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Elemental Analysis of Fresh Water Green Algae *Zygnema Leiospermum* & Its Antifungal Activity on Various Fruit Spoiling Fungi

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Abstract: This study was carried out to assess the elemental analyses of fresh water green algae *Zygnema* l*eiospermum* De Bary and their effect on fungal growth. The algae were analyzed for five elements including zinc (Zn), iron (Fe), potassium (K), sodium (Na) and calcium (Ca). The antifungal activity of green algae was also examined for various fruit spoiling fungal species. The results showed Calcium (Ca) content (45.682±5.552) was relatively higher than other four elements. The other elements such as Fe, K, Na content were recorded 16.019±1.946, 12.120± 1.473, 11.282±1.371 respectively. The algal specie *Zygnema leiospermum* had lower zinc content (4.373±0.531). The extract such as ethanol, ethyl acetate, chloroform, methanol and water extract were obtained from alga. The fruit spoiling fungi were collected from various fruits and their culture was grown on potato Dextrose medium. The fungal colonies grown in medium were applied with various extracts obtained from alga. The results showed that the antifungal activity of *Zygnema leiosperm* fraction of ethanol extract showed maximum inhibition over fruit spoiling fungi *Aspergillus flavus* Link ex Gray.

INTRODUCTION

Water covers over two thirds of earth surface; undoubtedly which is the most precious natural resource. It is divided into fresh water and marine water. Marine water available in biosphere is 99%, and approximately 1% fresh water is available in this biosphere. The fresh water habitat of biosphere occupies small area of earth, but flora and fauna are very large (Brassard, 1996; Ungate, 1996; MacDonnell, 1996; Rao, 2010). It is distributed in ponds, lakes, rivers, streams and fresh water wetlands. The genus *Zygnema* is in the class of freshwater (green) algae that covers more than 100 species (Guiry and Guiry, 2008). *Zygnema* grows as a free-floating filament mass; where yellow green to bright green tangled mat are formed by these filaments; where elongate barrel shaped cells are composed with two star shaped chloroplasts arranged along cell axis (Guiry, 2013). Similarly, another of freshwater green algae is known as *Microspora* in the algae family Microsporaceae. *Microspora* are autotrophic fungus like sprotists that often are characterized by their many segments. They are group of spore-forming unicellular parasites and are known to be fungi; there are some 1500 species(NCBI, 2007). Algae are a great diverse group of photosynthetic eukaryotic organisms having no close relationship and are polyphyletic. A range of organisms in algae is included from unicellular microalgae genera which generally contain diatomsand the *Chlorella* (*Lee, 2008; Nabors, 2004).* Most are autotrophic and aquatic that lack numerous types of distinct cells and tissues, which mainly comprised of xylem, stomata and phloem found in surface plants*(Allaby, 1992; Butterfield, 2000).* A polyphyletic group is constituted by Algae since excluding common ancestor, while their plastids have similarity in origin from cyanobacteria; they were acquired in different ways *(Keeling, 2004; Palmer et al., 2004).* Green algae is primarily chloroplasts and derived from endosymbiotic cyanobacteria; diatoms; while on the other hand brown algae are considered as derivative chloroplasts resultant of endosymbiotic red alga. An extensive range of reproductive stratagem is exhibited by Algae including simple asexual cell division to complex forms of sexual reproduction *(Tartar et al., 2003; Jonathan et al., 2007).*

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MATERIAL AND METHOD

The study was conducted through 2015-2016 years to conclude elementology of some species of fresh water algae, which included four species of the genus *Zygnema* and *Microspora*. The work was performed at the research-lab of the Institutes of Plant Sciences University of Sindh (IPS-UOS) Jamshoro and Soil Science Laboratory of NIA, Tandojam. The elementology of the following algal species *Zygnema Leiospermum* De Bary was carried out in NIA, Tandojam and antifungal activity studies in IPS, university of Sindh, Jamshoro.

Collection of algae

Algal specie was collected in the month of February 2018 from following districts Almanzar, River Indus (Jamshoro, Kot Ghulam Muhammad (Mirpurkhas , TandoAllahyar (Mirpurkhas), Tando Muhammad Khan (Hyderabad) and different fresh water pools of Hyderabad City. The purpose of collection of algal material was to analyze the elemental level found in the districts of central and southern parts of Sindh. Following elements were studied in the *Zygnema Leiospermum* De Bary such as Zinc (Zn), Iron (Fe), Potassium (K), Sodium (Na), Calcium (Ca). Moreover, the antifungal activity of *Zygnema Leiospermum* De Bary algal species against fruit spoil fungi were also carried out.

Elemental extraction from collected algae

Extraction is defined as a separation of material from medium. More examples include extraction from liquid to liquid, and extractions of a solid phase.

Partion theory is described that in an equilibrium condition a solute is divided into two phases. There were five compounds or fractions 1. Ethanol 2. Ethyl acetate 3. Chloroform 4. Methanol 5. Aqueous solution, were used in layering method or layer by layer technique of antifungal activity of different algal plants against different fruit fungal pathogens. One-kilogram dried fresh water algal material was collected. Then one kilogram of each was dipped in 10-liter ethanol in a bottle for 20 days for cold purification. The extract was filtered through whattsman filter paper No.42. The extract was residue concentrated under reduced pressure below 40^{o}c using rotary evaporator.

The residue was completely dried as syrupy liquid from the residue five different fractions i.e.: ethanol, ethyl acetate, chloroform, methanol and aqueous fractions were obtained by using separating funnel. The extract was left at room temperature. The solvent completed evaporation under vacuum. The extracts that obtained were mixed with the sterilized water (1g: 5ml) each extract sample was applied for its antifungal activity to fresh water extracts fractions by different solvents.

Digestion of fresh water green algal material and analysis of elements from fresh water green algal material by Atomic Absorption Spectrophotometer (AAS)

The collected material was washed with tap water then with distilled water and leaves them for 15 to 20 days for dry at normal temperature and then on 60 to 80^{o}C in an oven for 1 hour then used for preparing sample. After 15 to 20 days 1-gram dried plant substance was dissolved into 10ml nitric acid (HNO_3) for 8 hours. Then add 5ml of hydrogen peroxide (H_2O_2) with 10ml of nitric acid (HNO_3). Then keep them at 100^{o}C to120^{o}C on hot plate until 75% of the sample evaporated in the form of fumes and in the result only 1% sample remains. Finally add 24ml double distilled water in order to make 25ml solution. The bright yellow color should be observed in diluted sample. Then the whatman filter paper no.42 was used to filter the solute

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The sample was before softly kept on warm bowl at 100 to 120 ^{o}C until 75% of the sample evaporated in the form of fumes. Though, straight air acetylene flame technique was implemented for sample/solution fortitude on Atomic Absorption Spectrophotometer (Analytic-Jena-Germany, Model AAS-Vario-6) (Marry and Franson, 1992).

Elemental assay

It can be defined as a process where a sample of some material is analyzed, elemental analysis and testing include identification and quantification of elements in a sample. The samples were investigated for elemental analysis by using Atomic Absorption Spectrophotometer (AAS) at soil science division of Nuclear Institute of Agriculture (NIA) Agriculture, Tando Jam Hyderabad, Sindh. The proper and applicable standard solution was used for each element. Analytic Jena-Germany, Model AAS-vario-6 However, direct air acetylene flame method was adopted for sample/solution determination on Atomic Absorption Spectrometer (Marry and Franson, 1992). All elements were determined in fresh water algal species under this investigation process.

Percentage recovery test

The extraction method validity was tested by conventional digestion method (CDM). The duplicate sample of each part of the spike with known quantity of metal stands (FlukiaKamica) previous to digestion as defined above. Sample blanks were also made in method. Each result value is mean of at least 3 independent beaches restored in duplicate and both sample analysis at twice of every element. The medium of stands and sample solution was alike by utilizing 4N Nitric acid. The percentage recovery tests for element by this method.

Collection points of different spoiled fruits

Different spoiled fruits were collected from Hyderabad and Mirpurkhas Districts (Sindh, Pakistan) fruit markets. Fruits such as Amlok /Japanese persimmon (*Diospyros Kaki* L.), Grapes (*Vitis vinifera* L.), Pomegranate (Punica granatum L.), Grape Fruit (Citrus paradise Macfad)

Culturing of pathogenic fungi on potato dextrose

For culturing fungi, agar medium was made with the help of potato extract 4.0-gram, Agar 15 grams, Glucose 20 grams, and 1-liter distilled water with pH value 5.6 ± 02. In distilled water the whole materials were combined and dissolved. This solution was autoclaved for 20 minutes at 120^{o}C and 15 LB/ sq inch pressure.

Bioassay

It is defined as analytical method to determine concentration or potency of a substance by its effect on living cells or tissues. The sterile assay medium at 40-45^{o}C, was poured into sterilized petri dish then it was cooled. The highly absorbent paper strips (12.5 to 10mm diameter) were soaked with requisite quantity (80 to 100ml) of various extracts like ethyl acetate, methanol, chloroform, ethanol and aqueous of the samples of selected fresh water algae were put on the test agar medium. For the determination of inhibitory action of the fungi, the examining organism was incubated at 30^{o}C for 72 hours. With the help of controlled plates, the growth of examine fruit fungi was matched: The calculations for determination of mycelia inhibition percentage is given below (Usmani&Shameel, 1986).

% mycelial inhibition= [(dc-dl)/dc] *100

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Dc=colony diameter in control,

Dl=colony diameter in treatment

Statistical analyses

The information in this manner gathered were exposed to factual investigation utilizing ANOVA method and LSD (Least Significant Difference) test was utilized to separate the prevalence of treatment implies utilizing Mstat-C Micro-Computer Statistical Software, subsequent study of Gomez and Gomez (1984).

RESULTS AND DISSCSSION

The ANOVA describes that *Zygnema leiospermum* De Bary varied significantly (DF=19, F=270.82, P<0.01, CV=10.93%) for concentrations of certain elements. The elemental analyses of *Zygnema Leiospermum* algal specie showed that calcium was found in highest quantity followed by Iron (Fe), Pottasium (K)and Sodium (Na). The lowest concentration of element Zinc (Zn) was found in the alga under this study (Table 1). Macroalgae are potentially ridiculous source of nutrients and elements for human foods and differences in the concentrations of certain elements are commonly reported. The results determined highly significant (P<0.05) differences in concentrations of different elements in *Zygnema Leiospermum* De Bary species. Contained substantially highest amount of Calcium [Ca] (45.682±5552 %); followed by Iron [Fe] (16.019±1946 %), Potassium [K] (12.120±1473 %) and Sodium [Na] (11.282±1371 %). However, the Green algae species *Zygnema leiospermum* De Bary contained lowest concentration of Zinc [Zn] (4.373±0531 %). It was observed that green algae species *Zygnema leiospermum* De Bary contained highest concentration of calcium, followed by Iron, Potassium and Sodium; while *Zygnema leiospermum* De Bary contained least Zinc [Zn] concentration. In addition to improve total dietary zinc content, the fresh water species appeared to act synergistically to facilitate nutrients uptake and convert to the more readily absorbed form of nutrients. The nutrients content of wild algae varies depending on the metal contents of water, in addition to being species-specific. Wild harvesting must then be optimized for each locale known to produce algae for optimal concentrations of inorganic nutrients

The study conducted in the *Zygnema* genus of fresh Water algae showed that the maximum Zinc (Zn) content was possessed by *Zygnema cruciatum* (Vaucher) C. Agardh; while among the green algal species of the *Microspora, Microspora stagnorum* (Kutzing) Lagerheim contained the maximum Zinc (Zn) content. There was no comparison of different species in the *Zygnema* and *Microspora* genus for their Zinc (Zn) contents; because the Zinc (Zn) content was exceptionally higher in *Zygnema* species; while a tiny content of Zinc (Zn) was determined in the species of *Microspora* genus. In accumulation of improve whole dietary zinc content, Fresh Water particularly in species in the *Zygnema* genus seemed to turn synergistically to simplify zinc acceptance & convert zinc to the more gladly fascinated method of zinc. The zinc content of wild algae varies depending on the metal content of waters, in addition to being species-specific. Wild harvesting must then be optimized for each locale known to produce algae for optimal concentrations of inorganic nutrients. (Iqbal *et al*., (2006)

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(Table 1) Concentration of certain elements in *Zygnema Leiospermum* De Bary

SNo.	Elements	Symbol	Samples (1 g each sample)				Mean
			S1	S2	S3	S4	
1.	Zinc	Zn	4.330	5.023	3.724	4.417	4.373±0.531[d]
2.	Iron	Fe	15.860	18.398	13.640	16.177	16.019±1.946[b]
3.	Potassium	K	12.00	13.92	10.32	12.24	12.120±1.473[c]
4.	Sodium	Na	11.170	12.957	9.606	11.393	11.282±1.371[c]
5.	Calcium	Ca	45.230	52.467	38.898	46.135	45.682±5.552[a]

We also applied various extracts such as ethnol, ethylene acetate, chloroform, methanol and aqueous extract of these elements on fruit spoiling fungi. It was found that antifungal activity of *Zygnema leiosperm* fraction of ethanol extract showed maximum inhibition over fruit spoil fungi *Aspergillus flavus* Link ex Gray (Table 2).

Antifungal activity of *Zygnema leiospermum* DeBary was maximum (86.11%) against fruit spoil fungi *Aspergillus flavus* Link ex Gray in the fraction of ethanol extract, followed by *Aspergillus niger*van Tieghem(77.80%) in the fraction of Chloroform extract, *Penicillium funiculosum* Thom (72.40%) in the fraction of Methanol extract, *Aspergillus ochraceous* (70.35%) in the fraction of Ethyl acetate extract and *Pencillium funiculosum Thom* (68.44%) in the fraction of Aqueous extract. The maximum inhibition activity was observed against test organism *Aspergillus flavus* Link ex Gray*;* while minimum inhibition activity of *Zygnema leiospermum* DeBary against *Phytophthoraci tricola* Sawada was noticed under the fraction of Ethyl acetate extract.significant antifungal activity in the fresh water green algae against fruit and vegetable spoilage.(Sharma *et al*., (2013), (Tavares and Dias (2014), (Vijayan and Ray (2015), Philippsen *et al*., (2016), Mark *et al*., (2017) and Bilal *et al*., (2018) found that the antifungal activity of different species of algae in various genus varied significantly.

(Table 2): Antifungal activity of *Zygnema Leiospermum* De Bary against fruit spoil fungi (%) as affected by different extracts

Extract	Highest		Moderate		Lowest	
Ethanol extract	*Aspergillus flavus*	86.11	*Aspergillus ochraceus*	61.12	*Phytophthoraci tricola*	51.16
Ethyl acetate extract	*Aspergillus ochraceous*	70.35	*Penicillium funiculosum*	59.26	*Phytophthorac itricola*	32.22
Chloroform extract	*Aspergillus niger*	77.80	*Aspergillus ochraceus*	71.10	*Penicilliumfunicul osum*	58.58
Methanol extract	*Penicillium funiculosum*	72.40	*Aspergillus ochraceus*	67.32	*Aspergillus flavus*	52.23
Aqueous extract	*Penicillium funiculosum*	68.44	*Phytophthoraci tricola*	60.60	*Aspergillus flavus*	59.60

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