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Introduction

In recent days, nanotechnology has arrived as a leading-edge trove of knowledge having multidimensional applications due to its unique structures and physical properties. The application of nano-scale structures is an emerging area of nanoscience and nanotechnology. Nanomaterials are defined as particulate dispersions or solid particles with a size range of 1-100 nm [1 – 3]. Metal NPs are very extensively studied for their pharmaceutical, biomedical and industrial applications but are comparatively least studied for their potential in agricultural sector [4]. Most reported metal NPs are usually prepared from silver, gold, platinum, palladium titanium, copper, zinc *etc* [5, 6]. Such NPs are widely studied for their catalytic and antimicrobial applications [7 – 16]. However, in recent days metal NPs are also explored for their agricultural applications such as in seed germination, plant growth, formulating pesticides or insecticides, nanosensors *etc.* but are not adequately studied as compared to their pharmaceutical and antimicrobial applications [4, 17]. Conversely, the world population is estimated to attain approximately 9.6 billion by the end of the year 2050 which means food production should have to be increased by 70-100% to mitigate the food crisis [18]. Therefore, there is an urgent need to develop eco-friendly technologies which can increase the efficiency of crop production [4, 17]. Although a handful of recent findings demonstrate the probable applications of

metal NPs in enhancing the seed germination rate, plant growth and development but their mechanism of action and possible cytotoxic effect on the environment are yet to be clarified [20, 21]. Out of all the platinum group metals, Ag NPs are maximally explored mostly for their industrial, biomedical, pharmaceutical and catalyzing applications since decades but least studied for their role in seed germination [21 – 25]. Researchers have proved that ZnO NPs can be used in boosting crop production by enhancing the germination rate, sugar contents and catalysing the antioxidant activities [26]. Moreover, zinc oxide nanoparticles are used as a potent antimicrobial agent due to its nano-size which is less than 100 nm and large surface area [27]. Therefore, the use of zinc oxide nanoparticle has drawn its desirability towards biomedical research and also food industry [28].

Researchers have demonstrated increase in the water uptake in nano-primed seeds followed by the catalysis of the α -amylase activity inducing starch hydrolysis leading to enhanced germination rate in seeds [21, 29]. Recent studies have shown that exposure to specific concentrations of various metal NPs such as Ag, Zn, Au, Cu *etc.* could enhance plant growth compared with non-exposed plants [30–34]. However, the uptake mechanism of metal NPs by seeds or plant root is not completely understood [35].

Germination begins with water uptake by dry seed through a phenomenon called imbibition. The α -amylase is one of the key enzymes involved in degradation of starch during germination of cereal seeds and in subsequent seedling establishment [21]. This is the only enzyme which initiates hydrolysis of native starch granules and is *de novo* synthesized during the germination of cereal seeds and catalyzes the hydrolysis of α -1, 4 linked glucose polymers to release fragments that can be further broken down by other amyolytic enzymes [21]. On the other hand, metal NPs exhibit catalytic activities hence it is also hypothesized by researchers that such NPs may catalyze the activity of α -amylase by entering through the cellular pores of the seeds by taking the advantage of their nano-size and thus enhances the seed germination rate [21]. Besides, dehydrogenase activity is an indicator of root vitality and assessment of which may give a snapshot on the metabolic activity level and root's ability to absorb nutrients and water [36, 37]. Some researchers also hypothesize that

treatment with metal NPs may also increase the expression levels of aquaporin genes responsible for water uptake in germinating seeds [21]. In addition to these, metal NPs also shows antioxidant potential and it is well established that antioxidant protects and prevents oxidative deterioration of lipids and maintains structural and functional integrity of cells. Plants possess very efficient scavenging systems for reactive oxygen species that protect them from destructive oxidative senescence. Antioxidants are the substances that even when present in low concentration effectively protects the cell membrane against the oxidative damage induced by oxidants. The first enzyme involved in the antioxidant defence is the superoxide dismutase, a metalloprotein found in both prokaryotic and eukaryotic cells. Superoxide dismutase (SOD) in conjunction with catalase may act as an enzymatic oxidant detoxification system. Nano priming treatment of seeds may stimulate the superoxide dismutase and other reactive oxygen species scavenging enzyme activity in seeds [38].

Metal NPs are usually synthesized using diverse physical and chemical complex processes generating and introducing environmental pollutants. The most popular method is the chemical reduction method, in which the metals salts are converted to metal atoms by using reducing agents such as citrate, hydrides, ethylene glycol and hydrazine, all of which pose environmental and health risks [3]. Most of the available techniques are capital intensive, produce hazardous waste, and unstable NPs with reduced targeted activity. The continuous use of traditional methods, *i.e.*, physical and chemical methods for the synthesis of NPs ends up making the environment toxic [39, 40] which may be solved by following different eco-friendly and cost-effective methods based on green chemistry [39, 40]. Hence clean, nontoxic, size-controlled, and environmentally benign synthesis procedures are inevitable to promote large-scale synthesis of NPs for industrial, medical, and environmental applications [1]. Consequently, during the last decade, the biosynthesis of metal NPs has emerged and is undergoing development as an alternative environmentally benign procedure. The biological methods of NPs synthesis belong to new green generation processes, which are eco-friendly and are designed as credible alternatives to chemical and physical methods often called “green-synthesis” or “green chemistry” procedures [41]. Nanobiotechnology has recently emerged as an area of interest and

result of an active integration between microbial biotechnology and nanotechnology [42]. Microbial biotechnology has traditionally used microorganisms and their products in various applications such as medicine, industry, food production, agriculture, and remediation technologies [43]. It has been reported that NPs can be synthesized by biological sources such as plant extracts, fungi, algae, cyanobacteria, bacteria, yeasts, actinomycetes, virus and even standalone biomolecules such as proteins, amino acids, enzymes, glucose, biosurfactants *etc* [44 – 61]. Even though, green synthesis of metal NPs using various plant extract have now become a common practice but the possibility of hampering the biodiversity of indigenous plant species due to the extensive use of plants cannot be ruled out. Reverse micelle technique for the synthesis of various metal NPs have been widely used in recent days [62 – 65]. However, most of the surfactants that are used were chemical surfactants, which are toxic, economically nonviable and pollute the environment. Therefore, researchers have started looking into the synthesis of metal NPs by using microbial biosurfactants as the capping agent for better aggregation and stabilisation [66]. It is worth mentioning that, microbial biosurfactants are the surface active glycolipids or lipopeptides secreted by various microorganisms, the potential of which has also been explored in recent times for the synthesis of NPs [45]. They are amphiphilic compounds with both hydrophobic and hydrophilic moieties. They are produced by a variety of microorganisms including bacteria, fungi, yeasts *etc* [67]. They are intra or extracellular product of microbial cells containing hydrophilic moiety comprising carbohydrate, amino acid, cyclic peptide, phosphate, carboxylic acid or an alcohol and the hydrophobic moiety is mostly either, a long-chain fatty acid, hydroxyl fatty acid or α -alkyl β -hydroxy fatty acid [68]. Moreover, microbial production of biosurfactant is easy, commercially viable, environmental benign and also do not possess threat of hampering the biodiversity due to large scale production. It is also important to mention that petroleum contaminated soil and water serves as a rich source of biosurfactant producing microorganisms as microbes produce biosurfactant to reduce the surface tension of petroleum oil leading its further degradation inside the cell [69].

Aim and scope of the present study

Food security and health management are the most important issues of global concern. Rice, maize and wheat are considered as the most important and revenue generating crops worldwide which feed the world since time immemorial (**Fig. 1.1**) [70, 74]. Out of these, rice production is of national and regional importance. Moreover, production of some pulses such as chickpea (*Cicer arietinum*) and mung bean (*Vigna radiata*) are of great importance in both regional as well as national level [71 – 73].

The estimated global market size of rice was valued at USD 287.45 billion in 2021 [75]. In Southeast Asian countries, the revenue in the rice segment amounts to USD 77.09 billion in 2023 [76]. It is estimated that India will generate US\$ 93.10 billion by the end of the year 2023 with per person revenue of USD 65.58 [77]. Additionally, chickpea (*Cicer arietinum*) is one of the highest produced and revenue generating pulses in India with a global market size of 19.6 million tons in 2022 [72]. Therefore, developing smart agricultural techniques using bio-inspired nanotechnology to increase the production of these globally important cash crops which can help in reducing the food crisis is the need of the hour. On the other hand, plant pathogens such as *Pseudomonas syringae pathovars*, *Ralstonia solanacearum*, *Agrobacterium tumefaciens*, *Xanthomonas oryzae pv. oryzae*, *Xanthomonas campestris pathovars*, *Xanthomonas axonopodis pathovars*, *Erwinia amylovora*, *Xylella fastidiosa*, *Dickeya dadantii*, *Dickeya solani*, *Pectobacterium carotovorum*, and *Pectobacterium atrosepticum etc.* are commonly known for causing diseases in various economically important plants [78]. Even though, metal NPs are known for their antimicrobial activities, but their potential against plant bacterial pathogens are not adequately studied [79 – 82].

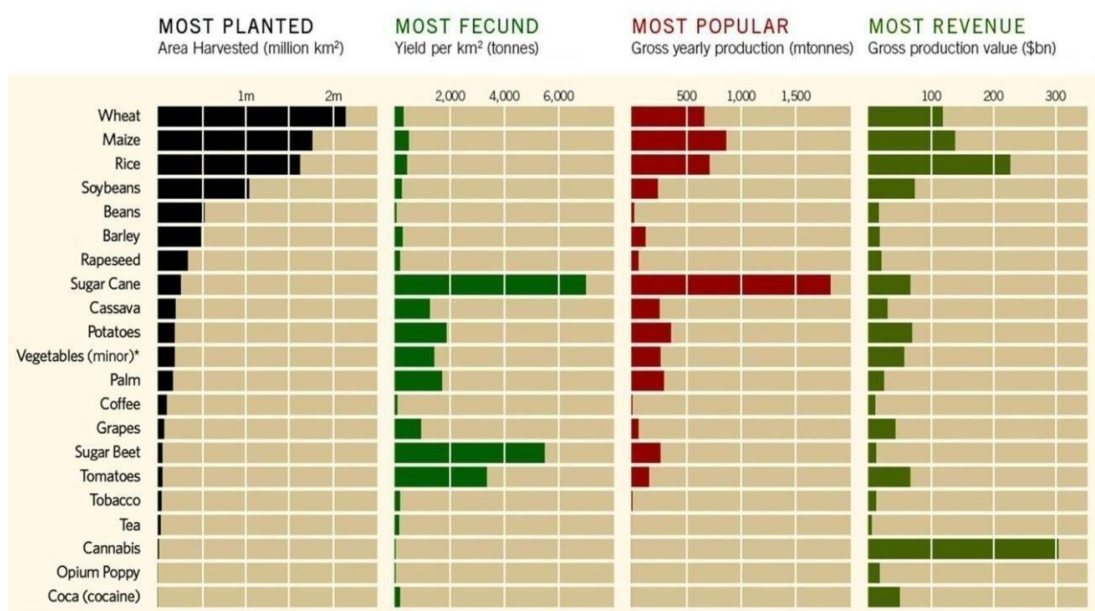


Fig. 1.1 Statistics shows commercial status of most money making crops in the world [70]

Northeast India is one of the major biodiversity hotspots in the world with numerous unexplored microbes. Hence, we propose to isolate biosurfactant-producing microbes from oil-logged soil samples from various locations in Guwahati, Assam. The biosurfactant produced by the best isolate will be used to reduce silver and zinc salts into Ag and ZnO NPs. It will be further extended to study the potential of Ag and ZnO NPs to enhance the germination rate of *Oryza sativa* and *Cicer arietinum* seeds. The study has been further extended to evaluate their antimicrobial activity against plant pathogens, cytotoxicity, and environmental toxicity assay.

Research objectives

The research objectives of the proposed study are:

1. Isolation and screening of biosurfactant producing microorganisms from petroleum contaminated soil samples.
2. Production, partial purification and characterization of biosurfactant from the best isolate.
3. Biosurfactant mediated synthesis of Ag and ZnO NPs and their extensive characterization.

4. Nano-priming of selected crop seeds and their in-vitro germination/growth assessment.
5. Antimicrobial and toxicity assessment of the nanoparticles.

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