THE POTENTIAL EFFECT OF TWO CYANOBACTERIAL SPECIES; *ANABAENA SPHAERICA* AND *OSCILLATORIA AGARDHII* AGAINST GRAIN STORAGE FUNGI

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Abstract

Cyanobacterial secondary metabolites have a diverse antagonistic activity that lead to disintegration of microbial growth. The present study was undertaken to explore the inhibitory effect of three different solvents extracts (methanol, acetone and water) of two cyanobacteria species: *Oscillatoria agardhii* and *Anabaena sphaerica* extracts against grain storage fungi. *In vitro* fungal growth, mycotoxins production of grain storage fungi (*Fusarium equiseti; F. moniliforme; F. solani; F. proliferatum , F.* graminearum*; Penicillium digitatum; Aspergillus terreus; Aspergillus niger; Aspergillus flavus; Aspergillus oryza; Alternaria alternate and Alternaria triticina)* were significantly($p<0.01$) inhibited by cyanobacterial extracts. HPLC analysis revealed that compelate reduction of Aflatoxin B1, Zearalenone and Fumonisin B1 with acetone extract of *Oscillatoria agardhii* and minimum concentration (0.3µg/L) was found with Ochratoxin A. Acetone extracts of both cyanobacteria species have potential for the suppression of all grain storage fungi and increased seeds germination of maize, wheat, barley and rice. It was also observed that when wheat seeds treated with water extract for one hour soaking, significant ($p<0.01$) increased in seed germination and reduced the frequency of seeds storage fungi during 180 days was also observed**.** These results statements be a necessary (or) recommended that the both species of Cyanobacterial having a potential capacity as a source of antifungal activity, which makes them interesting for screening for natural and highly bioactive secondary metabolites products and it may leads to development of new pharmaceutical agents.

Keywords: Antifungal activity, *Anabaena sphaerica*, inhibition zone, *Oscillatoria agardhii*, grain storage fungi

Introduction

One of the most important effects of post-harvest decays seed and feed deterioration by fungi is the induction of mycotoxicoses. This is a disease of animals and humans following consumption of feeds and foods invaded by fungi that produce toxic substances called mycotoxins. The storage fungi, mainly comprising several species of *Aspergillus* and *Penicillium,* do not invade grains to any appreciable degree or extent before harvest (Mehrotra, 1983), but they can cause severe dis-coloration of seed in storage resulting in germination failure, dis-coloured or otherwise damaged embryos or whole seeds, and production of mycotoxins that constitute a health hazard for man and animals (Dharam Vir, 1974 and Malaker *et al.*, 2008). Each species or group of species of *Aspergillus* has its own rather sharply defined lower limit of moisture content usually between 13 and 18% for invasion of stored grains. Some common mycotoxicoses caused by common and widespread fungi such as *Aspergillus, Penicillium, Fusarium* and *Stachybotrys* result in severe illness and death. *Aspergillus* and *Penicillium* produce their toxins mostly in stored seeds, hay or commercially processed foods and feeds although infection of seeds usually takes place in the field. Aflatoxin is about the most popular and widespread mycotoxin. Its name derives from the fact that it was originally found to be produced by *Aspergillus flavus* (Agrios, 1978 and Haggag, 2013 a & b), but is now known to be produced by other species of *Aspergillus*. Aflatoxin B1 is produced by *Aspergillus terreus,* though it may also be produced by *Aspergillus flavus* as well as *Aspergillus oryzae.* It is the most toxic, carcinogenic and most prevalent of the different aflatoxins (Haggag *et al.*, 2014 a, b). Algae are one of the chief biological agents that have been studied for the control of plant pathogenic fungi particularly soil borne disease. Cyanobacteria are characterized by their capacity to perform biological nitrogen fixation and oxygenic photosynthesis. As cyanobacteria are very resistant to extreme environmental conditions, they are assuming increasing importance in frontier areas of biotechnology. The typical anabiosis and rapid restoration of activity under favorable conditions are characteristic of them (Pankratova, 1987). Cyanobacteria have the capability to produce metabolites both intracellular and extracellular. Secondary metabolites from cyanobacteria are associated with toxic, hormonal, antineoplastic and antimicrobial effects (Shweta *et al.*, 2011; Abd El-Aty *et al.*, 2014). 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 (Mundt *et al*., 2001). Biologically active compounds found in plants appear to be more adaptable, acceptable and safer than synthetic compounds and display a wealthy source of potential pathogens control agents. Moreover, Cyanobacteria are also reported to produce a wide range of plant growth regulators such as abscisic acid, ethylene, jasmonic acid, auxin, and cytokinin-like substances as well as the cytokinin isopentenyl adenine (Kim, 2006). Fungal pathogens cause many diseases in plants leading to destruction of resources in agriculture. Traditional methods to circumvent fungal pathogens involve the application of synthetic fungicides and growing resistant cultivars. The use of crude extract from algae may be an alternative to conventionally used as antimicrobial to control microbe growth, due to their secondary metabolites compounds (Abd El-Aty *et al.,* 2014). 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 (Fish and Codd, 1994 & Schlegel et al., 1999). Therefore, the objective of the present work was to study the *in vitro* antifungal activity of three different extracts (methanol, acetone and water) of *Oscillatoria agardhii* and *Anabaena sphaerica* against grain storage fungi.

MATERIALS AND METHODS

Algae growth and different extracts preparation:

Two algal species were selected to evaluate their antifungal activity against some species of fungi. These algae belong to cyanobacteria, *Anabaena sphaerica* (N2-fixing blue algae) and *Oscillatoria agardhii.* The tested algal species were previously isolated from phytoplankton community structure of River Nile at Ismailia canal, Egypt. BG11 medium was used for maintenance of *Oscillatoria agardhii* (Carmichae, 1986) and Nitrogen free formula for *Anabaena sphaerica* (Abd El-Aty *et al.,* 2014). The cultured media were incubated at 30 ± 2 °C without aeration and under continuous illumination of fluorescent lamps with intensity 2500 lux. The cultures were shaken every day to prevent algal cell clumping and adherence of algal cells to the containers. *Oscillatoria agardhii* and *Anabaena sphaerica* were characterized for their antifungal activity as follow:

Extracts preparation

The algal pellets (37gm) were extracted using serial Exhaustive Extraction Method (Das *et al*., 2010) with methanol, acetone, and water. All extracts were dried and weighed to estimate the concentration in 1 ml. Dried extracts were either reconstituted in ethanol or deionized water.

Isolation and purification of pathogenic fungal strains

Fungi were isolated from stored maize, wheat, maize and rice grains. The grains were surface-sterilized in NaOCl for 2 min and rinsed in two changes of sterile distilled water. The grains were plated on sterile potato dextrose agar (PDA) at the rate of 10 grains per plate. Thirty grains were plated per crop and incubated at room temperature of 25 ± 1^{o}C. Sub-cultures were made from emerging colonies and pure cultures obtained for subsequent studies. After incubation the growth characters as well as percentage of infection were recorded. In order to isolate these fungi into pure culture, Potato Dextrose Agar (PDA) was prepared and the fungi were inoculated onto the sterile PDA and incubated for 7 days at the end of which the fungi were identified based in their colour, spore morphology and mycelia growth using the light microscope (Begum *et al*., 2004). The fungi identification was done according to the methods of Barnett and Hunter (1972).

Test fungi

Twelve species of *Fusarium equiseti; F. moniliforme; F. solani; F. proliferatum , F.* graminearum*; Penicillium digitatum; Aspergillus terreus; Aspergillus niger; Aspergillus flavus; Aspergillus oryza; Alternaria alternate and Alternaria triticina* isolated from above seeds, selected and used as test fungi for antifungal activity assay.

Antifungal activity assay of Cyanobacteria extracts

The antifungal activity was tested by using the filter paper disc diffusion method (Saha *et al*., 1995) employing 24 hours cultures of the above mentioned organisms.

The agar media were seeded by fungal suspensions adjusted to 10^5 CFU/ml, sterile filter paper discs (6 mm diameter) were saturated with 250 µg/ml of each crude extract. The Petri dishes were placed for 5 h at 4 °C till the metabolites release into the medium. The plates were incubated at 37 °C for 24 h. The antifungal activity was determined by measuring the diameters of the clean inhibitory zone around each paper disc.

Effect of algal extracts on dry weight and aflatoxin production of toxigenic fungi

One ml of conidial suspension of fungi (1xl0^7 conidia/ml) was inoculated into 100 ml medium amended with each extracts to obtain the previous concentration. They were then incubated at room temperature for 10 days. Three replications were used for each treatment (including the control). Observations were made on the dry weight of mycelia of fungi and aflatoxin production. The dry weight of mycelia was determined by drying

the fungal colonies in an oven at 70^0C for 4 days until a constant weight was attained. Aflatoxin B1 content was determined using a High Performance Liquid Chromatography method (Christian, 1990).

The contents of individual fungal culture were transferred into separate glass bottles and extracted with methanol (85%). Bottles were capped and shacked on a rotary shaker at 120 rpm for 90 min and the suspensions were centrifuged at 3500 rpm for 20 min. Supernatants were transferred to glass bottles and kept at room temperature for analysis. Aflatoxin levels were measured using high performance liquid chromatography (HPLC) (model: Perkin Elmer series 200 UV/VIS) with a C18 column that had an internal diameter of 300 x 3.9 mm. The HPLC apparatus was equipped with a UV detector, and fluorescence was measured using 365-nm excitation and 430-nm emission wavelengths. The mobile phase consisted of methanol: acetic acid: water (20:20:60 v/v/v). The total run time for the separation was approximately 30 min, and the flow rate was 1 ml/min (Christian, 1990).

Seed treatment with algal extracts: Selected seed samples of grains were treated following dipping method. The seeds were dipped in each algal extracts with the same concentration for 1 hour in algae previously prepared extracts (Acetone, water, and methanol). After 1 hour, extracts were drained out from the petri dishes. The treated seeds were allowed to be dried up on filter paper for some time and were tested following the standard blotter method (ISTA. 1976), to observe the growth of different fungal colonies on the seeds. For each treatment 100 seeds with three replications were placed on six petri dishes.

Determination of seed germination (%) of fresh and stored wheat seeds
Blotter method: Three pieces of blotting paper were placed in fold in each Petri dish 9 cm diameter and incubated at 25±2°C. Three replicates were prepared. The percent germination of wheat seed was calculated after one week (De Tempe, 1953). The experimental data were recorded from fresh seeds as well as those stored, after every three months (0, 90 and 180 days) of storage at room temperature.

Statistical Analysis The data collected from the experiment were analyzed for test of significance and compared the treatment means following Completely Randomized Design (CRD) by using Duncan's Multiple Range Test (DMRT) at 5% level of probability.

RESULTS AND DISCUSSION

Tested fungi

The study shows that maize, wheat, barley and rice grains were infested to various degrees with storage fungi. A total of twelve species of

Fusarium equiseti; F. moniliforme; F. solani; F. proliferatum , F. graminearum*; Penicillium digitatum; Aspergillus terreus; Aspergillus niger; Aspergillus flavus; Aspergillus oryza; Alternaria alternate and Alternaria triticina* isolated from grain seeds. Maize and wheat were the most infested of all fungi (Table 1). Mycotoxin assay revealed that several toxins were produced i.e. aflatoxin, fumonisin, Ochratoxin A and zearalenone were identified in this study. Most the fungi produced one toxin or the other (Table 2).

Table (1). Frequency of grain storage fungi associated with grains

Isolates	Percentage %			
	Maize	**Wheat**	**Barley**	**Rice**
Fusarium equiseti	7.2	3.2	2.1	-
F. moniliforme	9.2	1.4	0.7	-
F. solani	8.4	3.1	3.2	-
F. proliferatum	11.4	2.4	2.4	-
F. graminearum	4.2	3.3	6.6	-
Penicillium digitatum	6.4	8.4	1.3	-
Aspergillus terreus	6.7	11.4	2.4	
Aspergillus niger	15.3	5.4	4.2	1.3
Aspergillus flavus	12.4	6.4	3.2	1.3
Aspergillus oryza	3.2	-	-	12.3
Alternaria alternate	11.5	15.3	2.4	-
Alternaria triticina	-	4.6	2.4	-

Table (2). Mycotoxins produced by fungi isolated from stored grains in liquid media.

Isolates	Amount	Mycotoxin (µg/L)
A. flavus	Aflatoxin B1	20.42
A. terreus	Aflatoxin B1	11.21
A. oryzae	Aflatoxin B1	7.54
Fusarium sp.	Zearalenone	4.41
A. niger	Ochratoxin A	18.6
P. italicum	Fumonisin B1	12.71

Antifungal activity

Antifungal potentiality of solvents and aqueous extracts of cyanobacterial extracts of *Oscillatoria agardhii* and *Anabaena sphaerica* were tested against twelve species of *Fusarium equiseti; F. moniliforme; F. solani; F. proliferatum , F.* graminearum*; Penicillium digitatum; Aspergillus terreus; Aspergillus niger; Aspergillus flavus; Aspergillus oryza; Alternaria alternate and Alternaria triticina in vitro* as growth reduction and mycotoxins production (Table 3, 4 and 5). It was concluded

Table (3). Antifungal activity of *Oscillatoria agardhii* and *Anabaena sphaerica* extracts against fungal grains pathogens

Isolates	Diameter of inhibition zone (mm)					
	Oscillatoria agardhii			*Anabaena sphaerica*		
	Acetone	**Methanol**	**Water**	**Acetone**	**Methanol**	**Water**
Fusarium equiseti	23.3	13.4	9.4	16.2	10.3	7.7
F. moniliforme	24.1	16.7	7.9	15.5	12.4	6.6
F. solani	20.7	17.9	6.5	16.3	11.4	5.5
F. proliferatum	22.5	18.8	7.7	15.2	12.4	5.8
F. graminearum	18.7	15.6	8.1	14.2	11.3	9.1
Penicillium digitatum	11.4	8.8	5.5	9.8	7.6	6.6
Aspergillus terreus	13.5	10.8	7.3	11.3	9.8	6.6
Aspergillus niger	11.3	8.8	6.3	9.7	6.4	5.5
Aspergillus flavus	10.8	8.1	6.6	8.7	6.6	5.5
Aspergillus oryza	9.8	8.1	6.4	7.7	6.2	6.6
Alternaria alternate	23.4	16.8	9.9	10.8	8.8	9.5
Alternaria triticina	22.4	17.6	8.6	12.5	9.8	9.3
LSD	1.6	1.5	0.8	1.3	1.2	0.7

Table (4). Antifungal activity of *Oscillatoria agardhii* and *Anabaena sphaerica* extracts against fungal grains pathogens

Isolates	**Mycelial inhibition (Dry Mycelial Weight method mg/l)**							
	Oscillatoria agardhii				***Anabaena sphaerica***			
	Acetone	**Methanol**	**Water**	**Control**	**Acetone**	**Methanol**	**Water**	**Control**
Fusarium equiseti	0.00	0.9	6.3	39.8	09.0	2.1	8.8	39.8
F. moniliforme	0.02	0.9	6.3	38.7	09.0	3.3	8.7	38.7
F. solani	0.01	1.0	6.4	35.3	1.1	3.3	9.6	35.3
F. proliferatum	0.04	2.4	8.3	30.3	1.2	3.4	8.3	30.3
F. graminearum	0.02	2.3	8.4	36.6	09.0	2.4	8.3	36.6
Penicillium digitatum	0.04	2.4	7.3	23.3	09.0	3.2	10.8	23.3
Aspergillus terreus	0.03	2.3	8.2	22.3	08.0	2.3	11.3	22.3
Aspergillus niger	0.04	2.0	8.2	24.4	09.0	2.2	12.2	24.4
Aspergillus flavus	0.03	2.0	9.6	20.3	09.0	2.5	12.8	20.3
Aspergillus oryza	0.05	1.3	8.8	21.3	07.0	1.5	11.1	21.3
Alternaria alternate	0.04	2.2	4.8	34.4	09.0	2.4	10.1	34.4
Alternaria triticina	0.05	1.1	4.3	33.3	09.0	1.4	10.4	33.3

Table (5). Antifungal activity of *Oscillatoria agardhii* and *Anabaena sphaerica* extracts against mycotoxins produce

Isolates	***Mycotoxin***	**Mycotoxins production (µg/l)**							
		Oscillatoria agardhii				***Anabaena sphaerica***			
		Acetone	**Methanol**	**Water**	**Control**	**Acetone**	**Methanol**	**Water**	**Control**
A. flavus	**Aflatoxin B1**	0.0	0.4	3.4	20.42	0.5	1.2	6.4	20.42
A. terreus	**Aflatoxin B1**	0.0	0.5	3.2	11.21	0.5	1.5	7.2	11.21
A. oryzae	**Aflatoxin B1**	0.0	0.5	1.3	7.54	0.5	0.9	5.3	7.54
***Fusarium* sp.**	**Zearalenone**	0.0	1.0	1.4	4.41	0.8	1.9	4.3	4.41
A. niger	**Ochratoxin A**	0.3	2.3	2.4	18.6	1.5	2.7	5.4	18.6
P. italicum	**Fumonisin B1**	0.0	2.0	1.4	12.7	0.4	2.5	2.4	12.71

that the diameter of inhibition zone depends mainly on the type of the algal species, type of solvent used and the tested fungal species. The experimental analysis of antifungal effects indicated that all tested fungi showed higher sensitivity to the acetone extract of *Oscillatoria agardhii* with the higher zone with *Fusarium* spp. and *Alternaria* spp. as shown in Table (3). Whereas the methanol extract showed moderate activity against all fungal species.

The same results were also shown in liquid medium, significant ($p<0.01$) inhibited of mycelial growth was observed in *Oscillatoria agardhii* extract followed by *Anabaena sphaerica* (Table 4). The highest growth reduction was recorded with *Fusarium* spp. and *Alternaria* spp. Acetone extract is the best solvent in reducing fungal growth and mycotoxin production (Table 5). Complete reduction of Aflatoxin B1, Zearalenone and Fumonisin B1 with acetone extract of *Oscillatoria agardhii* and minimum concentration was found with Ochratoxin A. Biological natural products isolated from cyanobacterial extracts of *Oscillatoria agardhii* and *Anabaena sphaerica* extracts are recommended as potential as antimicrobial (Abd El-Aty, *et al.*, 2014). Broad ranges for the *in vitro* anti-fungal activities of different cyanobacterial extracts of *Oscillatoria agardhii* and *Anabaena sphaerica* extracts. The results obtained recorded the higher antifungal activity for acetone extract of *Oscillatoria agardhii* as indicated by inhibition in dry weight and mycotoxin production. The phenolic compound released from dried crudes of cyanobacterial extracts may be answerable for their antifungal properties as confirmed earlier by (Abd El-Aty, *et al.*, 2014) who found that phenolic compounds responsible for the antibacterial activities of these extracts. Other scientists have also reported that phenolic compounds from different plant sources could inhibit various food-borne pathogens (Plaza *et al*., 2009; Osman *et al*., 2011). The lack of activity in other extract in the disc diffusion assay doesn't mean that the extracts have no active ingredients. Though the screening by disc diffusion assay has its own advent as it is simple, less time consuming and requires only a small quantity of material.

Efficacy of different extract on seed germination and stored wheat seeds

In this experiment different extracts were used in controlling seed-borne fungi of four grain seeds. In all grain seeds, the highest germination was found when the seeds were treated with cyanobacterial of *Oscillatoria agardhii* and *Anabaena sphaerica* extracts and it increased germination compared with untreated control (Table 6). Among the extracts, best performance in terms of reducing percent seed-borne infection was obtained through treating the seeds of acetone extract of *Oscillatoria agardhii.* This may be due to the impact of these antifungal compounds on seeds germination. Reduction in germination of wheat seeds during storage might

be due to activity of the storage fungi. It was also observed that wheat seeds of the aqueous extract soaked for one hour duration soaking, significant ($p<0.01$)increased in seed germination. It was also observed that when wheat seeds treated with water extract for one hour soaking, significant($p<0.01$) increased in seed germination and reduced the frequency of seeds storage fungi during 180 days was also observed (Table 7 & 8)**.** Among the extracts, best performance in terms of reducing frequency of seed-borne fungi was obtained through treating the seeds of acetone extract of *Oscillatoria agardhii.*

These results are similar to those reported by Caire *et al.* (1993), Fish and Cood (1994), Borowitzka (1995) and Rizk, (2006) who reported that the extracts of *Nostoc muscorum* significantly($p<0.01$) inhibited the growth of *Candida albicans , Sclerotium rolfsii Sacc., Rhizoctonia solani Kühn. and Fusarium verticillioides* and *Sclerotinia sclerotiorum*. Cyanobacteria produce exopolysaccharides that can function as energy sources for fungi and produce plant growth regulators, which are abscisic acid, ethylene, jasmonic acid, auxin, and cytokinin-like substances, the cytokinin isopentenyl adenine. These substances can influence fungal growth (Ördög and Pulz, 1996). Also, Abo-Shady *et al.* (2007) also reported that cyanobacteria filtrates strongly inhibited the phytopathogenic fungi isolated from leaves, stems, and roots of *Faba bean*. Moreover, mycelial growth of several phytopathogenic fungi such as *Fusarium oxysporum, Penicillium expansum, Phytophthora cinnamomi, Rhizoctonia solani, Sclerotinia sclerotiorum, and Verticillium albo-atrum* were inhibited by the methanol extracts of the cyanobacterium Nostoc strain ATCC 53789 (Biondi *et al*., 2004).

The field fungi like *Alternaria,* and *Fusarium* invade seeds as they are developing on the plants in the field or after they have matured, but before they are harvested. These fungi usually do not continue to grow in grains after harvest, but may remain alive for years in grains stored at low moisture content and low temperature (Christensen and Kaufmann. 1979). In general, the damage caused by field fungi is done by the time the grains are harvested, although invasion of the developing or mature embryos of cereal seeds by *Fusarium* may result in development of discoloured embryos during storage. The storage fungi, mainly comprising several species of *Aspergillus* and *Penicillium,* do not invade grains to any appreciable degree or extent before harvest, but they can cause severe discolouration of seed in storage resulting in germination failure, discoloured or otherwise damaged embryos or whole seeds, and production of mycotoxins that constitute a health hazard for man and animals.

Table (6). Effect of *Oscillatoria agardhii* and *Anabaena sphaerica* extracts on germination of grain sedes

Seeds	*Oscillatoria agardhii*								*Anabaena sphaerica*							
	Acetone		Methanol		Water		Control		Acetone		Methanol		Water		Control	
	Seeds germination	% infected seeds	Seeds germination	% infected seeds	Seeds germination	% infected seeds	Seeds germination	% infected seeds	Seeds germination	% infected seeds	Seeds germination	% infected seeds	Seeds germination	% infected seeds	Seeds germination	% infected seeds
Maize	96.4	4.3	91.6	8.5	78.4	11.3	56.5	33.2	91.4	8.8	87.6	9.4	77.4	19.5	56.5	33.2
Whet	98.4	3.2	93.3	7.5	79.5	10.3	69.8	24.2	89.7	7.6	89.6	8.4	80.7	18.5	69.8	24.2
Barley	98.6	2.4	96.6	6.6	80.1	9.4	74.8	21.4	91.7	6.7	90.6	7.6	83.3	16.4	74.8	21.4
Rice	98.4	2.5	95.5	4.4	77.8	8.3	86.5	18.8	89.8	9.6	86.6	8.4	85.4	17.6	86.5	18.8
LSD	0.4	0.7	0.6	0.6	0.7	0.6	1.3	2.4	0.5	0.6	0.7	0.8	0.8	0.9	1.3	2.4

Table (7). Fungi isolated from seeds of *Triticum aestivum* treated with *Oscillatoria agardhii* extract by Agar Plate Method during different period of storage

Isolates	Fungi isolated from seeds											
	Acetone			Methanol			Water			Control		
	Fresh seeds	Day 90	Day 180	Fresh seeds	Day 90	Day 180	Fresh seeds	Day 90	Day 180	Fresh seeds	Day 90	Day 180
Fusarium equiseti	0.2	0.4	1.3	0.6	1.8	3.8	1.5	3.2	5.5	3.2	4.5	8.7
F. moniliforme	0.4	0.4	1.4	0.8	1.7	2.4	2.8	2.9	4.2	1.4	8.8	9.6
F. solani	-	-	-	-	-	-	-	-	-	3.1	6.9	8.8
F. proliferatum	-	-	-	-	-	-	0.7	1.4	11.4	2.4	7.7	15.4
F. graminearum	1.0	1.4	2.7	1.8	2.8	3.6	2.1	3.3	5.6	3.3	11.5	17.8
Penicillium digitatum	1.2	1.4	4.1	1.4	3.1	4.4	4.3	5.3	7.3	8.4	14.6	24.2
Aspergillus terreus	2.1	2.5	4.7	3.3	3.7	6.4	4.8	7.7	9.6	11.4	10.8	16.7
Aspergillus niger	2.4	2.4	5.6	2.8	3.6	4.4	5.4	6.2	8.4	5.4	12.4	21.4
Aspergillus flavus	1.4	1.5	5.7	1.9	2.1	3.2	6.8	7.1	9.8	6.4	10.8	18.8
Alternaria alternate	0.3	-	1.0	1.3	1.4	1.5	4.8	6.7	8.4	2.5	9.8	14.6
Alternaria triticina	0.6	-	1.8	-	1.5	2.0	2.8	3.8	6.2	2.4	7.8	13.4
LSD	0.2	0.4	0.6	0.5	0.7	0.9	0.7	0.9	1.2	1.2	1.4	1.6

Table (8). Fungi isolated from seeds of *Triticum aestivum* treated with *Anabaena sphaerica* extract by Agar Plate Method during different

Isolates	Fungi isolated from seeds											
	Acetone			Methanol			Water			Control		
	Fresh seeds	Day 90	Day 180	Fresh seeds	Day 90	Day 180	Fresh seeds	Day 90	Day 180	Fresh seeds	Day 90	Day 180
Fusarium equiseti	0.8	1.9	3.2	1.7	3.0	4.5	1.9	3.8	6.9	3.2	4.5	8.7
F. moniliforme	0.9	1.6	2.0	2.2	2.7	3.2	2.8	3.5	5.2	1.4	8.8	9.6
F. solani	-	-	-	-	-	-	-	-	-	3.1	6.9	8.8
F. proliferatum	0.4	1.0	1.8	0.9	1.8	7.4	1.7	2.9	12.1	2.4	7.7	15.4
F. graminearum	1.9	2.5	3.7	2.2	3.7	5.3	2.9	3.8	5.9	3.3	11.5	17.8
Penicillium digitatum	1.7	2.1	3.4	2.3	4.2	8.3	5.3	7.3	9.9	8.4	14.6	24.2
Aspergillus terreus	2.3	2.7	5.4	2.8	5.4	8.4	6.3	6.7	9.9	11.4	10.8	16.7
Aspergillus niger	2.7	3.6	4.9	2.4	5.3	7.1	7.4	7.2	9.4	5.4	12.4	21.4
Aspergillus flavus	1.3	2.6	3.8	2.8	5.3	8.8	8.8	8.1	11.8	6.4	10.8	18.8
Alternaria alternate	1.1	1.9	1.9	3.8	5.7	8.1	5.3	7.7	9.1	2.5	9.8	14.6
Alternaria triticina	0.4	1.1	1.7	3.8	6.8	7.4	4.3	5.8	7.2	2.4	7.8	13.4
LSD	0.5	0.6	0.9	0.7	0.9	1.1	0.9	1.2	1.4	1.2	1.4	1.6

CONCLUSION

Cyanobacteria are wonderful organisms with diverse range of potentials. Extraction with different solvents could affect the antifungal activities of *Oscillatoria agardhii* and *Anabaena sphaerica*. The most efficient solvent for antifungal activity is acetone. The present results lead to the suggestion that Cyanobacterial algal compounds could be considered as promising seed treatment against plant pathogenic fungi. This applicable study characterized as clean, cheep, and fungicide alternative and being friendly environment.

The acetone extract which exhibits high antifungal activity against seeds storage fungi should be purified and characterized completely to explore its potential application as grain preservative.

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