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

Project title: Casein-based nano-composites for sustainable crop plant protection and promotion of growth

1.1. Project goals

This project aims to synthesis of metal nanoparticles (Zn, Cu, Fe, Mg), important in plant protection and growth (micro and macro-nutrients), by green route (biosynthesis with the use of microorganisms) and their use for formation of complexes with casein micelles. Such nanoparticles and their complexes after assessment of physical and chemical properties will be evaluated for antimicrobial activity against plant pathogens and germination and promotion of growth of world leading crop plants such as maize or wheat, as well as the impact of such nanocomplexes treatment on the structural and functional biodiversity of the microbiome of rhizosphere.

1.2. Outline

The rapidly growing world population simultaneously requires an increase in agricultural production to meet the demand for food. Considering the limited resources for additional arable land, scarce water resources in the world and the adverse effects of climate change (e.g. drought, salinity), effective control of plant pathogens and fertiliser management is required to achieve the desired increase in agricultural production.[2]

The use of conventional pesticides and chemical fertilisers in large quantities and for a long period in the agricultural sector has caused serious problems such as loss of soil fertility, reduction of soil organic content etc.[1] Nano-fungicides and nano-fertilisers in the form of complexes with polymers, such as casein, allow for slow and sustained release of active substances resulting in increased nutrient absorption, increased soil fertility and water availability, as well as saving fertiliser consumption and minimising environmental pollution.[5,6]

Therefore, there is a need to develop innovative nano-fungicides and -fertilizers to increase crop yield, enhance plant nutrient efficiency and plant protection and minimize environmental disturbances for global sustainable development.[3-6] Application of innovative nanotechnology in agriculture is considered as one of the promising approaches. In order to meet the global food production and demand in an environmentally and economically sustainable manner, it is necessary to explore nanomaterial approach in agriculture.[5]

Green synthesis of nanoparticles is economical and eco-friendly approach when compared to physical and chemical methods. Complexes of nanoparticles with casein micelles are considered as materials for slow, controlled release of microelements in low concentrations over time, which become available to plants without their rapid leaching from the soil and accumulation in the environment.

1.3. Work plan

Objectives	**Year**			
	I	**II**	**III**	**IV**
1. Literature survey				
2. Biosynthesis of metal nanoparticles (MNPs) and casein-based nanocomposites				

3. Characterization of physico-chemical properties of NPs and composites				
4. Antimicrobial activity of materials against selected plant pathogenic microorganisms				
5. Pots experiments on plants				
6. Study of the structural and functional biodiversity of the plant microbiome of rhizosphere				
7. Publication of review and original papers				

Nanoparticles will be synthesized using different molds and utilized for formation of casein-based nonocomplexes. Nanomaterials will be characterized using various techniques, including UV-vis, Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffraction, transmission electron microscopy (TEM)+EDX, potential Zeta etc. [3-4] Antifungal potential of materials will be evaluated against the most important plant pathogenic fungi responsible for selected crop losses around the world (e.g. different *Fusarium* species) using standard techniques [3-4] which allows for selection of NPs as nanofungicides. Formulated casein based nanomaterials will be evaluated on plants (e.g. maize) in pot experiments. Plant condition after treatment will be estimated by *in situ* measurement of chlorophyll fluorescence, and estimation of plant growth parameters (length of plant roots and shoots, wet and dry mass) as well as on the redox homeostasis (oxidative stress parameters, antioxidant system parameters).[5] Moreover, structural and functional biodiversity of rhizosphere microbiome will be evaluated after treatment with nanocomplexes using omics approach.[7]

1.4. **Literature** *(max. 7 listed, as a suggestion for a PhD candidate preliminary study)*

1 Ahmad MF, Ahmad FA, Alsayegh AA, Zeyaullah M, AlShahrani AM, Muzammil K, Saati AA, Wahab S, Elbendary EY, Kambal N, Abdelrahman MH, Hussain S. (2024). Pesticides impacts on human health and the environment with their mechanisms of action and possible countermeasures. Heliyon, 10(7):e29128. doi: 10.1016/j.heliyon.2024.e29128.

2 Kumar L, Chhogyel N, Gopalakrishnan T, Hasan MK , Jayasinghe SL, Kariyawasam CS, Kogo BK, Ratnayake S. (2022). Climate change and future of agri-food production, In: Bhat R (ed), Future Foods, Academic Press,pp 49-79, https://doi.org/10.1016/B978-0-323-91001-9.00009-8.

3 Trzcińska-Wencel J, Wypij M, Rai M, Golińska P. (2023a). Biogenic nanosilver bearing antimicrobial and antibiofilm activities and its potential for application in agriculture and industry. Frontiers in Microbiology, 14: 1125685.

4 Trzcińska-Wencel J, Wypij M, Terzyk AP, Rai M, Golińska P. (2023b). Biofabrication of novel silver and zinc oxide nanoparticles from *Fusarium solani* IOR 825 and their potential application in agriculture as biocontrol agents of phytopathogens, and seed germination and seedling growth promoters. Frontiers in Chemistry, 11: 1235437.

5 Trzcińska-Wencel J. Golińska P, Gade A, Ingle PU, Shende S, Rai M. (2025). Microbial Biosynthesis of Biostimulant Nanomaterials and Nanofertilizers, In: Juárez-Maldonado A, Benavides-Mendoza A, Barrios DLO, Tortella Fuentes GR, Seabra AB (eds), Plant biostimulation

with nanomaterials. From molecular biology to ecosystems, Springer (ISBN: 978-981-96-4648-7 in press).
6 Trzcińska-Wencel, J., Golińska, P., Rai, M. (2024). Toxicity of nanomaterials to plants. W: M. Rai & G. D. Avila-Quezada (eds.), Nanotechnology in Plant Health CRC Press, Taylor and Francis Group, pp. 369-389.
7 Wang N, Li H, Wang B, Ding J, Liu Y, Wei Y, Li J and Ding G-C (2022). Taxonomic and Functional Diversity of Rhizosphere Microbiome Recruited From Compost Synergistically Determined by Plant Species and Compost. Front. Microbiol. 12:798476. doi: 10.3389/fmicb.2021.798476

1.5. Required initial knowledge and skills of the PhD candidate

Knowledge on nanomaterials, notably nanoparticles and biological routes of their synthesis;
Knowledge and basic skills on methods of nanoparticle characterization;
Experience in general microbiology, notably media preparation, microorganisms cultivation, microorganism physiology, antimicrobial assays.
Experience in *in vitro* and *in vivo* plant cultivation, analysis of parameters of germination and plant growth.
Experience in manuscript preparation and 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 and skills on casein-based nanocomposites synthesis, complete physico-chemical characterization and antimicrobial properties of such complexes, as well as their potential to be used in agriculture. Candidate will achieve advanced skills related to microbiological techniques and analyses (microorganisms cultivation, antimicrobial assessment tests, molecular analyses), 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.