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Fungal Plant Diseases in Egypt

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The association of fungi and plants is ancient and involves many different fungi. Fungi are an important group of plant pathogens—most plant diseases are caused by fungi—but fewer than 10% of all known fungi can colonize living plants (Knogge, 1996). Plant pathogenic fungi represent a relatively small subset of those fungi that are associated with plants. Most fungi are decomposers, utilizing the remains of plants and other organisms as their food source. Other types of associations that will be discussed here include the role of fungi as decomposers, as beneficial symbionts, and as cryptic plant colonizers called endophytes.

Let's now consider the role of fungi as plant pathogens. There are thousands of species of plant pathogenic fungi that collectively are responsible for 70% of all known plant diseases. Plant pathogenic fungi are parasites, but not all plant parasitic fungi are pathogens. What is the difference between a parasite and a pathogen? Plant parasitic fungi obtain nutrients from a living plant host, but the plant host doesn't necessarily exhibit any symptoms. Plant pathogenic fungi are parasites and cause disease characterized by symptoms.

By the beginning of the 20th century, special attention was being given to phytopathogenic fungi on wild and domesticated plants of economic importance (e.g. Fletcher 1902, Reichert 1921, Fahmy 1923, Shearer 1924, Briton-Jones 1922, 1923, 1925, Bishara 1928, Melchers 1931, Sirag El-Din 1931, Abdel-Salam 1933). According to Abdel-Azeem (2010) records of phytopathogenic fungi in Egypt were scattered through the literature until 1921, when Israel Reichert carried out his pioneer study of Egyptian fungi. This was followed by a comprehensive checklist of plant diseases and fungi occurring in Egypt by Melchers (1931). Records concerning aspects of plant pathology in Egypt continued to be accumulated during many decades until El-Helaly *et al.* (1963, 1966) started to update the information, and another updated bibliography of agricultural studies conducted in Egypt between the period 1900 to 1970 appeared (Ali Hassanein *et al.* 1972). For more details please check, Natrass (1933), Abou El-Seood (1968), Ghoniem (1985), El-Desouky & El-Wakil (2003), Phillips *et al.* (2006), and Hafez (2008). In this speech I will discuss the most common fungal plant pathogens in Egypt.

Impact of Climate Change on Egyptian Fungi

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Climate changes considerably altering the fungi including mushrooms, toadstools and other fungi, new research has found to be interested in response of fungi to climate change. The number of the Egyptian fungi recorded is 2 420 taxa belonging to 760 genera. They provide vital ecosystem services for the welfare of native trees and other plants, and are the natural recyclers of the planet, but until now their response to global climate change has not been examined intensively.

Climate change can be studied in terms of changes in ultraviolet radiations and carbon dioxide levels. UV radiation from the sun has always played important roles in our environment, and affects nearly all-living organisms biologically. Yet UV radiation at different wavelengths differs in its effects, and we have to live with the harmful effects as well as the helpful ones. The sun radiates energy in a wide range of wavelengths, most of which are invisible to human eyes and the harmful potentiality is reversible proportional to the wavelength. Wavelengths of ultraviolet (UV) radiation that reaches the Earth's surface is in between 280 and 400 nm are of two ranges UV-B (from 280–320 nm) and UV-A (from 320–400 nm).

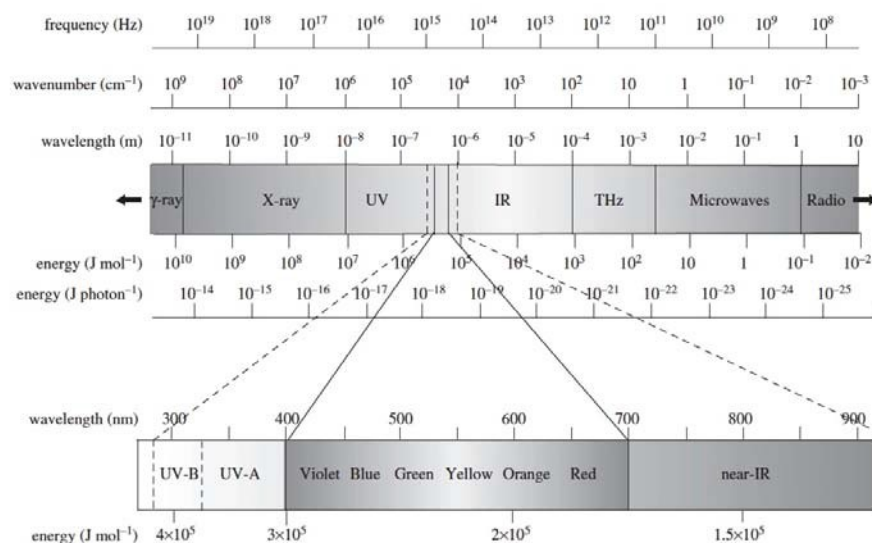


Figure 1 The electromagnetic spectrum: note that energy is given in J mol^{-1} (to convert to J photon^{-1} it is necessary to divide by Avogadro's number (6.022×10^{23}) so a photon of red light contains 2.84×10^{-19} J (from Jones & Vaughan, 2010).

A team from Suez Canal University's Faculty of science, Fungi lab in association with Plant physiology and photobiology lab is working on a research project to assess the impact of climate change especially enhanced levels of UV-A and UV-B on Egyptian fungal species (Abu-Elsaoud et al., 2016). The study involved monitoring of several morphological and growth parameters of fungi. Fungal consequences changed considerably in response to ultraviolet radiations. Moreover, some fungal species were found to accumulate small secondary metabolites called mycosporine-like amino acids (MAAs) as shown in figure 2.

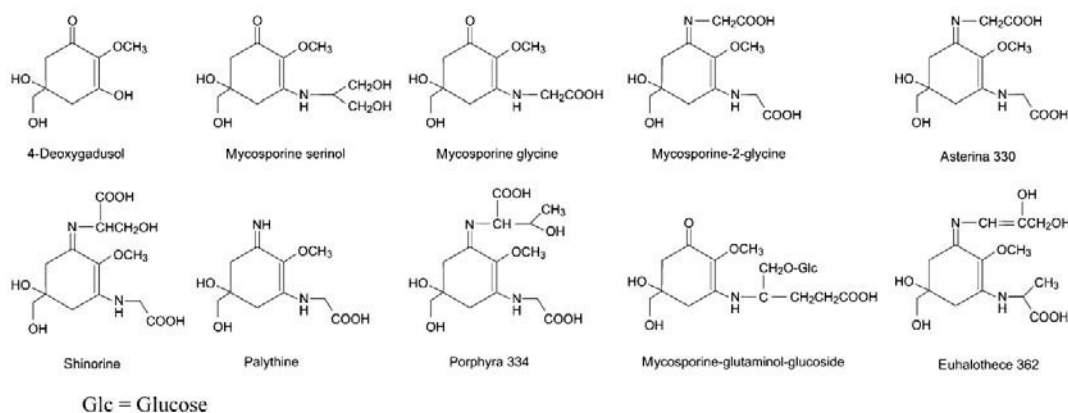


Figure 2. Chemical structures of representative mycosporines and MAAs (Oren and Gunde-Cimerman, 2007).

Mycosporines and mycosporine-like amino acids (MAAs) are low-molecular weight water-soluble molecules absorbing UV radiation in the wavelength range 310–365 nm. They are accumulated by a wide range of microorganisms, prokaryotic (cyanobacteria) as well as eukaryotic (microalgae, yeasts, and fungi), and a variety of marine macroalgae, corals, and other marine life forms. The role that MAAs play as sunscreen compounds to protect against damage by harmful levels of UV radiation is well established. MAAs are considered as a UV-absorbing compounds that accumulates in response to UVA, UV-B radiations (Abu-Elsaoud et al., 2016). The first thorough description of MAAs was done in cyanobacteria living in a high UV radiation environment (Garcia-Pichel et al, 1993). The major unifying characteristic among all MAAs is light absorption. All MAAs absorb UV light that can be destructive to biological molecules (DNA, Proteins, etc.). Though most MAA research is done on their photo-protective capabilities, they are also multifunctional secondary metabolites that have many cellular functions. MAAs are effective antioxidant molecules and are able to stabilize free radicals within their ring structure. In addition to protecting cells from mutation via UV radiation and free radicals, MAAs are able to boost cellular tolerance to desiccation, salt stress, and heat stress. The study found that the noticeable alteration

in fungal hyphal growth and fruiting mirrors changes in Egyptian environments and components of ultraviolet A and B.

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Fungal Enzymes Inhibitors: A good Research Area for Egyptian Mycologists

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Fungi this big world is unlimited biotic wealth. Its physiological properties and the huge amount of bioactive secondary metabolites put this kingdom in the scope of so many applications. One of these important bioactive secondary metabolites are enzyme inhibitors.

Enzymes are vital to all living organisms, maintaining the homeostasis of their lives by mediating/regulating numerous biochemical events, including metabolism, cell cycles, anabolism, catabolism, cellular signal transduction, , and development. Dysfunction, overexpression, or hyperactivation of the enzymes are often associated with disorder in cell metabolism.

Enzyme inhibitors are molecules that bind to enzymes and lower their activity. Many drugs are enzyme inhibitors due to the correction of metabolic imbalance may occur, as a result of blocking an enzyme activity. Therefore, the discovery of enzyme inhibitors is an active area of research in biochemistry and pharmacology.

To indicate the importance of fungal enzyme inhibitors (FEI) it is only required to mention penicillin. However, many other natural products from fungi have been described as the fungal compounds can be used against cancer, diabetes, poisonings, Alzheimer's disease, insecticides, herbicides etc.

The forms of inhibitions include acetylcholinesterase, nuclear factor- kappa B, protein kinase, tyrosine kinase, aromatase and sulphatase, matrix metalloproteinases, cyclooxygenase, DNA polymerase/topoisomerases, glycosidases and more. In Egypt, this area of fungal activity needs more extensive research in both academia and pharmaceutical industries.

Egyptian Fungi as a Source of Anti-leukemic Enzymes

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Enzymes are protein in nature they catalysis a specific reaction and act on specific substrates; they play various roles in medicine, industry, and agriculture. Enzymes classify into many categories; they would be excreted extracellular or intracellular. Acute lymphoblastic leukemia (ALL) considered one of the most common cancer diseases among children; it characterized by the overproduction of immature white blood cells (Neoplastic), such cells prevent the bone marrow from the formation of normal white blood cells. It cannot function properly toward any infections. ALL would occur at any age but much more common the age of (0-14 years) representing around 60% of the infected cases. Neoplastic cells lack L-asparaginase synthetase enzyme which leads to prevention of asparagine synthesis, in the mean neoplastic cells require high amounts of L-asparagine to proliferate. Thus they depend on external sources for asparagine, preventing the neoplastic of the asparagine will lead to the starving of the neoplastic cells and die off.

L-asparaginase (EC 3.5.11.) catalysis the hydrolysis of L-asparagine into L-aspartic acid and ammonia and thus classified under amidohydrolases. L-asparaginase has been subjected to many studies for its enormous clinical and non-clinical applications. The enzyme is widely distributed among plants, animals, and microorganisms. The antitumor properties of guinea pig serum reported by (KIDD 1953), which was in return (Broome 1963) stated that the antitumor activity was due to the activity of L-asparaginase. *E.coli* discovered to contains two L-asparaginases named afterward as; L-asparaginase I and L-asparaginase II, were L-asparaginase I used clinically for the first time. After the discovery of *E. coli* asparaginases, the bacterial L-asparaginases widely utilized for the treatment of Acute Lymphoblastic Leukemia (ALL), but unfortunately, several complications accompanied with the treatment in most of the cases even lead to death, complications reported are toxicity, allergy, hypersensitivity and drug instability. Furthermore a contamination with L-glutaminase combined with bacterial L-asparaginase which result in more side effects like; homeostasis, abnormal lipid metabolism, severe neurological disorders, and hypoglycemia.

Mammalian L-asparaginase extraction was found to be a complicated and expensive process. So the researchers started to target the L-asparaginases of

microbialsources. Where, *Achromobacteriaceae*, *Vibrio succinogenes* and *E.coli* classified among the best L-asparaginase producers.

The complications mentioned before made it necessary to find another alternative as an efficient and safe source for the production of L-asparaginase.

Several studies recently carried on fungal endophytes. Fungal endophytes are a diverse group of fungi living inside plant tissue which leads to some diseases or does not cause any harm to the plant. Studies showed that endophytic fungi could produce the same active metabolites the plants produce with same or much more activity (Pandey et al. 2014; Suryanarayanan et al. 2009).

L-asparaginases extracted from fungi have been recorded to possess many benefits than any other preparations. Researches revealed that the extracted fungal preparations have no immunological problems, unlike bacterial and other preparations, also being extracted from eukaryotic source that solved the instability issues. Regarding the contamination with L-glutaminase, not all of the fungal sources found to be contaminated with L-glutaminase. Therefore, the discovery of extracellular safe and stable fungal L-asparaginase gave the attention to scientists to carry more researches on it.

Our present study is subjected towards screening of fungal endophytes for the production of L-Asparaginase followed by chemical, molecular characterization and studying its anti leukemic activity.

A total number of 2760 plates from the were used for isolation of endophytic mycobiota, Identified species were screened qualitatively for the production of L-Asparaginase enzyme using modified Czapek's Dox media (Patil et al., 2012) using modified test in the presence of bromothymol blue as an indicator and asparagine as substrate, Species were recovered and identified by phenotypic and molecular means, Zygomycota represented by One species, teleomorphic Ascomycota by Two species, and anamorphic Ascomycota by 23 species, Out of 26 species, 7 Species produced L-asparaginase. L-Asparaginase producing isolates were belonging to genera of *Aspergillus*, *Fusarium*, *Ulocladium* and *Lasiodiplodia*

Bioinformatics and Fungal Conservation

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'Omic' technologies adopt a holistic view of the molecules that make up a cell, tissue or organism. They are aimed primarily at the universal detection of genes (genomics), mRNA (transcriptomics), proteins (proteomics) and metabolites (metabolomics) in a specific biological sample in a non-targeted and non-biased manner. This can also be referred to as high-dimensional biology; the integration of these techniques is called systems biology.

Bioinformatics is an interdisciplinary field that develops and improves on methods for storing, retrieving, organizing and analyzing biological data. A major activity in bioinformatics is to develop software tools to generate useful biological knowledge. Bioinformatics has become an important part of many areas of biology. In experimental molecular biology, bioinformatics techniques such as image and signal processing allow extraction of useful results from large amounts of raw data. In the field of genetics and genomics, it aids in sequencing and annotating genomes and their observed mutations. It plays a role in the textual mining of biological literature and the development of biological and gene ontologies to organize and query biological data. It plays a role in the analysis of gene and protein expression and regulation.

Bioinformatics tools aid in the comparison of genetic and genomic data and more generally in the understanding of evolutionary aspects of molecular biology. At a more integrative level, it helps analyze and catalogue the biological pathways and networks that are an important part of systems biology. In structural biology, it aids in the simulation and modeling of DNA, RNA, and protein structures as well as molecular interactions. Researchers affiliated with our program conduct research in systems biology, genomics, and proteomics.

Conservation Biology, which is sometimes also known as Biological Conservation is a field of scientific study whose practitioners seek to understand patterns of existing biodiversity, and how the processes that generate and maintain biodiversity are influenced by human environmental impacts, and then use this knowledge to properly manage biological resources to allow their sustainable use by humans in a way that conserves that biodiversity. Now, Conservation Biology as a whole, is a multidisciplinary field. One sub-discipline of the field relevant to bioinformatics is conservation genetics, which is the application of genetic concepts and tools to the study

and conservation of genetic biodiversity in the context of anthropogenic change. Bioinformatics Tools, many of which are computer programs to analyze genetic data like DNA and protein sequences have become essential tools for this work. Therefore, appropriate data mining of these omic data in depth and the obtained information can benefit our understanding of the complex fungal biological processes from genotype and physiology to phenotype, including cell-cell (microbial) communications and pathogen-host interactions and beyond.

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Using Egyptian Fungi to Clean Up Pollutants

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Mycoremediation is a type of bioremediation where fungi serve as bioremediants. Biosorption of heavy metal by different native fungal taxa have been the target of many studies carried by several investigators worldwide during the last three decades. **Biosorption** is defined as a physio-chemical interaction which occurs between metal (loid) s and extra-cellular compounds such as polysaccharides, proteins and lipids with amino, carboxyl, phosphate and sulphate groups (Volesky 2003; Febrianto *et al.* 2009).

As a generic term, sorption is used for both absorption and adsorption. In an excellent review, Gadd (2008) explains the two processes, relative to various pollutants: "Absorption is the incorporation of a substance in one state into another of a different state (e.g. liquids being absorbed by a solid or gases being absorbed by water), i.e. into a three-dimensional matrix. Adsorption is the physical adherence or bonding of ions and molecules onto the surface of another molecule, i.e. onto a two-dimensional surface. In this case, the material accumulated at the interface is the adsorbate and the solid surface is the adsorbent. If adsorption occurs and results in the formation of a stable molecular phase at the interface, this can be described as a surface complex. According to the dependence on the cells metabolism various researchers (Volesky, 1990; Ahalya *et al.*, 2003; Vijayaraghavan and Yun, 2008) divided biosorption mechanisms into two types:

- 1- Metabolism dependent.
- 2- Non-metabolism dependent.

In addition, according to the location where the metal removed from solution is found, biosorption can be classified as:

- 1- Extra cellular accumulation/precipitation.
- 2- Cell surface sorption/precipitation.
- 3- Intracellular accumulation.

The distinction between **bioaccumulation** and **biosorption** is important in attempts to use (microbial) biomass for metal sequestering/concentration purposes. It is important for biosorption process design and often has been poorly understood and described by many researchers. Bioaccumulation and biosorption have often been combined into the single heading of metal uptake because the predominant mechanism in effect is not known. Biosorption possesses certain inherent advantages over bioaccumulation processes, which are listed in (Table 2) (Vijayaraghavan and Yun, 2008; Hlihor and Gavrilescu, 2009).

Biosorbents are complex and variable materials. The uptake of contaminants by biological materials occurs via various physico-chemical mechanisms including ion exchange, sorption, complexation, chelation, micro precipitation etc. (Volesky, 1999; Gadd, 2008). The composition of cell wall, to which metal ions are bound, depends not only on biosorbent species, but also on environmental conditions of its growth. In fact, cell walls of microorganisms, consisting mainly of polysaccharide, protein, lipid, teichoic acid, extra-cellular and surface polysaccharides, offer many anionic functional groups such as phosphoryl, carboxyl, amino, sulfhydryl, hydroxyl groups containing ligands (Arief *et al.*, 2008). Deprotonated ligands, e.g. $-RCOO^-$, behave as Lewis bases and adsorption of metal cations can be interpreted as competitive complex formation (Gadd, 2009; Stumm and Morgan, 1996).

The mechanism of binding by inactivated biomass may depend on the chemical nature of pollutant (species, size, ionic charge), type of biomass, its preparation and its specific surface properties, and the environmental conditions (pH, temperature, ionic strength, existence of competing organic or inorganic ligands in solution) (Lin and Juang, 2009). Quantitative structure-activity relationships (QSARs) have been used extensively to predict the bioactivity and toxicity of classes of organic compounds (Zamil *et al.*, 2009).

The goal of a QSAR approach is to relate characteristics of a compound to observe toxicity (or bioavailability), and account for variation in toxicity in a related group of chemicals. Once the relationship has been established, one can also predict the additional effects of molecular substitutions. On the other hand, quantitative ion character-activity relationships (QICARs) have been developed by Newman and coworkers as a useful tool to predict, for example, the relative toxicity of metal ions based on metal–ligand binding tendencies. From then, QICARs have been employed successfully to various effects, species, and media for prediction of metal toxicity (Newman *et al.*, 1998; Tatara *et al.*, 1998; Ownby and Newman, 2003).

Biosorption is a cost effective way of removing toxic metals from industrial wastewaters in a concentration range below 100 mg/L, where other techniques are ineffective or costly, by passive cation binding by dead or living biomass (Volesky, 1990; Schiewer and Volesky, 1995). Certain types of microbial biomass can retain/bind relatively high quantities of metal ions on their cell wall due to its structural characteristics (Fig.1).

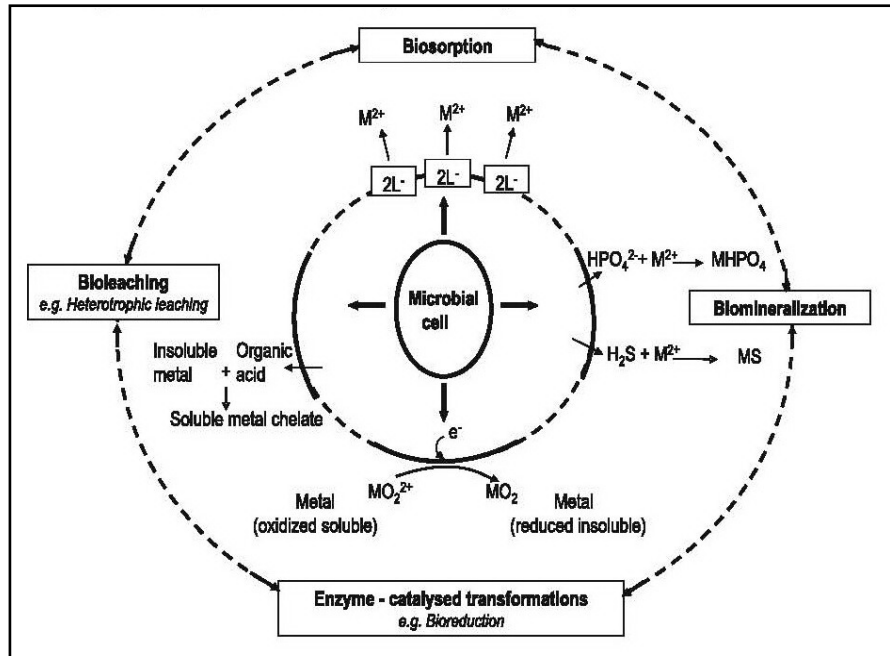


Figure 1. Mechanisms of metal-microbe interactions that can be harnessed for bioremediation applications (Lloyd, 2002; Gavrilescu, 2004).

Egyptian Fungi as a Sustainable Source of Industrial and Pharmaceutical Enzymes Production

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Endophytic fungi are microfungi, which inhabit in healthy living plant tissues for all or part of their life cycle without causing apparent harmful symptoms to the host. Fungal enzymes are one of them which are used in pharmaceuticals, food, beverages, confectionaries, textiles and leather industries. They are often more stable than enzymes derived from other sources. Twenty one fungal species, isolated from medicinal plants (*Achillea fragrantissima*, *Artemisia herba-alba*, *Chiliadenus montanus*, *Origanum syriacum*, *Teucrium polium*, *Tanacetum sinaicum*) were collected from Saint Katherine Protectorate were screened for extracellular enzymes such as amylase, cellulase, chitinase, esterase, laccase, lipase, pectinase, protease and tyrosinase on solid media. Sixty one percent of fungi (13 isolates) screened for enzymes showed positive for amylase, 92% for cellulase, 30% chitinase, 23% esterase, 53% laccase, 46% lipase, 53% pectinase, 76% protease, and only 30% showed positive for tyrosinase. The array of enzymes produced differs between fungi and often depends on the host and their ecological factors. This work investigated the powerful capability of the Egyptian endophytic fungi to produce a large number of pharmaceutical and industrial enzymes and more investigation and production on commercial scale is urgently needed.

Ancient Egypt is the cradle of mycology, what about modern Egypt?

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Documentation of the world fungi may be dated back to 4500 B.C., when ancient Egyptians produced a number of hieroglyphic depictions of plants (many of which are psychedelic) on walls and within texts throughout Egypt (Abdel-Azeem 2010). Conservation of nature by ancient Egyptians was recorded on walls of temples and papyrus sheets. Egypt, known as the cradle of civilisation, has documented its fascination with fungi, when ancient Egyptians produced a number of hieroglyphic depictions of psychedelic mushrooms on temple's walls and through hieroglyphic texts throughout the country (Abdel-Azeem 2010; Abdel-Azeem and Salem 2017). The majority of temples with countless pillars, e.g. Philae temple, are like huge mushrooms with caps, tall stipes, and mushroom imprintings (Fig. 1) and paintings distributed all over Egypt (Abdel-Azeem 2010, 2014).



Figure (1): A- Wall relief of a mushroom basket in Temple of Hathor, Dendera, B- A pillar with a fan or oyster-shaped top in Temple of Philae in Aswan, Egypt, C- Mushroom relief in Edfu Temple © Abdel-Azeem 2016.

In the Egyptian Book of the Dead, the Papyrus of Ani (Budge 1967), mushrooms are called “the food of the gods,” or “celestial food” and “the flesh of the gods.” Berlant (2005) theorized that both ancient Egyptian crowns, white and triple, were inspired from the primordia of *Psilocybe cubensis*. Many old dynastic Egyptian ear studs and other structures that obviously resembled mushrooms found in this era (Aldred 1971; Fine Arts Catalogue of Boston Museum 1982). The Hearst (1550 B.C.) prescriptions 89-92 deal with the treatment of skin abrasions or contusions, recommend the application of moldy bread crumbs, salt, and rags. Here one may assume an ancient observation on the possibility of molds being helpful in preventing skin infections in connection with abrasions. This is a long way from the modern antibiotics, but it suggests the same sort of observational skill (Leake 1952). The orchil dyes have a longer recorded history and were known to the ancient Egyptians. Orchil dyes (including cudbear and litmus) derived from orcinol depsides, especially erythrin, lecanoric acid and gyrophoric acid, obtained from species of *Ochrolechia*, *Roccella*, *Umbilicaria* and *Lasallia* lichens (Coppins and Watling 1995).

An exhaustive revision of all the available literature and sources mentioned since 1931 and after the omission of duplicate names, name correction, allowance for synonyms and taxonomic assignments of all reported taxa from Egypt, the number of the Egyptian fungi recorded is 2452 taxa. On the kingdom level Fungi came first by 1962 species followed by Chromistan fungal analogues (186) and Protozoan fungal analogues (61) respectively. The Ascomycota form the single largest group within this checklist, with about 1595 species, of which about 157 are lichen-forming, and about 1000 are known only from their conidial (asexual) states, 89 belonging to Chytridiomycota, 27 to Blastocladiomycota, 70 to Zygomycota, 47 to Glomeromycota, and 220 to Basidiomycota had been recorded up to the present time. Database of 210,000 records of the Egyptian fungi coming soon to the cyberlife “<http://fungiofegypt.com/index.html>”, site hosting funded by Arab Society for fungal Conservation (ASFC).

Egyptian fungi are sustainable source for production of antioxidants, anticancer, antitumor, antirheumatoids...etc. In the past decades, species of the genus *Chaetomium* have been revealed to be a rich source of fascinating and structurally complex natural products. To date, more than 200 compounds have been reported from this genus (Zhang et al. 2012). A huge number of new and bioactive secondary metabolites associated with unique and diverse structural types, such as chaetoglobosins, epipolythiodioxopiperazines, azaphilones, depsidones, xanthenes, anthraquinones, chromones, terpenoids, and steroids, have been isolated and identified. Many of the compounds have been reported to possess significant biological activities, such as antitumor, antimalarial, cytotoxic, enzyme inhibitory, antimicrobial, phytotoxic, and other activities (Blunt et al. 2011, Zhang et al. 2012). *Chaetomium* species are frequently

reported to be cellulase and ligninases producers with the ability to degrade cellulosic and woody materials (Longoni et al. 2012; Nagadesi and Arya, 2013).

Dietary antioxidants, including polyphenolic compounds, vitamin E and C are effective nutrients in the prevention of these oxidative stress related diseases (Ames et al. 1995). Polyphenolic compounds are the secondary metabolites commonly found in plants, mushrooms and fungi (Miller et al. 1995). A great number of naturally occurring substances have been recognized to have antioxidant abilities, such as flavonoids and other phenolic compounds (Kang et al. 2003). Other active compounds include fiber, conjugated isomers of linoleic acid, some vitamins, calcium, uric acid, glutathione, and protease inhibitors were reported (Kaur and Kapoor, 2001), also these compounds may work independently or synergistically. A number of plants and macrofungi are commonly known to produce antioxidants and there are few reports on microfungi.

The biodiversity and conservation of fungi and public perceptions in Egypt remains very low, and more education on fungal conservation is urgently needed. This work has to be considered as an important contribution to the nature conservation program in Egypt. One important theme is the sustainable use of fungal resources, enforcement of legislations and conventions concerning the fungal diversity in different habitats in Egypt. Environmental education is an important point in the activity concerning fungi conservation and application of appropriate protected area management systems. There is a need to separate fungi from plant kingdom in educational courses in Egypt and to integrate mycodiversity and fungal conservation into the science (biology) and environmental curricula and extra-curricular activities. Egyptian mycologists carry the responsibility to discuss these issues and communicate with public and politicians. This is a very heavy burden, as even the majority of scientists deny the true importance of fungi and their essential role in the conservation, recycling and protection of biomes. A hard mission for the Egyptian mycologist is to get the attention of national politicians and decision makers and is even more difficult as national legislation is strongly focused on protecting of plants and animal and ignoring 'orphans of Rio'. Egypt's Mycologists Network (EMN) founded by Abdel-Azeem in 2016 (Fungi of Egypt - Network) is a structured network of Egyptian mycologists with a steering committee to guide and promote best practices and to solve such problems through collaboration between decision makers, mycologists, fungal conservation societies/ individual enthusiasts, national protectorates and environmental agencies.