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Review Article

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A REVIEW ON NANOPARTICLES SYNTHESIS USING ENTOMO PATHOGENIC FUNGI

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Abstract

The increasing demands on nanoparticles have wide pertinent in almost all the fields. The ecosystem has variety of living resources, which includes prokaryotes like microorganism to eukaryotic organism like higher plants and animals. The present review deal with the application of fungus in nano technology. Our discussion mainly focused on the entamopathogenic fungi are involved in nanosynthesized. The present review is focusing on the following topics, antifungal, antibacterial, antimicrobial, anticancer and mosquito larvicidal activity of fungus.

Key Words: Nanotechnology, Entamopathogenic fungi, antimicrobial, antifungal and larvicidal activities.

INTRODUCTION

In modern ecosystem fungi play an important role in almost every nutrient cycle, either as sources of food, decomposers of organic materials, or essential symbiosis of plants and animals, providing necessary nutrient or protecting their hosts. Although Fungi are primarily terrestrial, recent studies have emphasized that a diversity of fungi are found in fresh water, seas and ocean bottoms, ranging from tropical to Arctic and Antarctic regions, Although the precise roles of fungi in these ecosystems are still poorly understood, it is clear that they are essential.

Fungi that produce active metabolites have been obtained from various marine substrates, such as animals (predominantly sponges or fishes) but also primary producers, most importantly mangroves, sea grasses and algae. Accordingly this group of organisms has attracted considerable attention from natural product chemists, and numerous studies dealing with diverse and unique compounds of marine fungi have been reported, with pertinent biological activities including antimicrobial, anticancer, anti-inflammatory and antiviral properties (Liberra and Lindeguist, 1995; Biabani and Laatsch, 1998; Bugni and Irelant, 2004; Ebel, 2006; Pan *et al*., 2008). Primary metabolites many of fungi produce low-molecular weight, often biologically active compounds known as secondary metabolites. Although compounds may be chemically diverse, they are usually produced via common biosynthetic pathways often related to morphological development some secondary metabolites may have potent physiological activities that provide fungi with fitness benefits in nature, for instance, for competing with other microorganisms, for protection from consumption, for attracting a mate or by facilitating their distribution by attracting vectors. Attraction involves some gasteroid fungi (e.g. truffles) that form their fruiting bodies underground and have therefore no traditional means for spore dispersal.

The metabolites of other fungi such as certain species of *Psilocy* be may cause hallucinations when consumed and have been used not only recreationally but also for spiritual experiences other fungi have been shown to produce beneficial compounds such as antibiotics (e.g. *penicillium spp.*) inhibitors of virus reproduction, and inhibitors of cancer cell growth (e.g. metabolites of *Gonaderma lucidum.*) At present, numerous studies are aimed at uncovering new fungal secondary compounds of benefit to humans.

Entamopathogenic Fungi

Entomopathogenic fungi are considered to play vital role in biological control agent of insect population. A very diverse array of fungal species is found from different classes that infect insects. These insect pathogenic species are found in a wide range of adaptations and infecting capacities including obligate and facultative pathogens. (Hafiza Tahira Gul *et al*., 2014) Entophytic fungi exhibit complex interaction with their hosts. This fact has recently triggered several studies exploring their utilization as sources of novel bioactive natural products. The production of metabolites depends on several factors; fungi employ molecular pathways resulting in specific compounds. The products of these metabolic processes include important drugs such as *penicillin* and stains and toxic substances such as aflatoxins (Keller *et al*., 2005).

Bio Nanotechnology is a new and rapidly advancing field of research lies at the interface between biology and nanotechnology (Sahayaraj and Rajesh, 2011). The field of Nanotechnology is a science and technology to control at the Nano scale level (Bala and Arya, 2013).

Nanotechnology and Nano science could be used across all the other science fields, such as chemistry, biology, physics, material sciences and engineering (Rajasekhar Reddy *et al*., 2009). The fabrication and application of materials at the nanometer scale in biology is a great concern in the field of nanotechnology (Safekordi *et al*., 2011).

Nanotechnology involves synthesis of nanoparticles of size ranging from 1 to 100 nm which can be suitably manipulated for the desired applications. There have been impressive developments in the field of nanotechnology in the recent past, with numerous methodologies formulated to synthesize nanoparticles of particular shape and size depending on specific requirements. Nanoparticles and Nanospheres have considerable utility as controlled drug delivery systems when

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suitably encapsulated, a pharmaceutical can be delivered to in appropriate size, its concentration can be maintained at proper levels for long periods of time, and it can be prevented from undergoing premature degradation. Nanoparticles have the advantage that they are small enough that they can be injected into the circulatory system (Manjuchandra prabha *et al*., 2012). Insecticides synthesized from natural products such as silver, gold or silicon nanoparticles (Priya and Santhi, 2014).

Fungi and fungus-derived products are highly toxic to mosquitoes, yet have low toxicity to non-target organisms. Accordingly the use of endomophagous fungi and their derived products may be a promising approach for biological control of mosquitoes (Kirschbaum, 1985). The use of endomopathogenic fungi against a range of mosquito larvae has been the subject of various studies (Clark *et al*., 1988; Alves *et al*., 2002). Studies demonstrated that endomopathogenic fungi can be effective at killing mosquito larvae under laboratory conditions. Vector control is an essential requirement in control of epidemic diseases such as malaria, filariasis, dengue that are transmitted by different species of mosquitoes. Emergence of insecticide resistance and their harmful effects on non-target organisms and environment has necessitated an urgent search for development of new and improved mosquito control methods that are economical and effective as well as safe for non-target organisms and the environment.

Properties of fungi

Antimicrobial activity

Aspergillus niger AgNPS was synthesized as extracellular nanoparticles at room temperature. The antimicrobial activity of spherical shape nanoparticles ranged in excellent antimicrobial activity against *S. aureus* and *E. coli*. *A. niger* organisms are highly potential for the green, sustainable production of AgNPs (Gaikwadsagar *et al.,* 2012). Synthesized using *Aspergillus terreus* showed inhibition against dermatophytic fungi. *T. rubrum*. *E.floccosoum* source of silver nanoparticles suggests the future use as antidermatophytic drugs / agents. Antimicrobial activity of AgNPs synthesized using fungi are effective various pathogenic bacteria fungal synthesized AgNPs were attached to the surface of the microbial cell membrane and disturb the power function of permeability and respiration.

Antibacterial activity

Antimicrobial resistance has been a major health issue and still presents threat to health care system globally (Ferri *et al.,* 2015). Studies have shown that microbes have developed resistance to antibiotics through various molecular mechanisms such as prevention of access to drug targets and modification of the drug (Blair *et al.,* 2015). Thus, this global problem has led to the increase in researches featuring endophytic fungi, particularly those isolated from medicinal plants for their potential as source of new antibiotics (Radu *et al.,* 2009; Liang *et al*., 2012).

Several C*innamomum* species has been studied for their associated endophytes because of their promising antibacterial activity (Kharwar *et al.,* 2012; Homy *et al.,* 2012).

An Ascomycota isolate, Fusarium sp.2, is being reported to have a broad spectrum and promising antibacterial activity five entophytic fungi showed highest antibacterial activity. *Cunninghamella*, *Rhizoclonia* sp.2, *Fusarium* sp.1, *Fusarium* sp.2 and *Mycelir sterilia* were further evaluated for their antibacterial activity using the liquid fermentation assay. Antimicrobial activity was exhibited by some endophytic fungal isolates from C.*burmani*, C. *camphora* and C. *insularimontanum* and C. *zeylanicum* (Kharwar *et al*., 2012; Strobel *et al*., 2001).

All endophytes showed growth inhibitory activity against at least one of the test pathogens. Most of the isolated fungi had antibacterial activity only against the gram positive bacteria. The activity exhibited by the endophytes from C. *mercadoi* can be said to be in agreement with antibacterial activity reported in previous studies using its bark extract (Torres *et al.,* 2003; Fuentes *et al*., 2010). preliminary study had demonstrated that C. *mercadoi* and a host to fungi is potential source of antibacterial compounds. Among the fungi *Fusarium sp* showed the most promising antibacterial activity (Jia *et al.,* 2017).

Antifungal activity

Recently as the resistance of fungal pathogens to anti fungal drugs has been has become increasingly serious. There is a growing demand for new antifungal compounds. Natural products from fungi are considered an important source for novel antifungal because of their abundant fungal species diversity their rich secondary metabolites and the improvements in their genetic breeding and fermentation processes. The antimicrobial activities of increasing number of fungi living in distinctive environments are being investigated for the discovery of new antifungal compounds (Rateb *et al.,* 2011).

Algae, sponges and mangroves are the most common materials for the isolation of fungal strains that can produce antifungal compounds. The fungi associated with those algae, sponges and mangroves are expected to produce compounds with novel or special skeletons because of the special interactions between the fungi and the algae, sponges or mangroves. Antifungal compounds were identified and approximately 50% of them were identified based on their DNA sequence. New antifungal compounds were purified from five strains from hypocreales. Two *Aspergillus sp*. (of the 3 total *Aspergillus sp*). and one *Penicillium sp*. (of five) were also good sources for new antifungal compounds (Thomas *et al*., 2010).

Anticancer activity

Paclitaxel is one of the best known and most curious examples of anticancer medication derived from endophytic fungi (Porras-Alfaro and Bayman, 2011; Strobel and Daisy, 2003). Paclitaxel is one of the most commonly used anti-tumors remedies eternally developed and is active against a wide range of human cancers, such as breast, lung and ovarian cancers, however it demonstrates poor water solubility, which results in the difficulty challenging the development of paclitaxel parenteral formulations, so its medical applications is seriously limited. These significantly promote its anticancer efficacy.

The entophytic fungus *Trametes hirsute*, is able to produce podophyllotoxin from *Podophyllum hexandrum* and other linked aryl tetralinlignans with effective anticancer properties. There is a group of substances known as the cytochalasins, these drugs have antitumour activity, but they also have cellular toxicity was produced by *Chaetomium globusum*, *Chalara*, *Hypoxylon*, *Phoma* and *Xylaris*. Anticancer agent, vincristine has been collected from mycelia sterilis, inhibiting Catharanthus roseus and also induced antitumor activity.

Larvicidal activity

Entamopathogenic fungi are preferred as they exhibit selective toxicity, do not persist and do not need to be ingested as other microbes as reported by Maurya *et al.* (2011) and have low toxicity to non-targed organisms. There is worldwide interest in the use of endomopathogenic fungi as biological control agents and a significant advance in development and manufacturing.

The toxicity of the late third instar larvae of *Culex. quinquefasciatus* to culture filtrates of five fungi *Aspergillus flavus, A. parasiticus, Penicillium falicum, Fusarium vasinfectum* and *Trichoderma viride*. Among the five fungal species tested. *A. flavus* was highly toxic to larvae of *Cx. quinquefasciatus* (Govindarajan *et al.*, 2005). Priyanka *et al.,* (2001) found that culture filtrate of *Chrysosporium tropicum* toxic to larvae of *Anopheles stephensi*. The evaluation of the role of fungus extracts in the larvicidal bioassay against *Aedes aegypti* and *Anopheles nuneztovari* has demonstrated promising larvicidal activity (Xiaoqing *et al.*, 2001). *Pithium carolinianum* killed mosquito larvae in two ways. The fungus penetrated the larvae mostly at anal and rear part of abdomen through exploitation cuticular nutrients and eventually growing extensively over all of the body. After death of the larvae, the fungus grows throughout the carcase and again penetrate the cuticle to produce external zoosporangia which released zoospores to infect other larvae. The second infection method involved clusters of mycelia developing at annual gill and siphon, sewing new and old cuticles together. This caused the failure of molting at these parts and larvae, then, died by suffocating. The infection of larvae *Culex quinquefasciatus*. The mosquito-controlling effects of the fungus not only reduced larval density but also stunted their development.

A number of Endomopathogenic fungi have been so far used effectively to control mosquito vector for the last decades are unique because fungi have the ability to directly infect the host by penetration into the cuticle and do not need to ingest by the insect to cause disease. *Metarhizium anisopliae* has so far not been tested in Algeria and this is the primary report on it as mosquito larvicide (Benserradj and Mihoubi, 2014).
Endomopathogenic fungi are naturally pathogens of mosquito larvae, although no products based on fungi are currently available for the control of vector species. Entomopathogenic fungi have an advantage over other insect pathogens as they can infect all developmental stages of their hosts such as eggs, larvae, pupae and adults. Entomopathogenic fungi for the control of disease vectors such as *Culex, Anopheles* and *Aedes*. (Laerciana *et al.*, 2013).

The extracellular secondary metabolites from many fungi have been screened for larvicidal activity against mosquitoes. (Priyanka *et al.*, 2001; Prakash *et al.*, 2010).

Nanoparticle synthesis by fungi:

The biosynthesis of nanoparticles has received increasing attention due to the growing need to develop safe, cost, effective and environmentally friendly technologies for Nano-materials synthesis. In this report, silver nanoparticles (AgNPs) were characterized by synthesized using a reduction of aqueous Ag^+ ion with the of *Aspergillus terreus*. The reaction occurred at ambient temperature and in a few hours. The bioreduction of AgNPs was monitored by ultraviolet-visible spectroscopy and the AgNPs obtained were characterized by transmission electron microscopy and X-ray diffraction (Guangquan *et al.*, 2012).

The use of eukaryotic organisms for nanoparticle synthesis was first demonstrated *Verticillium* sp. for the synthesis of gold nanoparticles. (Mukherjee *et al.* 2001) studied the synthesis of intracellular AgNPs using the fungus *Verticillium*. Electron microscopy analyses of thin sections of the fungal cells revealed that the AgNPs were formed below the cell wall surface, possibly due to the reduction of the metal ions by enzymes present in the cell wall membrane.

The authors speculated that trapping of $AuCl_4^-$ ions on the surface, possibly due to the reduction of the metal ions by enzymes present in the cell wall membrane. The authors of fungal cells could occur by electrostatic interaction with positively charged groups such as lysine residues in enzymes that existed in the *mycelia* cell wall (Mukherjee *et al.*, 2001). Indeed, when the site of nanoparticle synthesis is intracellular, downstream processing becomes difficult and often defeats the purpose of developing a simple and cheap process. In this regard, (Bhainsa and D.Souza 2006) have reported extracellular biosynthesis of AgNPs using the filamentous fungus *Aspergillus fumigatus* (Bhainsa and Souza, 2006). A majority of the filamentous fungi (*Aspergillus fumigatus*) that have reportedly been used for the purpose of extracellular biomass free synthesis of AgNPS are pathogenic to plants and / or humans. This makes handling and disposal of the biomass a major inconvenience toward commercialization of the process. Thus, there is a need for developing a newer/novel approach of testing a nonpathogenic fungus for the successful synthesis and capping of Nano sized silver particles.

Mukherjee *et al.* (2001) demonstrated “green synthesis” of highly stabilized Nano crystalline silver particles by a nonpathogenic and agriculturally important fungus, *Trichoderma asperellum* (Mukherjee *et al.*, 2008). An interesting aspect of this study is the mechanism of formation of AgNPs. The process of growing nanoparticles comprises two key steps, bio reduction of $AgNO_3$ to produce AgNPs followed by stabilization and / or encapsulation of the same by a suitable capping agent. Fourier transforms infrared spectroscopy (FTIR) spectroscopic studies were carried out to investigate the plausible mechanism behind the formation of these AgNPs.

The biosynthesis of silver nanoparticles was achieved by *Epicoccum nigrum* an endophytic fungus isolated from the cambium of *Phellodendron amurenso*, AgNPs showed considerable activity against the pathogenic fungi. The gold nanomaterial’s (AuNPs) with particle have been synthesized by bio reduction of chloroauric acid ($HAuCl_4$) using the fungal culture filtrate (FCF) of *Alternaria alternata*. The mycosynthesized silver nanoparticles (AgNPs) using an endomopathogenic fungi *Trichoderma harzianum* against developmental stages of the dengue vector *Aedes aegypti* showed effective larvicidal and pupicidal activity.

Table1 Overview of biological activity and biosynthesis by fungi

Year	Name of the species	Biological activity of biosynthesized	Authors
2001	*Verticillium*	Nanoparticle synthesized	Mukherjee *et al.* (2001)
2001	*Mycelial*	Nanoparticle synthesized	Mukherjee *et al.* (2001)
2001	*Chrysosporium tropicum*	Larvicidal activity	Priyanka *et al.* (2001)
2001	*Pithium carolinianum*	Larvicidal activity	Xiaging *et al.* (2001)
2004	*Catharanthus roseus*	Anticancer activity	Yang *et al.* (2004)
2005	*A. flavus*	Larvicidal activity	Govindarajan *etal.* (2005)
2006	*Aspergillus fumigatus*	Nanoparticle synthesized	Bhainsa and Souza (2006)
2006	*Trametes hirsute, Piodophyllum hexandrum*	Anticancer activity	Puri *et al.* (2006)
2008	*Trichoderma asporellum*	Nanoparticle synthesized	Mukherjee *et al.* (2008)
2010	*C. hercadoi*	Antibacterial activity	Fuentos *et al.* (2010)
2011	*Paclitaxel*	Anticancer activity	Porras-Alfaro and Bayman (2011)
2012	*Aspergillus terreus*	Nanoparticle synthesized	Guangquan *et al.* (2012)
2012	*Alternaria alternate*	Nanoparticle synthesized	Sarkar *et al.* (2012)
2012	*Cinnamomum*	Antibacterial activity	Kharwar *et al.* (2012)
2012	*C. burmani, C. camphora, C. insularimontanum* and *C. zeylanicum*	Antibacterial activity	Kharwar *et al.* (2012)
2012	*Aspergillus niger*	Antimicrobial activity of nanoparticles synthesized	Gaikwadsagar *et al.* (2012)
2012	*Aspergillus* sp.	Antifungal activity	*et al* 2011
2012	*Penicillium* sp.	Antifungal activity	*et al* 2008
2013	*Trichoderma harzianum*	Larvicidal and pupicidal activity	Sundaravadivelan and Padmanabhan (2013)
2014	*Metarhizium anisopliae*	Larvicidal activity	Benserradj and Mihoubi (2014)
2017	*Fusarium* sp. 2	Antibacterial activity	Jia *et al.* (2017)

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