1. PHD PROJECT DESCRIPTION (4000 characters max., including the aims and work plan)

Project title:

Search for antifungal activity in biogenic nanoparticles against selected plant pathogenic fungi with special reference to formulation of nanofungicides

1.1. Project goals

As far as the biotic factors are concerned, plant pathogenic fungi are considered as most important pathogens leading to severe yield loss in a variety of crop plants. To date, number of chemical fungicides have been developed and effectively used in the control of plant diseases caused by a wide-range of fungi. Universal application of broad spectrum fungicides definitely helps in controlling the fungal diseases in plants, but at the same time these fungicides have been posing severe hazardous effects on environment and human health for several years. Moreover, extensive use of chemical fungicides has also resulted in the development of resistance in many plant pathogenic fungi against such fungicides. Besides, loss in the soil fertility is an another major concern [1].

In this context, search of novel, most effective and environmentally friendly fungicides is a prime goal of proposed research project. Several efforts have been made to develop such novel fungicides. Among these, nanotechnology is one of the most promising platform found to have enormous applications in different sectors including agriculture, particularly in the management of plant diseases and their causative agents.

Moreover, to date, different hypothetical mechanisms have been proposed, but actual mechanism behind the mode of action of different nanoparticles (NPs) in fungi is still not clear [2].

Considering all these facts, the proposed research plan will be of great importance because it is focused on most important issues which involve screening of different NPs against important plant pathogenic fungi responsible for huge crop losses in Poland and understanding the actual mechanism(s) behind the mode of their action so as to develop novel and efficient nano-based fungicides (nanofungicides).

1.2. Outline

To achieve the goal, silver, copper, zinc, chitosan and sulfur nanoparticles will be biologically synthesised using some specific fungi (e.g. *Fusarium* sp*., Rhizoctonia* sp., *Aspergillus* sp., *Aureobasidium* sp.). Fungi are proposed to be used for synthesis of NPs as they are simple in cultivation, give large amount of produced biomass and possess high metal tolerance, extracellular binding (cell wall, secreted metabolites) and intracellular metal uptake capabilities [3-4] that result in an efficient and cost-effective production of NPs. The extensively secreted various components are involved in the reduction and capping of NPs [5-7]. Moreover biosynthesis of NPs by fungi is eco-friendly process when compared with physical and chemical ones [8].

Bio-NPs will be characterized for physico-chemical properties using standard techniques such as transmission electron microscopy (TEM; size and shape study), potential Zeta (stability),

nanoparticle tracking (NTA; size, concentration, aggregation) and Fourier Transform Infrared Spectroscopy (FTIR; capping agents) [9]. These features determine activity of NPs on biological systems, including antifungal activity [10-11].

Further, antifungal potential of NPs will be evaluated against the most important plant pathogenic fungi responsible for major crop losses in Poland (e.g. *Fusarium avanaceum, F. oxysporum*, *Alternaria solani* or fungus-like *Phytophtora plurivora* or *P. infestans*). Antifungal activity of bio-NPs will be performed using disc diffusion and dilution methods in agar plates [12] which allows for selection of NPs as nanofungicides.

Formulated nanofungicides will be evaluated against selected fungal pathogens *in vivo* (experiments in pots) on plants (e.g. tomato, cucumber, pepper or cabbage seedlings). Plant condition after treatment with fungi or fungi and nanofungicides will be estimated by *in situ* measurement of chlorophyll fluorescence (Fv/Fm ratio determination, PEA) and estimation of plant growth parameters (length of plant roots and shoots, wet and dry mass). This ratio is widely considered to be a sensitive indication of plant photosynthetic performance (maximum quantum efficiency of Photosystem II) and decreases in plants under abiotic or biotic stress [13].

In addition, actual mechanism of action will also be investigated. Fungi treated nanofungicides will be observed using TEM to check changes in their morphology and/or cytology.

The proposed research plan will definitely help to develop effective and environment friendly fungicides having great potential towards management of most important plant pathogenic fungi and of course, crop losses in Poland.

1.3. Work plan

Objectives	Year			
	I	II	III	IV
1. Literature survey				
2. Biosynthesis of Ag, Cu, Zn and S NPs				
3. Characterization of NPs				
4. Evaluation of antifungal activity NPs against selected plant pathogenic fungi				
5. Development of nanofungicide for management of plant fungal diseases				
6. Unravelling the inhibition mechanism of plant pathogenic fungi by NPs				
7. Publication for the benefit of the researchers and society				

1.4. Literature

1. Gaur R, Singh R, Gupta M, Gaur MK. *Aureobasidium pullulans*, an economically important polymorphic yeast with special reference to pullulan. African J Biotechnol, 2010; 9(47): 7989-7997.
2. Huang W, Yan M, Duan H, Bi Y, Cheng X, Yu H. Synergistic antifungal activity of green synthesized silver nanoparticles and epoxiconazole against *Setosphaeria turcica*. J Nanomater, 2020: 9535432.
3. Gade A, Ingle A, Whiteley C, Rai M. Mycogenic metal nanoparticles: progress and applications. Biotechnol Lett 2010; 32:593–600.
4. Velusamy P, Kumar GV, Jeyanthi V, Das J, Pachaiappan R. Bio-Inspired green nanoparticles: synthesis, mechanism, and antibacterial application. Toxicol Res 2016; 32(2):95–102.
5. Gudikandula K, Maringanti SC. Synthesis of silver nanoparticles by chemical and biological methods and their antimicrobial properties. J Exp Nanosci 2016; 11(9):714-721.
6. Singh P, Kim Y-J, Zhang D, Yang D-C. Biological synthesis of nanoparticles from plants and microorganisms. Trends Biotechnol 2016; 34(7):588-599.
7. Gahlawat G, Choudhury AR. A review on the biosynthesis of metal and metal salt nanoparticles by microbes. RSC Adv 2019; 9:12944.
8. Golinska P, Rathod D, Wypij M, Gupta I, Składanowski M, Paralikar P, Dahm H, Rai M. Mycoendophytes as efficient synthesizers of bionanoparticles: nanoantimicrobials, mechanism, and cytotoxicity, Critical Reviews in Biotechnology, 2017; 37:6, 765-778.
9. Wypij M, Swiecimska M, Czarnecka J, Dahm H, Rai M, Golinska P. Antimicrobial and cytotoxic activity of silver nanoparticles synthesized from two haloalkaliphilic actinobacterial strains alone and in combination with antibiotics. J Appl Microbiol 2018;124(6):1411-1424.
10. Jeevanandam J, Barhoum A, Chan JS, Dufresne A, Danquah MK. Review on nanoparticles and nanostructured materials, history, sources, toxicity and regulations. Beilstein J Nanotechnol, 2018;9:1050–1074.
11. Rai M, Gupta I, Ingle AP, Biswas JK, Sinitsyna OV. Nanomaterials, what are they, why they cause ecotoxicity, and how this can be dealt with? In: Rai, M., Biswas, J.K. (Eds). Nanomaterials, Ecotoxicity, Safety, and Public Perception. Springer Nature Switzerland AG, 2018; pp. 3-18.
12. Wayne PA. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, Approved standard, 9th edn. CLSI document M07-A9, 2012.
13. Murchie EH, Lawson T. Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. J Exp Botany 2013; 64(13):3983-3998.

1.5. Required initial knowledge and skills of the PhD candidate

Knowledge on nanomaterials, natoably nanoparticles and biological routes of their

synthesis;

Knowledge and/or basic skills on methods of nanoparticle detection and characterization;

Knowledge and experience in microbiology, notably media preparation, microorganisms cultivation, microorganism physiology, antimicrobial assays.

Skills in manuscript preparation and/or public results presentation will be very welcome.

Skills in working alone and in team is required.

1.6. Expected development of the PhD candidate's knowledge and skills

Phd candidate will gain advanced knowledge on mechanisms of biological routes of metal and non-metal synthesis of nanoparticles, mechanisms of nanoparticle action on microbial cells and their potential use in agriculture. Candidate will achieve advanced skills related to microbiological techniques and analyses (microorganisms cultivation, antimicrobial assessment tests), techniques used for characterization of nanomaterials such as TEM, EDX, NTA, potential zeta, FTIR. PhD candidate will also gain the skills of planning research work and its independent performance, making research hypotheses and their verification as well as independent data analyses and manuscript writing.