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Endophytic fungal research in Egypt: Present status

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ABSTRACT

Plant endophytes are a potential source for the production of bioactive compounds that can fight against devastating diseases in both plants and humans. Among these endophytic microorganisms, endophytic fungi are one of the dominant group of microorganisms with a potential role in plant growth promotion and the discovery of noble bioactive natural products. Endophytic fungi possess several bioactivities like anticancer, antimicrobial, anti-rheumatoidal, insecticidal, plant growth stimulants, crop protection, phytoremediation, etc. In 2010, Abdel-Azeem mentioned endophytic fungi as a group needing more exploration in Egypt among the other overlooked fungal groups such as algicolous fungi, invertebrate associated fungi, mycorrhizas, lichens, wood deteriorating, and coprophilous fungi. Presently, main target of many studies in Egypt on endophytic fungal research is coupled with the ability of these micro-organisms to produce and accumulate novel bioactive metabolites as these are potent source of novel natural products useful for anticancer, anti-rheumatoid, antimicrobial, antioxidants, industrial and pharmaceutical enzymes…etc. This mini-review illustrates the role of most brilliant Egyptian researchers to generate potentially valuable products by using native endophytic fungi and the present status of endophytic fungal research in Egypt.

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Introduction

Bioprospecting is generally described as the search for naturally occurring chemical compounds and biological material, especially in extreme or biodiversity-rich environments (Abdel-Azeem et al. 2012). Biologically active metabolites are produced by a great number of Fungi and most bioprospecting programs have been limited to certain ecological groups of Egyptian fungi (Abdel-Azeem 2010).

Pupo (2006) mentioned that endophytic fungi have been shown to be a promising source of novel natural bioactive agents. Several crude extracts from different fungal culture broths showed that plants growing in unique environmental setting and have ethanobotanical uses with endemic location produce novel endophytic microfungi of which the secondary metabolite are usually unique and may have applicability in medicine (Salem and Abdel-Azeem 2014 ; Abdel-Azeem et al. 2016, 2018, 2019). Many investigations focused on antioxidant activities of the endophytic fungi isolated from ethnomedicinal plants (Ranjan and Joshi 2012; Abdel-Azeem et al. 2018; Abo Nahas 2018, 2019).

Endophytic fungi as anti-rheumatoid agents

In 2016, Abdel-Azeem et al. studied the antirheumatoid activity of secondary metabolites produced by the endophytic *Chaetomium globosum* recovered from

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Adiantum capillus-veneris from the arid Sinai.

Khalil et al. (2016) studied the effects of *Chaetomium globosum* and *Curvularia lunata* on Rheumatoid Arteritis (RA) and the results revealed that the effects of crude extracts of both taxa were similar to the effects observed from Leflunomide, the well-known drug in the treatment of RA. Although it is obvious that Leflunomide is a clinically effective drug, it is well known that it results in bone marrow hypocellularity; thus, it would not be a desirable therapeutic agent in RA patients.

Endophytic fungi as biocontrol agents

Plants immune response to some pathogens may be absent, however the endophytes within the plant tissue improve the plants' immune response to the invasion of harmful disease-causing agents (Rajamanikyam et al. 2017).

Hassanein et al. (2016) reported 30 endophytic fungal species have been recovered from 8 healthy medicinal plants grown in their Egyptian natural habitat and assessed for their biocontrol capability against wheat root rot disease triggered by *Fusarium oxysporum*. Of all thirty species *Alternaria alternata* and *Cochliobolus lunatus* exhibited the maximum inhibitory action (71.43 percent inhibition value) versus the examined *Fusarium oxysporum* then *Fusarium oxysporum* and *Cladosporium cladosporioides* with 68.57 percent and 60 percent, respectively.

On the other hand, 8 strains of fungi namely: *Alternaria alternata*, *Aspergillus fumigatus*, *Drechslera hawaiiensis*, *Fusarium solani*, *Penicillium citrinum*, *Neoscytalidium dimidiatum*, *Thyrostromella myriana* and *Ulocladium chartarum* were recovered from *Hyoscyamus muticus* (Abdel-Motaal et al. 2010). Inhibition activity of recovered taxa have been investigated against plant pathogens, *Gibberella zeae* and *Thanatephorus cucumeris* and six strains of non-pathogenic taxa namely, *Alternaria alternata*, *Cladosporium cladosporioides*, *Cladorrhinum foecundissimum*, *Curvularia clavata*, *Penicillium janthinellum* and *Ulocladium chartarum*. The results showed antifungal activity versus all the tested fungal strains (Abdel-Motaal et al. 2010).

Furthermore, 132 strains of endophytes have been recovered from eighteen medicinal plant genera collecte from Saint Katherine Protectorate (SKP). 55 of 99 tested endophytes exhibited a wide-ranging of inhibition action versus diverse pathogenic yeasts and bacteria (Selim et al. 2011).

Recently, Yasser et al. (2020) revealed 280 isolates representing twenty fungal species isolated from root, leaf, and stem of *Pelargonium graveolens* collected from Beni-Suef area. Most of the isolates show inhibition action versus one or more of the examined pathogenic fungi. The maximum inhibition rate recorded for *Emericella nidulans* (E6658) against *Microsporum audouinii* (80%).

Aspergillus niger (E6657) and *Penicillium* sp. (E6651) exhibit powerful antagonistic activities versus all the examined pathogens.

Kamel et al. (2019) recovered twenty-two isolates belonging to twenty-one fungal species representing fifteen fungal genera as well as one variety from *Euphorbia geniculate*. The isolated fungi displayed inhibition activity versus 6 strains of plant harmful disease-causing fungi namely, *Eupenicillium brefeldianum*, *Penicillium echinulatum*, *Alternaria phragmospora*, *Fusarium oxysporum*, *Fusarium verticilloid*, and *Alternaria alternata* in dual culture assay.

Endophytic fungi with antiviral activities

Another interesting application of endophytic fungal antibiotic products is the suppression of viruses. Over 100 endophytic fungal strains representing 29 diverse taxa from eighteen Egyptian medicinal plants recovered from Saint Katherine Protectorate, have been isolated and identified and their metabolites have been extracted with ethyl acetate by Selim et al. (2018).

Their antioxidant and antiviral functions were also investigated out of 99 extracts and only 15 crude extracts illicit the replication of HSV-2 virus. Instead, the replication of VSV-virus was prevented through 16 fungal crude extracts.

The successful anti-(HSV-2 and VSV) native endophytic *Pleospora tarda* isolate extract; which was primarily recovered from the *Ephedra aphylla* revealed 40.7 percent and 15.2 percent virus inhibition action, separately. Also, *Pleospora tarda* able to yield alternariol and alternariol-(9)-methyl ether for the first time in this study (Selim et al. 2018).

The study carried by Abou El-Kassem et al. (2019) have been isolated and identified 48 endophytic fungal strains from 10 medicinal plants then their broths have been investigated for potential Anti-HCV protease cytotoxic action. Their results showed that ethyl acetate extracts of *Alternaria alternata* PGL-3, *Cochlibolus lunatus* PML-17, *Nigrospora sphaerica* EPS-38, then *Emerecilla nidulans* RPL-21 exhibited the most potent inhibitory action of HCV NS3/4A protease with IC50 17.0, 20.5, 33.6, and 54.6 μg/ml, respectively, with quiet cytotoxicity excluding the later.

Alternariol and alternariol -9-methyl ether have been extracted from the ethyl acetate extract of *Alternaria alternata* PGL-3, while the ethyl acetate extract of *Emericella nidulans* RPL-21 extracted *Emericellin*, shamixanthone, arugosin C. The findings indicate that *Alternaria alternata* PGL-3 native endophyte is a source of antiviral lead from the *Punica granatum* peel (Abou El-Kassem et al. 2019).

Endophytic fungi with antioxidant and hepatoprotective activities

In 2018, Abdel-Azeem et al. studied the hepato-curative effects of endophytic fungi hosted medicinal plants in SKP. During their study, 36 species belonging to 21 genera were isolated from 7 medicinal plants. Ascomycota was represented by 35 species and only 1 for Zygomycota. The dominant and most frequently isolated taxa were Aspergillus flavus and A. niger (they were omitted during our study due to their ability to produce mycotoxins), followed by *Alternaria alternata*, *Curvularia lunata*, *Penicillium chrysogenum*, *Chaetomium globosum* and *Trichoderma viride*.

The previously mentioned five species were surveyed for their H_2O_2 scavenging activity. The results showed that, among the five species, *Chaetomium globosum* recovered from Adiantum *capillus-veneris* and *Curvularia lunata* isolated from *Verbascum sinaiticum* have the higher radical scavenging activity as recorded 75.31% and 73.44% respectively. So, both taxa were chosen for determination of their flavonoid and phenolic content. *Chaetomium globosum* and *C. lunata* recorded 92.5 and 106 µg/ml of total flavenoid and 3.594 and 3.172 mg/ml of total phenolic content respectively.

Depending on the results of antioxidant and biochemichal studies, *Chaetomium globosum* (CG) and *Curvularia lunata* (CL) were selected to survey their metabolites curative potentiality against paracetamol induced-liver injury in mice. Both taxa were cultivated on potato dextrose broth (PDB) medium for 15 days at 28°C, followed by extraction with ethyl acetate (EtOAc). For each species two extracted were examined watery (W) and ethyl acteate (E).

Abo Nahas (2019) collected the studies carried by Abdel-Azeem's team in Suez Canal University in her review titled" Endophytic fungi: A gold mine of antioxidants.

Antioxidant activity of thirty-five endophytic fungal species representing 14 genera recovered from the leaves of two artichoke varieties viz., French Hyrious and Egyptian Baladi were evaluated for their total antioxidant ability (TAA), total phenolic contents (TPCs) and total flavonoid contents (TFCs). The TAA, TPCs and TFCs of the fungal cultures ranged from 163 to 681 mg AAE/g DW, 10.38 to 40.30 mg GAE/g DW, and 13.92 to 173.55 mg QE/g DW, respectively. Moreover, in the methanol extract of *A. alternata*, liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) reported the existence of 1,3-dicaffeoylquinic as well as 1,5-dicaffeoylquinic acids (Seddek et al. 2018).

Selim et al. (2018) reported that *Chaetomium globosum* recovered from *Adiantum capillus-veneris*, showed an auspicious scavenging action of DPPH (1, 1-diphenyl-2-picrylhydrazyl) with percentage 99% at 100 µg/mL from 99 extracts.

Production of Kojic Acid

Moharram et al. (2015) reported 24 endophytic fungal isolates including: *Aspergillus* (5 isolates of two species and two species varieties), *Petromyces* (9 isolates of two species), *Penicillium* (6 isolates of four species), *Chaetomium globosum* (2 isolates) and 1 isolate from each of *Emericella nidulans* and *Pleospora allii* were able to produce kojic acid, from 214 endophytic fungal strains isolated from leaves of 11 common medicinal plants in Egypt. They were investigated for acid production using static cultivation at 28 ° C and all positive isolates were tested for their acid making using submerged technique.

Enzymatic activity

In 2015, Abdel-Azeem MA et al. surveyed Egyptian endophytic fungi hosted medicinal plants in Saint Katherine Protectorate for production of some pharmaceutical and industrial enzymes.

The study that carried by Moharram et al. (2016) reported 7 endophytic fungal species and two different sterile mycelia from healthy apparent leaves of *Withania somnifera* then they have been examined for their L-asparaginase enzyme yielding ability. Of all isolates *Alternaria alternata* was the most common fungal isolates. The results revealed that all examined isolated fungi were able to create L-asparaginase enzyme excepting Eurotium rubrum. 6 isolated endophytic fungi showed elevated amount of l-asparaginase enzyme in their filtrates of culture with $1.1 \pm 0.03 – 1.98 \pm 0.16$ IU range (Moharram et al. 2016).

Endophytic fungi with anticancer agents

Salem and Abdel-Azeem (2014) surveyed anticancer metabolites produced by some endophytic mycobiota isolated from some medicinal plants in Saint Katherine Protectorate, South Sinai. Throughout their study, 75 endophytic taxa with 32 genera were isolated and identified from eight dominant plant species occupying different altitudes in Saint Katherine Protectorate. To explore the anti-cancer activity of endophytic metabolites two taxa namely *Stachybotrys chartarum* (4E-SCUF) and *Trichothecium roseum* (23A-SCUF) were selected and cultivated on potato dextrose broth (PDB) medium for 14 days at 28°C, followed by extraction twice ith ethyl acetate (EtOAc). Fungal extracts were tried in vivo against Ehrlich

Ascites Carcinoma (EAC) cells in female Swiss albino mice. Parameters used to estimate the therapeutic effects of EtOAc and aqueous extracts of fungi on EAC bearing mice include: body weight gain (BWG), tumor volume (TV),

median survival time (MST) and percentage increased life span (%ILS). Moreover, their effects on some liver and kidney biochemical parameters and several tumor markers were also investigated.

Both EtOAc and aqueous extracts of *T. roseum* significantly decreased BWG and TV, but significantly increased MST and %ILS as 23-27 and 48-71% respectively. Biochemically, *T. roseum* metabolite extracts did not alter liver or kidney functions, while significantly reduced the tumor markers for breast cancer (CA 15.3), ovarian cancer (CA 12.5), pancreas cancer (CA 19.9), carcinoembryonic antigen (CEA) and -fetoprotein tumor marker (AFP).

Also as proven in the aforementioned study carried by Abou El-Kassem et al. (2019), the extracts of *Emericella nidulans* RSL-24, *Fusarium oxysporum* SML-41, *Emericella nidulans* RPL-21, and *Penicillium* sp. RSL-43 showed robust cytotoxic activity versus human breast cancer cell lines (MCF-7) with IC50 10.8, 11.0, 12.5, and 13.7 μg/ml, respectively. Furthermore, *Emericella nidulans* RSS-22, *Emericella nidulans* RSL-24, and *Fusarium oxysporum* SML-41 exhibited a powerful cytotoxicity on human liver cancer cell lines (HEP-G2) with IC5014.8, 20.3 and 24.0 μg/ml, respectively.

Balbool and Abdel-Azeem (2020) reported that L-asparaginase synthesized by seven strains; representing 4 different species, of all 25 endophytic fungal species which isolated and purified from a 23 plant species recovered from Protectorate of Saint Katherine, South Sinai. These positive results have enzymatic activities within the range from 44.5 ± 1.66 to 152.58 ± 0.63 Uml^{-1}. The maximum activity with 152.58 Uml^{-1} was recorded for *Lasiodiplodia theobromae* isolated from *Teucrium polium*. Also *L. theobromae* in this study promised to be used as an alternate and dependable resource of L-asparaginase for effective cancer inhibitory agents.

Endophytic fungi with insecticidal activities

In this study, to investigate an effective insecticide against S. littoralis, 15 different medicinal plants were chosen, and their potential endophytic fungi have been isolated, and their insecticidal activity were resolved. 45 isolates were collected from these plants, *Sarocladium strictum*, endophyte of *Cynancum acutum*, and *Aspergillus nidulans*, endophyte of *Lantana camara*, exhibited the maximum insecticidal activity versus 2nd larval instar of *S. littoralis* (El-Sayed et al. 2020).

Biogenesis nanoparticles by endophytic fungi

Synthesis of fungal-derived silver nanoparticles (AgNPs) by endophytic fungi is being of major interest and applied recently in various fields. Abu-Elsaoud et al. (2015) aimed to green synthesis of AgNPs by some endophytic native taxa isolated from six medicinal plants from arid South Sinai and optimizing production conditions combined with photostimulation. Thirteen species out of twenty-two entophytic fungi were screened for production of AgNPs. Reaction conditions such as silver nitrate concentration, pH, temperature and efficiency of photostimulation using monochromatic red polarized light and UV radiations were optimized and assed for high production of AgNPs. High concentrations of AgNPs were produced by *Chaetomium globosum* and *Trichoderma viride* recovered from *Tanacetum sinaicum* and *Chiliadenus montanus* respectively. Both fungi showed significantly different response to photostimulation by either red polarized or red LED light. *T. viride* showed a promising results and significant increase in AgNPs production after photostimulation by monochromatic red polarized light. Application of monochromatic red polarized light in the field of bionanotechnology for enhancing green synthesis of AgNPs would be recommended.

During continuous bioprospecting of endophytic fungi in Saint Katherine Protectorate carried by Abdel-Azeem et a. (2020), fungal endobionts hosted four wild medicinal plants were isolated and surveyed for their capability to green synthesize AgNPs. *Trichoderma atroviride* hosted in *Chiliadenus montanus*, the most potent taxon for production was selected. The mycosynthesized AgNPs were characterized using UV-vis (UV-vis), Raman spectroscopy, X-ray diffraction (XRD) and high-resolution transmission electron microscopy (HRTEM). The antibacterial and antifungal efficacy of the mycosynthesized AgNPs was studied against phyto and human pathogenic bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella flexneri*, and *Salmonella typhimurium*), yeast (*Candida albicans*) and filamentous fungi (*Aspergillus brasiliensis*, *A. niger* and *Fusarium oxysporum*).

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