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Bioactivity of *Nostoc linckia* Isolated from the Desert of Saudi Arabia against Fungi Responsible for the Post Harvest Diseases

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The bioactivity of methanol, acetone and water crude extracts of *Nostoc linckia* isolated from the desert of Saudi Arabia was evaluated against five post harvest disease causing fungi (*Aspergillus flavus, A. niger, Fusarium oxysporum, Penicillium chrysogenum* and *Cladosporium* sp.). The methanol extract of the *N. linkia* was observed to reduce the growth of all tested fungi significantly ((P ≤ 0.05). Maximum reduction in the mycilial growth was observed by methanol extract against *Cladosporium* sp. (40%). While *F. oxysporum* growth was reduced to 30.0%, the percent reduction in mycelial growth of *A. niger* was 24%, whereas, percent growth reduction in *P. chrysogenum* was 20% and in A. *flavus* it was 18.9%. Maximum growth reduction by acetone extract was 18.9% against *F. oxysporum,* where as water extract was not found much effective in reducing the growth of any tested fungi. The crude extracts of *N. linckia* were analyzed using gas chromatography-mass spectrometry (GC-MS). The main components in the crude extracts were Octadecanal (aldehyde) (86.8%); Boronic acid, Ethyl-, Dimethyl ester (84.8%). Beside these chemicals, Isopropyl Lactate was also detected in the crude extract; this component may be responsible for the antifungal activity of the extract. This study can be useful in the management of post harvest diseases caused by fungal pathogens. However, further studies are required to identify the compounds directly responsible for antifungal activities.

Key words: Bioactive compounds, *Nostoc linckia*, GC-MS, post harvest diseases, fungi.

Cyanobacteria are oldest photo-autotrophic vegetation in the world that occurs in freshwater, marine as well as terrestrial habitats[1]. Cyanobacteria have drawn much attention as prospective and rich sources of biologically active constituents and have been identified as one of the most promising groups of organisms to be able of producing bioactive compounds[2,3]. Cyanobacteria are known to produce metabolites with diverse biological activities such as antibacterial[4,5], antifungal[6,7], antiviral[8], anticancer[9,10], antiplasmodial[11], algicide[12], antiplatelet aggregation[13] and immuno-suppressive[14] activities. Cyanobacteria from local habitats seem to be a source of potential new active substances that could contribute to reduction of the number of bacteria, fungi, viruses and other microorganisms[15].

Plant pathogenic fungi are responsible for pre and post harvest diseases including yield losses in numerous economically important crops[16]. Losses due to postharvest disease may occur at any time from harvest to consumption. Losses due to postharvest disease are affected by a great number of factors including: host type, susceptibility of cultivars, the postharvest environment, duration of maturity and ripeness stage, treatments used for disease control, handling methods and postharvest hygiene. These post harvest disease also poses a potential health risk. A number of fungal genera such as *Penicillium,*

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Aspergillus Alternaria and *Fusarium* are known to produce mycotoxins under certain conditions. To reduce the use of chemical fungicide, extensive efforts have been made. Bioactive compounds isolated from various sources have been proved as the good biological control agents. Several reports suggest the antimicrobial effect of metabolites/compound extracted from algae[17] including against bacteria and fungi[18,19] .

Thus to fulfill the global need of the ecofriendly fungicides, we evaluated the antifungal activity of the methanol, acetone and water crude extracts of *Nostoc linkia*. Further the crude extract was analyzed by gas chromatography-mass spectrometry (GC-MS) to determine the chemical content of it.

MATERIALS AND METHODS

Cultivation and extraction of *N. linkia*

The culture of *Nostoc linkia*, previously isolated and identified from the desert soil of Saudi Arabia and maintained at the Department of Botany and Microbiology, King Saud University were used in the present study. *N. linkia* was sub cultured in BG 11 nutrition media[20] and allowed to flourish at 20-30 °C under constant light for 2-4 weeks. Cells of the active growing test cyanobacteria were harvested by filtering through Whatman no. 1 filter paper and the extracted biomass used for extraction.

To prepare the crude extract of *N. linkia* the solvents, acetone, methanol and water were used. Five gram biomass of *N. linkia* with 100 ml of methanol (Sigma Aldrich, USA) was kept on a shaker for 3 days at 20°C. After that mixture was filtered with Whatman no. 1 filter paper. The extract was kept in the water bath at 40 °C under the fume hood till the extract evaporated to dryness. The obtained residue was dissolved in 2 ml distilled water to get the final concentration of 50 mg/ml of the crude extract. The same process was followed with the solvent acetone and water.

Determination of inhibition potential of *N. linkia* by agar well diffusion method

The antifungal activity was evaluated by measuring diameter of the inhibition zone formed around the well. Pure cultures of fungal strains responsible for post harvest diseases, *Aspergillus flavus, A. niger, Fusarium oxysporum, Penicillium chrysogenum* and *Cladosporium* sp., were used in the present study. Stock cultures of test fungi were maintained on potato dextrose agar slants (PDA, Scharlau Chemie, Spain) and were stored at 4°C. Test fungi (1.0×10^6 spores/ml) were spread on PDA with the help of sterilized cotton swab. The extracts (50 µl) were placed in wells made on the pathogen inoculated agar plates. Wells containing only solvent served as the control. Plates were incubated for 3 days at 28 ± 2°C, and inhibition zones of mycelial growth around the wells were measured.

Inhibitory effect of the crude extracts of *N. linkia* on the growth of fungi

Four ml of crude extracts of *N. linkia* were placed in sterilized petri dish which was immediately followed by pouring 16 ml of PDA, so as to make the final concentration of culture filtrates 20%. After the agar solidified, mycelial discs of the tested fungi (5 mm) obtained from actively growing colonies were placed in the centre of the agar plates. The Petri dishes were incubated at 25°C for 4 days and after that the percent inhibition in the radial colony growth was calculated. The diameter of mycelial colony developed on the crude extract containing PDA plates was compared with the diameter of colony obtained on control plates (devoid of the crude extract). The inhibition of fungal growth was calculated by the following formula:

$$I = (C-T/C) \times 100$$

Where, I = inhibition (%), C = colony diameter in control plate and T = colony diameter in treated plate.

GC/MS analysis of crude extracts of *N. linkia*

To find out the chemical components of the crude extract of *N. linkia* the gas chromatography-mass spectrometry (GC-MS) was used. This was done by using Perkin Elmer (Clarus 500, USA) gas chromatography coupled with (Clarus 500, USA) mass spectrometer (MS) equipped with RTx-5 column (30x0.32nm). Oven temperature was initially held at 75°C for 2 min, then increased to 75 to 175°C at a rate of 50°C per min and finally held at 175°C for 7 min. Helium (3 ml/min) was used as a carrier gas. Neither internal nor external chemical standards were used in this chromatographic analysis. Interpretation of the resultant mass spectra were made using a computerized library-searching program (NIST database) and by studying the fragmentation

pattern of such compound resulted from mass spectrometry analysis. Concentration of such compound was calculated by the following formula:

Compound conc. percentage= [P1/P2] x 100

where, P1 is the peak area of the compound and P2 is whole peak areas in the fractionated extracts.

Statistical analysis

Data were analyzed by least significant difference (L.S.D.) test at probability of 0.05 to identify significant effect of a treatment. Duncan Multiple Range Test was used to evaluate the significant differences between treatments ($P \leq 0.05$). Analysis of variance (ANOVA) analysis was done with the SPSS statistics software.

RESULTS AND DISCUSSION

Results presented in Table 1 indicate that water, acetone and methanol extract of *N. linkia* have significant ($P \leq 0.05$) effect on the tested fungi. Several scientists have reported that the extracts of *Nostoc* species significantly inhibit the growth of phytopathogenic fungi[21,22]. Methanol extract had significant ($P \leq 0.05$) effect on the growth reduction of the tested fungi. The maximum zone of inhibition by methanol extract was observed against *Cladosporium* sp. (27.7 mm), next to it was *F. oxysporum* (22.7 mm) followed by *A. niger* (17.2 mm), *P. chrysogenum* (18.5 mm) and *A. flavus* (13.5 mm). Acetone extract was found moderately effective against *F. oxysporum* (17.0 mm), whereas, it showed weak activity against *Cladosporium* sp. (11.3 mm) and *P. chrysogenum* (9.0 mm) and the extract was not effective against *A. niger* and *A. flavus*. Water extract has showed weak zone of inhibition against *F. oxysporum* and *Cladosporium* sp., however, it was unable to inhibit the growth of, *P. chrysogenum, A. niger,* and *A. flavus*. It has been noted that all crude extracts had the potential to inhibit the growth of one or more than one tested plant pathogenic fungi but none of them were found to be fungicidal. All three crude extracts were effective in inhibiting the growth of most of the plant pathogenic fungi. However the affected fungal species were not equally susceptible to the bioactive compounds of crude extract; the reason for this may be the phylogeny of the microorganism species[23].

Fig. 1 depicts the effect of water, acetone and methanol extracts of *N. linkia* on the percent growth reduction of the tested plant pathogenic fungi. Methanol extract has showed the maximum potential to reduce the mycelial growth of all tested fungi. Maximum reduction in the mycelial growth was observed by methanol extract against *Cladosporium* sp. (40%); while *F. oxysporum* growth was reduced to 30.0%, the percent reduction in mycelia growth of other fungi was as follows; *A. niger* (24%), *P. chrysogenum* (20%) and A. *flavus* (18.9%). Acetone and water extract also able to significantly ($P \leq 0.05$) reduce the mycelial growth of fungi as compared to control. The percent reduction in the growth of plant pathogenic fungi by these two extracts ranges from 19 to 5 percent. Kim[24] reported the strong antifungal effect of

Table 1. Effect of water, acetone and methanol crude extracts of *N. linkia* against *A. flavus, A. niger, F. oxysporum, P. chrysogenum* and *Cladosporium* sp.

Plant pathogenic fungi	Crude extract		
	Water	Acetone	Methanol
	Zone of inhibition (mm)		
F. oxysporum	10.25 ±0.50^{a}	17.00 ±0.82^{d}	22.75 ±0.96^{a}
A. flavus	8.00 ±0.82^{b}	8.00 ±0.58^{a}	13.50 ±0.58^{b}
A. niger	5.75 ±0.50^{b}	8.25 ±0.50^{a}	17.25 ±0.50^{c}
Cladosporium sp.	10.33 ±0.96^{a}	11.33 ±0.50^{c}	26.67 ±0.50^{f}
P. chrysogenum	8.50 ±0.58^{b}	9.00 ±0.00^{b}	18.50 ±0.58^{d}

Â 8 mm: No activity; 8.0-15 mm: weak activity; 16-19 mm: moderate activity; 20-25 mm: strong activity

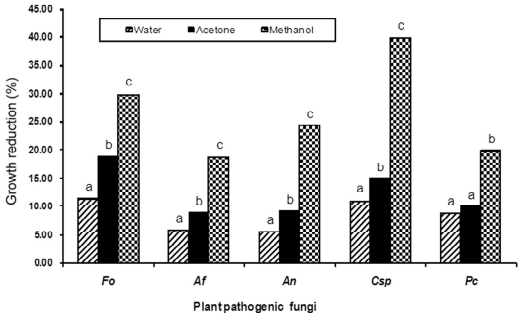
Data are means ± S.D of four replicates. Column with different letters are significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

Table 2. The GC-MS analysis of different components in the crude extract of *N. linckia*

S. No.	Compound	%
1	Boronic acid, Ethyl-, Dimethyl ester	84.8
2	(S)-Isopropyl Lactate	79.7
3	Octadecanal (Aldehyde)	86.8
4	3,4-Hexanediol, 2,5-Dimethyl-	77.2
5	2-Butanone, 3-Hydroxy-	82.8
6	Octatricontadiene-1,37	86.1

% of the compound concentration in the total extract

petroleum ether and methanol extracts of *N. commune* against some plant pathogenic fungi. Similarly, ether extract of *N. muscorum* inhibited *Sclerotinia sclerotiorum* infection on lettuce[25]. *In vitro* and *in vivo* fungal growth of *F. oxysporum* f. sp. *lycopersici* was significantly inhibited by methanol extract of *N. commune*[26]. The difference in antifungal activity was noticed between different extracts within the *N. linkia*. This suggested that the effectiveness of algal bioactivity depends on the type of solvents used in extraction[27,28] Antimicrobial activity of methanol, acetone and diethyl ether extracts of blue green algae and various algae have been reported earlier[29,30,31]. The methanol extract of *N. linckia* showed moderate zone of inhibition against *F. oxysporum* f. sp. *lycopersici* [32].



Fo: *Fusarium oxysporum;* Af: *Aspergillus flavus;* An: *Aspergillus niger;* Csp.: *Cladosporium* sp.; Pc: *Penicillium chrysogenum*

Each value is an average of four replicates. Bars with different letters are significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

Fig. 1. Effect of crude extracts of *N. linkia* on percent growth reduction of *A. flavus, A. niger, F. oxysporum, P. chrysogenum, Cladosporium* sp. and *A. alternata*

Chemical composition and concentrations of the analyzed fractions presented in Table 2 showed that the main component in the crude extracts were Octadecanal (aldehyde) (86.8%); Boronic acid, Ethyl-, Dimethyl ester (84.8%). Beside that (S)-Isopropyl Lactate was also detected in the crude extract. It has been reported that pressurized liquid ethanol extracts of *H. pluvialis* showed antifungal activity against the *C. albicans*, but not against *A. niger*. Butanoic acid and methyl lactate were claimed to be the main compounds responsible for such antifungal activity[33]. The antimicrobial activity of microalgae has been attributed to compounds belonging to several chemical classes – including indoles, terpenes, acetogenins, phenols, fatty acids and volatile halogenated hydrocarbons [34,35]. Cyanobacteria produce biologically active compounds that have antifungal activity[36,37] and antibiotic and toxic activity against plant pathogens[38,39].

Results revealed that methanol crude extract had the potential to inhibit the growth of the most of the tested fungi. This study can be useful in the management of post harvest fungal diseases. Thus, *N. linkia* should be explored further to find out the exact bioactive compound responsible for the antifungal activity, its mode of action and utilization of it for controlling the post harvest diseases caused by fungi.

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