 6. Linoleic acid is also used in paints, varnishes, coatings, vitamins (O'neil, 2013), beauty products, anti-inflammatory agents, and skin-lightening therapies (Ando et al. 1998; Darmstadt et al. 2002).

As summarized in Tables 2 and 3, there are different species of *Nostoc*; they have variations in the number, type, arrangement of predominance, and concentration area for each bioactive component. Some cyanobacterial strains have a high source of bioactive secondary metabolites, which can have therapeutic, industrial, and agricultural importance, including findings by Gupta et al. (2013); Rimsha et al. (2014). In the present study, octadecanoic acid, which acts as a plant defense response

against pathogens (Bihana et al. 2018), and glycine (a good antioxidant) were used to save the kidney and the liver from dangerous side effects of some drugs, alcohol, cancer prevention, memory enhancement, could have been used directly on the skin to treat leg ulcers and heal other wounds (Szabo and Nemeroff 2015) were among the identified compounds. 1-hexadecene, which possesses antibacterial activity (Beevi et al. 2014), propanoic acid, phenol, vitamin E, and K with antimicrobial and antitumor properties (Venkatachalam et al. 2013; Rangel-Sánchez et al. 2014) were also presented in the *Nostoc* spp. extracts.

Effect of cyanobacterial isolates on the mycelial growth of pathogenic fungi

Figure 7 shows that all the tested cyanobacterial species in the methanol extract and culture) have highly significant differences between the two cyanobacterial species. *N. calcicola* and *N. linckia* exhibited antifungal activity against *Fusarium oxysporum* (FOL) *in vitro* after 8 days of incubation.

The antifungal activities of the tested cyanobacteria could be arranged in the following sequence *N. calcicola* (extract) > *N. linckia* (extract) > *N. linckia* (culture) > *N. calcicola* (culture), which were 16.66, 16.16, 16.0, 15.33 mm respectively. The results revealed that extracts exhibited higher antifungal activity than the culture. These results agreed with Mostafa et al. (2009).

Pot experiment

The pot experiment shows the role of inoculation for some cyanobacteria, such as *N. calcicola* and *N. linckia*, on the root, shoot length, fruit number, and weight of tomato. The experiment was divided into two stages after 50 and 100 days. The differences between the two phases as differences between the extracts and the culture for each species were observed.

Cyanobacteria have phytochemical compounds, which enhance plant growth and increase its tolerance to stress conditions (Buzi et al. 2004; Faoro et al. 2008). The levels of phytoalexins, which were a large and structurally diverse group of antimicrobial plant defense compounds, increased following pathogen inoculation or elicitation (Echeverri et al. 1997; 2012). These substances affect the gene expression of the host plants, thereby bringing about qualitative and quantitative changes in the phytochemical composition of plants. The elicitors and signaling compounds involved in phytochemical responses have been identified as belonging to groups of carbohydrate, lipids, glycolipids, or glycoproteins (Ebel and Cosio 1994; Hahn 1996).

(A) Pot experiment after 50 days

The results in Table 4 show highly significant differences among the different species. The plant height of *Nostoc* spp. was arranged in the following sequence: *N. calcicola* (culture); 14.40 cm, *N. calcicola* (extract); 14.00 cm, *N. linckia* (extract); 13.87 cm, and *N. linckia* (culture); 13.50 cm compared with Control 1 (without any addition), which represented 14.17 cm, and the highest plant recorded for *Fusarium* (Control 2); 14.67 cm. From these results, it became clear that *Fusarium* achieved clear plant growth according to the plant height, and there was a similarity with Minerdi et al. (2011); Splivallo et al. (2007). Also, the pathogen increases the growth of the plant, but for a limited period, then there is a drop in the growth of the plant, due to the fact that the fungus secretes substances that encourage growth during this period and this agreement with (Mace 1965; Manners 1982) found that the auxin IAA is produced by *F. oxysporum* f. sp. and affect growth and development of the plant, or by affecting the production of plant growth hormones by the host or degradation of hormones in the tissues.

The plant fresh weight of the tested cyanobacteria was arranged in the following sequence: *N. linckia* (culture) > *N. calcicola* (culture) > *N. calcicola* (extract) > *N. linckia* (extract). On the other hand, the dry weight recorded for *N. linckia* extract was higher than its culture, but *N. calcicola* exhibited the same fresh weight. Both fresh and dry weights were compared with Control 2 (with *Fusarium* only), which represented 5.76 g and 0.67 g, respectively, and the highest weight recorded in Control 1 (without any addition) achieved 10.56 g and 0.90 g, respectively. These results were in agreement with Alwathnani and Perveen (2012).

Chlorophyll a

Figure 8A shows that after 50 days, chlorophyll a was arranged as *N. calcicola* (extract); 63.39 µg/g, *N. calcicola* (culture); 55.47 µg/g, *N. linkia* (culture); 54.74 µg/g, and *N. linkia* (extract); and 54.20 µg/g compared with Control 1 (without cyanobacteria) and Control 2 (with *Fusarium*) were recorded as 41.81 and 57.93 µg/g, respectively. After 60 days, chlorophyll a appeared in the following arrangement: *N. linkia* (extract); 34.30, *N. calcicola* (extract); 32.84, *N. linkia* (culture); 30.52, and *N. calcicola* (culture); 26.79 µg/g. All these were compared with Control 1 (without cyanobacteria), 23.09 µg/g, and Control 2 (with *Fusarium*), 28.68 µg/g. Application of cyanobacteria were improved the chlorophyll a, b, leaf area, gibberellin, carotenoids, and height of the plant (Yanni et al. 2020; El-Habet and Elsadany 2020; Geris and Elsadany 2021).

Chlorophyll b

After 50 days, the chlorophyll b contents were 25.54, 26.33, 25.62, 25.55, and 25.54 µg/g for *N. calcicola* (extract), *N. linckia* (culture), *N. calcicola* (culture), and *N. linckia* (extract), respectively, compared with the infected plants with *Fusarium* (Control 2), 25.89 µg/g, and the untreated plant with cyanobacteria (Control 1), 26.82 µg/g. On the other hand, chlorophyll b contents after 60 days were recorded in plants treated with *N. linckia* (culture), *N. calcicola* (culture), *N. calcicola* (extract), and *N. linckia* (extract), which

represented 16.31, 16.09, 15.58, and 14.45 µg/g, respectively, compared with Control 2 (for *Fusarium* only), 15.61 µg/g, and Control 1 (without any addition), 17.31 µg/g (Fig. 8B).

The maximum value of chlorophyll a + b peaked after 50 days, then started to decline. Chlorophyll a + b increased significantly in all treated plants as compared to the FOL inoculated Control 1. The maximum value was observed in *N. calcicola* (extract) treated plants due to its high contents of biochemicals that were concentrated in its extract. These results agreed with Alwathnani and Perveen (2012).

Carotene

Figure 8C showed that the maximum carotene content after 50 days was observed in plants treated with *N. calcicola* (extract) (3.479 µg/g) followed by *N. linkia* (extract) (3.11 µg/g), *N. linkia* (culture) (3.088 µg/g), and *N. calcicola* (culture) (3.054 µg/g). The carotene content after 60 days of tested cyanobacteria could be arranged in the following arrangement: *N. calcicola* (extract), > *N. linkia* (culture) > *N. calcicola* (culture) > *N. linkia* (extract), which were represented as 2.20, 2.19, 2.06, and 1.87 µg/g, respectively. These results were compared to plants that were not treated with cyanobacteria (Control 1) at 1.77 µg/g, or the Control 2. The case of plants infected with *Fusarium* at 1.64 µg/g. The carotene level increased in the tomato plant. These results are shown in Fig. 8C and Fig. 9; when treated by cyanobacteria, as reported by Saniewski and Czapski (1983).

(B) Pot experiment after 100 days

Plant height (cm) and dry weight (g) of tomato

Data in Table 5 shows a highly significant difference among samples. The maximum plant height was observed in plants treated with *N. linckia* (culture) (30.33 cm), followed by *N. calcicola* (culture) (29.33 cm), *N. calcicola* (extract) (29.33 cm), and *N. linckia* (extract) (28.00 cm). On the other hand, the maximum dry weight was observed in plants treated with *N. linckia* (culture), 34.27 g; followed by *N. linckia* (extract), 27.83 g; *N. calcicola* (culture), 26.84 g; and *N. calcicola* (extract), 26.73 g. The fungicidal activity of culture filtrates of *Nostoc* spp. was attributed to the presence of bioactive compounds, which are employed as natural defense mechanisms against pathogenic fungi and bacteria. These agreed with Zee Shan et al. (2010). The same observations were reported by Mostafa et al. (2009). Both plant height and dry weight were compared to those control 1 (plants un-treated with cyanobacteria) and those control 2 (plants infected with *Fusarium*). These results agree with Alwathnani and Perveen (2012), who said it is evident that plant height and fresh and dry weights have increased in all treated plants compared to those of the FOL inoculated control.

Number and weight (g) of tomato fruit

Figures 10A and B show a very high significant difference between samples in fruit weight and number after 100 days, respectively. They were recorded in the following order: *N. linckia* (culture) > *N. linckia* (extract) > *N. calcicola* (culture) > *N. calcicola* (extract) compared to Control 1 and Control 2. These results agreed with Mostafa et al. (2009).

Figure 11 shows a clear appearance in the two control plants. *Fusarium* appeared dead, and tomato fruits appeared dry. *Nostoc* spp. was successful as an antagonist against *Fusarium oxysporum lycopersici*, which agreed with Chaudhary et al. (2012). The inhibitory effect of antifungal compounds may be due to their ability to inhibit spore germination or the fungal cell wall component, which alters the permeability of fungal cell membranes, as reported by Gupta et al. (2013). Awad et al. (2009) also reported that antifungal substances might inhibit lipid synthesis in the tested pathogenic fungi due to a decrease in the ratio of unsaturated to saturated fatty acids.

Conclusions

The present work indicated that *N. calcicola* and *N. linckia* were the most predominant cyanobacterial species in the collected soil samples at the study sites. *N. calcicola* recorded higher dry weight parameters, chlorophyll a, carotenoid, and total phenol than those of *N. linckia*. On the other hand, using the extract for both species achieved higher results *in vitro* and at the beginning of the pot experiment (*in vivo*), but at the end of the experiment, the results of the culture were higher than that of the extract. There was an improvement in the yield of tomatoes treated with *Nostoc* spp. Consequently, *N. calcicola* and *N. linckia* were considered to be biological control agents against *Fusarium oxysporum f. sp. lycopersici* (FOL) on tomato plants, and it is a promising strategy for sustainable agriculture

Declarations

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Compliance with ethical standards

Conflict of interest:

The authors declare that they have no conflict of interest.

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Tables

Due to technical limitations, tables are only available as a download in the Supplemental Files section.

Figures

Figure 1

Samples sites at Kafr El-Sheikh centers (1,3,6,7,8 in Sidi Salim, and 4,9,10 in Baltim).

A. *N. calcicola*

B. *N. linkia*

Figure 2

Nostoc spp. under microscope, A. *N. calcicola*, B. *N. linkia*

Figure 3

Effect of A. Total dry wt. (mg.L-1), B. Chl-a (mg/g fresh wt.), C. Carotenoid (µg/g fresh wt.) on *Nostoc* spp. growth, ** very high significant $p \leq 0.01$.

Figure 4

Total phenol content of Nostoc spp. ** very high significant $p \leq 0.01$.

Figure 5

GC/MS chromatogram of N. Calicicola.

Figure 6

GC/MS chromatogram of *N. Linkia*.

Figure 7

Inhibition zone of *Nostoc* spp. extract and Culture, ** very high significant $p \leq 0.01$.

Figure 8

Total pigments contents (µg) of tomato plant after 50 and 60 days, A. Chlorophyll-a, B. Chlorophyll-b, C. Carotene, ** very high significant $p \leq 0.01$ - ns: non-significant.

Figure 9

Pot experiment after 50 days; A- Control 1 (plant without any addition), B- Control 2 (plant infected with FOL), C- *N. calcicola* extract, D- *N. calcicola* culture, E- *N. linkia* extract, and F- *N. linkia* culture.

Figure 10

A. Fruit number, B. Fruit weight (g) of tomato after 100 days, * highly significant $p \leq 0.01$, ** very high significant $p \leq 0.01$.

Figure 11

Pot experiment after 100 days; A- Control 1 (plant without any addition), B- Control 2 (plant infected with FOL), C- *N. calcicola* extract, D- *N. calcicola* culture, E- *N. linkia* extract, and F- *N. linkia* culture.

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