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A Review on Potentialities of Selenium Nanoparticles and Its Application Using Air Borne Fungus

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Abstract Airborne fungal pathogens are known as pathogens and cause number of diseases including infections of skin and severe respiratory tract diseases. The presence of mycotoxins in fungi are found responsible for causing infections and these mycotoxins degrade substances also. Keeping in view of this property, a number of researchers explored different fungal species to synthesize nanoparticles which exhibit promising therapeutic properties. Some of the examples of fungi used for nanoparticles include *Aspergillus* and *Trichoderma.* The biosynthesis of fungi based nanoparticles is safe, eco-friendly, biocompatible and low cost. Present review deals with the synthesis of selenium nanoparticles using air borne fungus. Selenium is one of the micronutrient required by plants in trace amounts also has therapeutic properties. But large amount of selenium is toxic and may be hazadorous when enters via food chain. Nanoselenium has similar bioactivity like other forms of selenium in humans and has many biological applications in the field of medical and pharmaceutical research to combat threats to number of diseases and for human health. Biogenic SeNPs have antimicrobial, anticancer (cytotoxic), antioxidant activity. The present review emphasizes on myconanotechnology and its application, synthesis of myconanoparticles. Application of selenium and its therapeutic properties as antimicrobial, anticancer and antiviral, whereas can be used as remedy for number of diseases. Collectively, self-assembly of SeNPs-fungal complexes affects their (patho) biological identity, which may impact human health and ecology.

Keywords: *selenium, air borne fungus, SeNPs, nanotechnology and mycogenic*

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1. Introduction

Myconanotechnology is a synthesis of nanoparticles by fungi, is one of the emerging branch of nanotechnology that has the potential to meet the crucial needs of disease control [1,2]. The filamentous fungi have capability to grow on readily available and inexpensive substrates, as well as their ability to produce a wide range of commercial metabolites [3]. The myco-synthesis of nanoparticles in an eco-friendly manner takes place in which bio-reduction of metal oxides to their elemental form is catalyzed mainly by the extracellular enzymes and metabolites released by the fungal organisms [4,5]. Selenium is one of the micronutrient required by plants in lesser amount and in large quantity it is found toxic. During the process of myco-nanostructures, the toxicity of the metal ions changes to non toxic metallic nanoparticles. Selenium is found less toxic in its reduced nano-form when compared to its other chemical forms such as sodium selenite and selenium sulfide [6]. Selenium nanoparticles (SeNPs) in their amorphous forms posses photoelectric, semiconductiong and X-ray sensing properties [7]. SeNPs are widely used in nutritional supplements, medical apparatus and nanotherapeutics and are explored by number of researchers for their anti-microbial [8], antioxidant, anti-cancerous and anti-inflammatory properties [9]. The researchers had reported that biological activities and good adsorptive ability of SeNPs may be due to interactions of nanoparticles with the functional groups present in proteins such as NH, C=O, COO– and C–N [5]. As well as biological activities reported due to micronutrient selenium, which is a main component of selenoenzymes, glutathione peroxidase, iodothyronine deiodinase, and thioredoxin reductase [10]. The application of nanotechnology in plant pathology in the form of nanofungicides, nanopesticides and nanoherbicides are being used extensively in agriculture [11].

This review provides overview of myconanotechnology and synthesis of nanoparticles using fungi, the role of fungi in the synthesis of nanoparticles, the mechanism involved in the synthesis, applications of fungi mediated SeNPs.

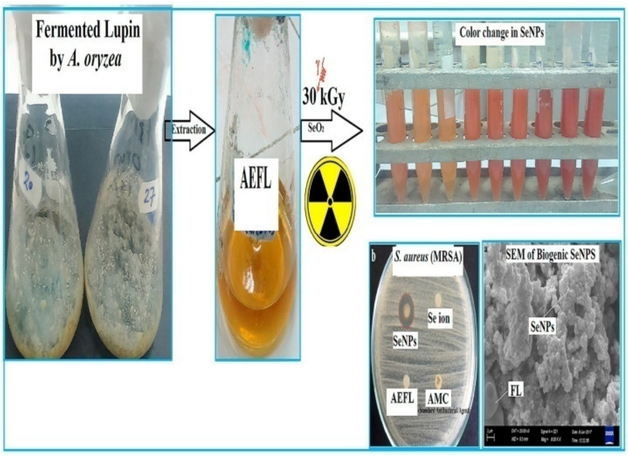

Figure 1. Synthesis of SeNPs using *Aspergillus oryzae*. (Source: Farag *et al.*, 2018 [33])

Figure 2. Synthesis of SeNPs using *Aspergillus oryzae*. Change in colour in SeNPs (Source: Farag *et al.*, 2018 [33])

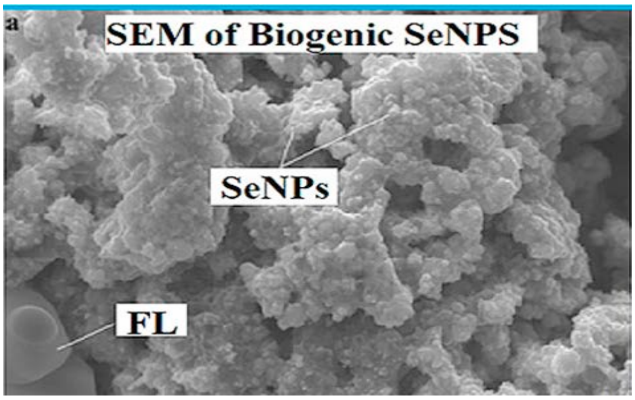

Figure 3. SEM picture of Biogenic synthesis of SeNPs using *Aspergillus oryzae* (Source: Farag *et al.*, 2018 [33])

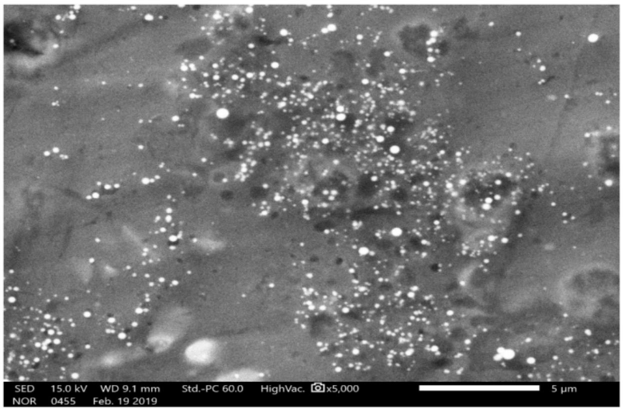

Figure 4. Scanning electron microscopic image of mycogenic SeNPs depicting their spherical shape. The white bar indicates 5µm (Source: Shreya *et al.*, 2019 [20])

2. Synthesis of Myconanoparticles

The synthesis of metal nanoparticles (NPs) using microbial cells has emerged as a new approach. Several fungal strains have been used for the synthesis of nanoparticles, for example *Fusarium*, *Aspergillus*, *Verticillium* and *Pencillium*. Both intra and extracellular production of metal NPs were proficiently done using different fungal species. Reduction and formation of selenium nanoparticles are sometimes indicated by change in colour. The improvement of methods for the controlled synthesis of metal NPs of well-defined size, shape and composition is a specific challenge. The use of fungi in the synthesis of NPs is potentially important since they produce large quantities of enzymes and are simpler to handle in the laboratory, thus it has number of advantages other than microbes and plant material for NP synthesis [12]. Since the NPs are produced outside the cell (extracellularly), they are easy to purify and can be directly used in various applications [13]. Fungal mycelium mesh can withstand flow pressure and other conditions in bioreactors or other chambers as compared to plant material or bacteria. Most fungi have a high tolerance towards metals and a high wall-binding capability, as well as intracellular metal uptake capabilities. Fungal biomass has enzymes (eg. NADH dependent nitrate reductase) which are present in fungal system, these enzymes are responsible of reducing the metal ions which are trapped at the surface of fungal cells thus producing the inorganic metal NPs when ions comes in contact with fungal cell wall [14]. A number of studies had been published and countable studies have been done on SeNPs synthesis by bacteria and fungus, but no specific studies done on air borne fungus, except few fungus including *A. terreus*, *Alternaria alternate*, *Tentinula edodes*, *Fusarium* sp, *Trichoderma veesei*, where SeNPs were synthesized between varying size ranges from 30 – 150 nm and were spherical in shape [15]. Figure 1 to Figure 4 shows images of synthesis of SeNPs using fungi by different researchers and change in colour during synthesis and SEM images also [20,33].

3. Fungi Mediated SeNPs

Synthesis of SeNPs were done by number of researchers by unicellular eukaryotic organisms, such as yeast *Saccharomyces cerevisiae* [16] and a genetically modified *Pichia pastoris*, [17] or even multicellular organisms, such as a fungus from the phylum of Ascomycota, *Aspergillus terreus* [18] were used. The fungi *Aureobasidium pullulans*, *Phoma glomerata*, *Mortierella humilis* and *Trichoderma harzianum* were also used for the formation of selenium nanoparticles and it is observed on fungal surfaces by change in colour by red and black. The hyphal matrix provided nucleation sites for metalloid deposition with extracellular protein and polymeric substances localizing the resultant SeNPs [19].

The broad spectrum antifungal activity of mycogenic selenium nanoparticles synthesized from *Trichoderma atroviride* (free living, asexually reproducing, root colonizing fungus, known to posses mycoparasitic and antimycotic activities against fungal pathogens) and characterization of bioactive nanoparticles were done using UV–VIS spectroscopy, dynamic light scattering (DLS), Fourier transform infrared (FTIR), X-ray difraction (XRD), scanning electron microscopy, dispersive X-ray spectroscopy (SEM-EDS) and high resolution transmission electron microscopy (HR-TEM). The nanoparticles inhibited the growth of *Colletotrichum capsici* and *Alternaria solani*, responsible for infection on chili and tomato at concentrations of 50 and 100 ppm, respectively and showed antifungal activity against *Pyricularia grisea [20]*. *Trichoderma* derived SeNPs recently have been demonstrated to control pearl millet downy mildew disease and this is attributed to the presence of ABC transporters involved in the secretion of antibiotics and cell wall degrading enzymes and improves plant growth under greenhouse conditions [21].

In another study *Trichoderma* mediated selenium nanoparticles were synthesized from cell lysate (CL), culture filtrate (CF) and crude cell wall (CW) using six species of *Trichoderma* namely, *T. asperellum*, *T. harzianum*, *T. atroviride*, *T. virens*, *T. longibrachiatum* and *T. brevicompactum*. SeNPs synthesized from all the three forms in which CF gave significant results than CL and CW by suppressing the growth, sporulation and zoospore viability of *Sclerospora graminicolai* and the biological activities of SeNPs were inversely proportional to the size of SeNPs and it varies from 49.5 to 312.5 nm with zeta potential of +3.3 mv to −200 mv [22].

The metabolism and accumulation of selenium in yeast cells *Saccharomyces cerevisiae* also showed that it can convert selenite and selenate to selenoamino acids and selenomethionine. SeNPs synthesized were of defined shape and size in the selenite and selenate reduction process and reported potent antimicrobial and anticancer activities [23].

The high concentration of Selenium in environment may be hazadorous, areas related to manufacturing of glass, ceramics, solar cells, and pharmaceuticals. The concentration of Se may reach up to 1.4 mg/L in irrigation or agricultural wastewaters, 4.9 mg/L in oil refinery wastewater, 7 mg/L in lead wastewater, and 33 mg/L in gold mine wastewaters and this may contaminate environment. Ascomycete, filamentous fungi can remove large quantities of both selenite [Se(IV)] and selenate [Se(VI)] in 9 days under optimal conditions and enhanced with supplementation of carbohydrate or glycerin based products and in addition with nitrogen and phosphorous [24].

The aerobic mycoremediation potential of Se (selenite or selenate) contaminated wastewaters of municipal (WWM) and industrial (WWI) were reduced by fungal isolates, *Alternaria alternata* strain SRC1lrK2f isolated from coal mine drainage treatment system and *Alternaria* sp. strain CMED5rs1aP4, an organism cultured from Se-enriched soil overlying the phosphoria formation at a reclaimed mining site in Idaho, United States [25].

4. Therapeutic Properties of SeNPs

The properties of Se nanoparticles varies with size and shape and it posses various biological properties. Researchers had investigated on different inorganic nanoparticles to induce toxicity in cancer cells and SeNPs showed promising results associated with chemotherapeutic agents and exhibit different activity against normal and malignant cells [26]. The toxicity and pharmacological effect depends on the concentration, redox state and type of selenium compound used. The anticancer effects of SeNPs are mediated through their ability to inhibit the growth of cancer cells through induction of cell cycle arrest at S phase, mediated by degradation of the eIF3 protein complex [27] and G2/M by mitochondrial pathway [28]. SeNPs can be internalized selectively by cancer cells through endocytosis and induce cell apoptosis by triggering apoptotic signal transduction pathways [29]. Biogenic SeNPs synthesized from *L. plantarum* strain showed immunostimulatory effect in BALB/c mice with 4T1 breast cancer cells. SeNPs oral treatment significantly enhances the proinflammatory cytokines such as Th 1, cytokines IFN-γ, IL-2, IL-12 and TNF-α and also increases the delayed hypersensitivity reaction [30]. The SeNPs was reported for its protective effect on progression of diabetic nephropathy. A stable long peptide BAY 55-9837 with 27 amino acids was found to be beneficial for type 2 diabetes mellitus (T2DM) [31]. The selenium is reported for its antiviral properties by number of researchers as well as used in conjugate with other drugs causes low toxicity also. An antiviral drug oseltamivir (OTV) was surface-modified SeNPs with superior antiviral properties and restriction on drug resistance. The drug prevented H1N1 from infecting Madin Darby canine kidney cell line (MDCK) by blocking chromatin condensation and DNA fragmentation and inhibited the entry of H1N1 into host cells inactivating virus glycoproteins – hemagglutinin and neuraminidase by inhibiting ROS generation as well as the activation of phosphorylation of cellular tumor antigen p53 and Akt [32].

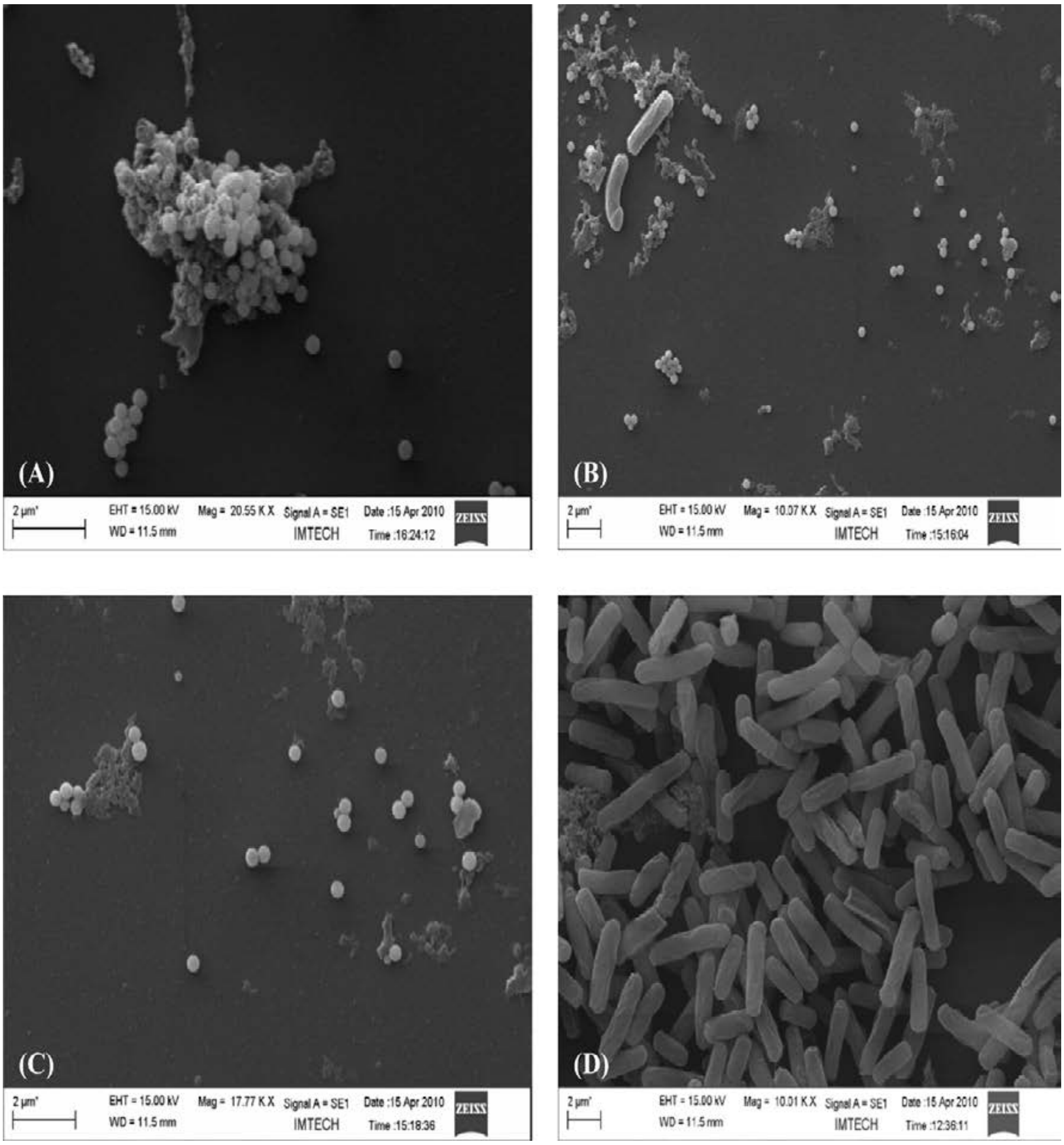

Figure 5. Picture shows effect of SeNPs on bacterial cells. Scanning Electron Micrographs of strain CM100B grown in the presence of 5 mM selenite. (A) Selenium nanospheres adhering to the bacterial cells. (B) Bacterial cells surrounded by selenium nanospheres. (C) Free selenium nanospheres. (D) Control cells grown in TSB without addition of selenite (Source: Dhanjal and Cameotra, 2010 [7])

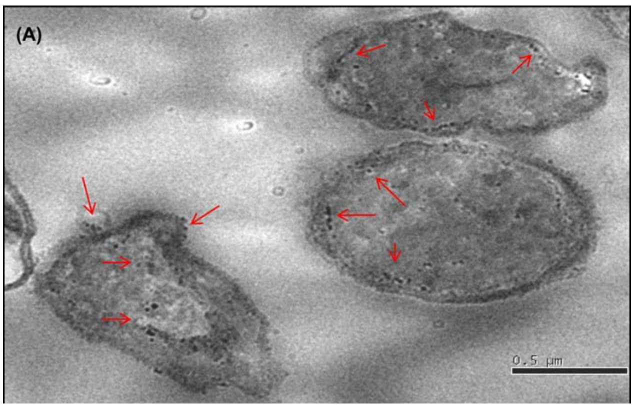

Figure 6. TEM images of localization of selenium nanospheres (indicated by arrows). Intracellular and surface localization of selenium nanospheres in strain CM100B under aerobic condition (Source: Dhanjal and Cameotra, 2010 [7])

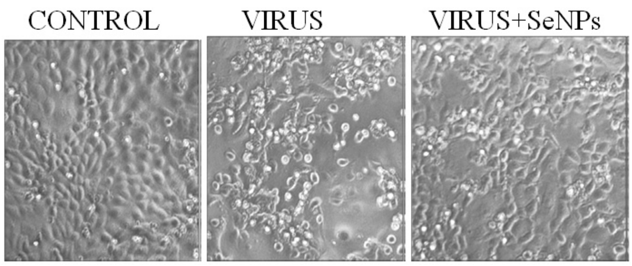

Figure 7. Effects of SeNPs on the growth of H1N1 infection of MDCK cells by MTT assay. The Images showed Control untreated, virus and SeNPS+virus treated cells of MDCK (Source: Li *et al.*, 2017 [32])

5. Conclusion

Selenium is reported for its immense therapeutic properties and is one of the essential trace element, but on the other hand it is hazadorous if it is consumed in high concentrations. Many researchers had developed its nanoparticles from different physical and chemical methods. The methods are costly and inconvenient sometime, therefore the need for green synthesis came into being. SeNPs were developed from bacteria, plant and fungi. This review highlights on synthesis of SeNPs using air borne fungus. There are number of researchers developed SeNPs using fungi which includes *Trichoderma* and *Aspergillus*. The synthesis is cost effective and convenient as well as gives pleomorphic pharmacological activities. The mycogenic SeNPs have shown promising therapeutic properties which include antimicrobial, cytotoxic and antioxidant properties. Though fungus is responsible for number of infections but mycogenic mediated SeNPs were found potent and posses multifunctional NPS based on the records and can be an important therapeutic agent.

6. Future Directions

Selenium is an essential element required as a cofactor for various enzymes and has emerged as an important tool for the remedy of wide range of maladies ranging from bacterial, fungal and viral infections, inflammation, neurodegenerative disorders, diabetes, drug induced toxicity, cancer, etc. Still there is a need to exploit therapeutic properties of selenium. Even though the literature is scarce on usage of air borne fungus mediated biosynthesis of SeNPs application. The drug delivery properties of SeNPs were scarcely used for air borne fungus and often suffer from the problem of poor pharmacokinetics though possess attractive pharmacological efficacy. In future multifunctional SeNPs may be designed with improved uptake by fictionalization and simultaneous drug loading and theranostic properties.

References

[1] Worrall, E.A., Hamid, A., Mody, K.T., Mitter, N. and Pappu, H.R, “Nanotechnology for Plant Disease Management,” *Agronomy*, 8 (285). Nov.2018.

[2] Jogaiah, S., Abdelrahman, M., Tran, L.S.P. and Ito, S.I, “Different mechanisms of *Trichoderma* virens-mediated resistance in tomato against *Fusarium* wilt involves the jasmonic and salicylic acid pathways,” *Mol Plant Pathol*, 19 (4). 870-882. 2018.

[3] Dhillon, G.S., Brar, S.K., Kaur, S. and Verma, M, “Green approach for nanoparticle biosynthesis by fungi: current trends and applications,” *Critical Reviews in Biotechnology*, 32 (1). 49-73. Jun.2011.

[4] Zhang, S., Luo, Y., Zeng, H., Wang, Q., Tian, F., Song, J. and Cheng, W.H, “Encapsulation of selenium in chitosan nanoparticles improves selenium availability and protects cells from selenium-induced DNA damage response,” *J Nutr Biochem*, 22 (12). 1137-1142. Dec.2011.

[5] Wang. H., Zhang, J. and Yu, H, “Elemental selenium at nano size possesses lower toxicity without compromising the fundamental effect on selenoenzymes: comparison with selenomethionine in mice,” *Free Radic Biol Med*, 42 (10). 1524-1533. May.2007.

[6] Nuttall, K.L, “Evaluating selenium poisoning,” *Ann Clin Lab Sci*, 36 (4). 409-420. 2006.

[7] Dhanjal, S. and Cameotra, S.S, “Aerobic biogenesis of selenium nanospheres by *Bacillus cereus* isolated from coalmine soil,” *Microb Cell Factories*, 9 (52). 2010.

[8] Tran, P. and Webster, T.J, “Enhanced osteoblast adhesion on nanostructured selenium compacts for anti-cancer orthopedic applications,” *Int J Nanomedicine*, 3 (3). 391-396. 2008.

[9] Khiralla, G.M. and El-Deeb, B.A, “Antimicrobial and antibiofilm effects of selenium nanoparticles on some food borne pathogens,” *LWT Food Sci. Technol*, 63 (2). 1001-1007. Oct.2015.

[10] Forootanfar, H., Adeli-Sardou, M., Nikkhoo, M., Mehrabani, M., Bagher, A.H., Ahmad, R.S. and Shakibaie, M, “Antioxidant and cytotoxic effect of biologically synthesized selenium nanoparticles in comparison to selenium dioxide,” *J Trace Elem Med Biol*, 28 (1). 75-79. Jan.2014.

[11] Mousa, A. A., Hassan, A., Mahindra, R., Ernest, S.G. and Kamel, A. A, “Myconanoparticles: synthesis and their role in phytopathogens management. Article; Agriculture and Environmental Biotechnology,” *Biotechnology & Biotechnological Equipment*, 29 (2). 221-236. Mar.2015.

[12] Mohanpuria, P., Rana, N.K. and Yadav, S.K, “Biosynthesis of nanoparticles, technological concepts and future applications,” *J Nanoparticle Res*, 7. 9275-9280. Mar.2008.

[13] Gaikwad, C. S., Birla, S.S., Ingle, A.P., Gade, A.K., Marcato, P.D., Rai, M.K. and Duran, D, “Screening of different *Fusarium* species to select potential species for the synthesis of silver nanoparticles,” *J Braz Chem Soc*, 24 (12). 1974-1982. 2013.

[14] Krishna, G. and Singara, C.M, “Synthesis of silver nanoparticles by chemical and biological methods and their antimicrobial properties,” *Journal of Experimental Nanosciencei*, 11 (9). 714-721. Jan.2016.

[15] Angamuthu, A., Venkidusamy, K. and Muthuswami, R. R, “Synthesis and characterization of nano-selenium and its antibacterial response on some important human pathogens,” *Current Science*, 116 (2). 285-290. Jan.2019.

[16] Zhang, L., Li, D. and Gao, P, “Expulsion of selenium/protein nanoparticles through vesicle-like structures by *Saccharomyces cerevisiae* under microaerophilic environment,” *World J Microbiol Biotechnol*, 28. 3381-3386. Sep.2012.

[17] Elahian, F., Reiisi, S., Shahidi, A. and Mirzaei, S.A, “High-throughput bioaccumulation, biotransformation, and production of silver and selenium nanoparticles using genetically engineered *Pichia pastoris*,” *Nanomedicine: Nanotechnology, Biology, and Medicine*, 13. 853-861. 2017.

[18] Zare, B., Babaie, S., Setayesh, N. and Shahverdi, A.R, “Isolation and characterization of a fungus for extracellular synthesis of small selenium nanoparticles,” *Nanomed J*, 1 (1). 14-20. 2013.

[19] Xinjin, L., Magali, A.M.P., Kenneth, C.N., Philipp, E. Joerg, F., Laszlo, C. and Geoffrey, M. G, “Fungal formation of selenium and tellurium nanoparticles,” *Applied Microbiology & Biotechnology*, 103. 7241-7259. July.2019.

[20] Shreya, M.J., Savitha, D. B., Sudisha, J. and Shin-ichi, I, “Mycogenic Selenium nanoparticles as potential new generation broad spectrum antifungal molecules,” *Biomolecules*, 9 (419). 11-16. Aug.2019.

[21] Lanzuise, S., Ruocco, M., Scala, V., Woo, S.L., Scala, F., Vinale, F. and Lorito, M, “Cloning of ABC transporter-encoding genes in *Trichoderma* spp. to determine their involvement in biocontrol,” *J Plant Pathol*, 84. 184. Jan.2002.

[22] Boregowda, N., Puttaswamy, H., Harischandra, S. P., Hunthrike, S. S. and Nagaraja, G, “Trichogenic-selenium nanoparticles enhance disease suppressive ability of *Trichoderma* against downy mildew disease caused by *Sclerospora graminicola* in pearl Millet,” *Scientific Reports*, 7 (2612). Jun.2017.

[23] Kieliszek, M., Bła, Z. S., Gientka, I. and Bzducha-Wróbel, A, “Accumulation and metabolism of selenium by yeast cells,” *Appl Microbiol Biotechnol*, 99. 5373-5382. May.2015.

[24] Mary, C. S., Carla, E. R., Todd, D., DeJournett, Katie, S., Karl, W. and Cara, M. S, “Fungal Bioremediation of Selenium-Contaminated Industrial and Municipal Wastewaters,” *Frontiers in Microbiology*, 11 (2105). Sep.2020.

[25] Rosenfeld, C.E., Kenyon, J.A., James, B.R. and Santelli, C.M, “Selenium (IV,VI) reduction and tolerance by fungi in an toxic environment,” *Geobiology*, 15 (3). 441-452. Jan.2017.

[26] Amit, K., Sravani, T., Mohd, A.S., Pooladanda, V. and Chandraiah, G, “Therapeutic applications of selenium nanoparticles,” *Biomedicine & Pharmacotherapy*, 111. 802-812. 2019.

[27] Luo, H., Wang, F., Bai, Y., Chen, T. and Zheng, W, “Selenium nanoparticles inhibit the growth of HeLa and MDA-MB-231 cells through induction of S phase arrest,” *Colloids Surf B Biointerfaces*, 94. 304-308. Jun.2012.

[28] Zeng, H. and Combs, G. F, “Selenium as an anticancer nutrient: roles in cell proliferation and tumor cell invasion,” *J Nutr Biochem*, 19 (1). 1-7. Jan.2008.

[29] Pi, J., Jin, H., Liu, R., Song, B., Wu, Q., Liu, L., Jiang, J., Yang, F., Cai, H. and Cai, J, "Pathway of cytotoxicity induced by folic acid modified selenium nanoparticles in MCF-7 cells," *Appl Microbiol Biotechnol*, 97 (3). 1051-1062. Feb.2013.

[30] Yazdi, M.H., Varastehmoradi, B., Faghfuri, E., Mavandadnejad, F., Mahdavi, M. and Shahverdi, A.R, "Adjuvant effect of biogenic selenium nanoparticles improves the immune responses and survival of mice receiving 4T1 cell antigens as vaccine in breast cancer murine model," *J Nanosci Nanotechnol*, 15 (12). 10165-10172. Dec.2015.

[31] Rao, L., Ma, Y., Zhuang, M., Luo, T., Wang, Y. and Hong, A, "Chitosan-decorated selenium nanoparticles as protein carriers to improve the in vivo half-life of the peptide therapeutic BAY 55-9837 for type 2 diabetes mellitus," *Int J Nanomed*, 9. 4819-4828. Oct.2014.

[32] Li, Y., Lin, Z., Guo, M., Xia, Y., Zhao, M., Wang, C., Xu, T., Chen, T. and Zhu, B, "Inhibitory activity of selenium nano-particles functionalized with oseltamivir on H1N1 influenza virus," *Int J Nanomedicine*, 12. 5733-5743. Aug.2017.

[33] Farag M.M., Gharieb S.E., Rasha M.F. and Ahmed I.E, "Biomolecules-mediated synthesis of selenium nanoparticles using *Aspergillus oryzae* fermented Lupin extract and gamma radiation for hindering the growth of some multidrug-resistant bacteria and pathogenic fungi," *Microbial Pathogenesis*, 122. 108-116. Sep.2018.