Nanoinformatics: Enhancing Crop Production with Application of Nanoparticles in Agriculture

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Abstract. Introduction: Nanotechnology has received a lot of attention in recent years because of its wide range of applications in all scientific fields. Agriculture could benefit from the use of nanoparticles. In today's world, sustainable agriculture is essential. Smartly designed nanoparticles, such as nanozeolites, nanoclays, and nanofertilizers, can be used to improve soil. Green synthesis of nanoparticles from various organic matter is a much more environmentally friendly method of producing nanoparticles.

Methodology: *Pleurotus* ostreatus is a common edible and medicinal mushroom species due to its ability to produce a wide range of effective compounds. The oyster mushroom ranks first among macrofungi in the production and application of metallic nanoparticles. *Pleurotus ostreatus* is used to produce nanoparticles of magnesium, nitrogen, silver, molybdenum, copper, and nickel. In addition, the nanoparticles interact with Chitosan. Chitosan and its derivative nanoparticles have many biomedical applications, including drug delivery, vaccine delivery, antibacterial agents, and wound healing. This mixture interacts with biofertilizers such as *Glucanoacetobacter dioazotrophicus (5KOH), Frankia (2RKO), and Clostridium pasteurianium (1CP2).*

Result: The synthesis of nanoparticles was confirmed by a significant colour change after a 15-day incubation period at room temperature in each of the metal nano solutions. When the Nano complex was docked with the selected biofertilizers, the In-silco studies of nanoinformatics revealed a good molecular docking score. Based on a successful interaction with a high docking score, it is possible to conclude that nanoparticles can improve biofertilizers in crop production.

Keywords: *Pleurotus ostreatus* · Nanoinformatics · Biofertilizers · Agriculture · Nanoparticles

1 Introduction

Pleurotus ostreatus (P. ostreatus), also known as oyster mushroom, is one of the most widely cultivated edible mushrooms in the world, second only to white button mushroom (*Agaricus bisporus, A. bisporus*) [1]. It could also grow on decomposing organic matter.

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Depending on the species, the fruit bodies of this mushroom are shell or spatula shaped and come in white, cream, grey, yellow, pink, or light brown. The common name "oyster mushroom" refers to the fruiting body's white shell-like appearance [2]. The primary function of oyster mushroom fruiting bodies is to absorb amino acids, proteins, vitamin B (niacin, thiamine, and riboflavin), vitamin D, carbohydrates, and mineral salts (iron, calcium, and phosphorus) [3, 4].

They are an excellent addition to any meat-free diet. They may also lower the risk of developing serious health problems such as Alzheimer's, heart disease, cancer, and diabetes [6]. *P. ostreatus* contains a variety of bioactive compounds, including -glucans, which may improve cardiometabolic health [5]. It contains the majority of the mineral salts needed by the human body. Oyster mushrooms contain folic acid, which aids in the treatment of anaemia. *P. ostreatus* contains more folacine, vitamin B1, and vitamin B3 but less vitamin B12. They're also high in selenium, copper, thiamin, magnesium, and phosphorus. They also have significant antifungal, anti-inflammatory, antibacterial, and immunomodulatory properties [7]. *Pleurotus ostreatus* attacks and kills nematodes and bacteria with impunity [8, 9] (Fig. 1).

Chitin and chitosan are natural biopolymers found in crustacean shells, insect and mollusk exoskeletons, and fungi cell walls. Chitosan is a linear polysaccharide that is easily derived by alkaline N-deacetylation of chitin. It is composed of randomly distributed -(14)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit) [10]. The percentage of the acetyl group in the chemical structure of chitin and chitosan differs significantly. If the percentage of N-acetylglucosamine is greater than 50%, the material is chitin; if it is less than 50%, the material is chitosan [11]. Chitosan improves plant growth, development, and defence induction when used in a controlled environment. It also protects plants from pathogenic fungi. Chitosan has been reported to have a positive effect on rhizobacteria growth [12], where Agriculture is currently facing a number of challenges, including climate change unpredictability, soil contamination from various harmful environmental pollutants such as fertilisers and pesticides, and significantly rising food demand as the world population grows. The use of chemical fertilisers has resulted in significant soil fertility degradation, environmental pollution, pest resistance, biodiversity loss, and economic losses. As a result, agricultural

Fig. 1. Oyester mushroom grown using straw as substrate

researchers and scientists are focusing on a more secure and productive method of fertilisation [13].

Biofertilizers are microorganism-containing substances that, when applied to soil, increase crop yield and promote plant growth [15]. Fertilizers increase soil fertility directly by adding nutrients, whereas biofertilizers add nutrients naturally by fixing atmospheric nitrogen (N), solubilizing phosphorus (P), and stimulating plant growth through the synthesis of various growth promoting substances [16]. Thus, the use of biofertilizer is required primarily for two reasons. First, increased fertiliser use leads to increased crop productivity; second, increased chemical fertiliser use causes soil texture damage and raises other environmental concerns [14].

Nanoparticles have the potential to be used in agriculture. Smartly designed nanoparticles can be used to improve soil in the form of nanozeolites, nanoclays, and nanofertilizers [19]. A nanoparticle is a small particle with a diameter of 1 to 100 nm. Nanoparticles, which are invisible to the naked eye, can have significantly different physical and chemical properties than larger material counterparts [20]. With the potential properties of nanomaterial and biofertilizer, nano-biotechnology aids in the development of novel, low-cost, eco-friendly nano-biofertilizes [18]. The use of nanotechnology in agriculture has grown in recent years, and it is a valuable tool for achieving the global goal of sustainable food production [17]. Many different types of nanomaterials have been used to develop bioactive compound delivery strategies with the goal of increasing crop production and crop protection [21].

Omics-association studies

A massive amount of scientific data has been generated over the last several decades, particularly in the fields of molecular biology and genomics. Bioinformatics, a multidisciplinary field that includes everything from physics, mathematics, and behavioural sciences to engineering biology, statistics, and computer science, has been critical in determining how data from biological experiments is used [23]. Bioinformatics is important for the development of the agricultural sector, agro-based industries, agricultural by-product utilisation, and. It is expected that in the next decade, we will make another giant leap in the field of bioinformatics, with computational models of system-wide properties serving as the foundation for experimentation and discovery [22].

Bioinformatics in Agriculture

Bioinformatics is becoming increasingly important in the collection, storage, and analysis of genomic data in the field of agricultural genomics, or agri-genomics [24]. Some of the various applications of bioinformatics tools and methods in agriculture, known collectively as agri-informatics, include improving plant resistance to both biotic and abiotic stressors and improving nutritional quality in depleted soils. Aside from these goals, gene discovery via computer software has enabled researchers to develop targeted methods for improving seed quality, incorporate added micronutrients into plants for improved human health, and engineer plants with phytoremediation capabilities [25].

2 Material and Methods

Materials. White species of *Pleurotus ostreatus,* spawns were collected from the local market from Kengeri, Bangalore, dried straw was sterilized to use as a substrate for growth of the mushroom. Additionally 150 ml and 250 ml conical flasks, jars with lid, motor and pestle were needed (Fig. 2).

Omics Tools

- **NCBI-** The National Center for Biotechnology Information advances science and health by providing access to biomedical and genomic information.
- **PubChem-** a public repository for information on chemical substances and their biological activities.
- **PDB file-** the PDB archive is a repository of atomic coordinates and other information describing proteins and other important biological macromolecules.
- **RCSB PDB-** Researchers, educators, and students use RCSB PDB resources to study the shape and interactions of biological molecules and their implications in molecular biology, medicine, biotechnology, and beyond.

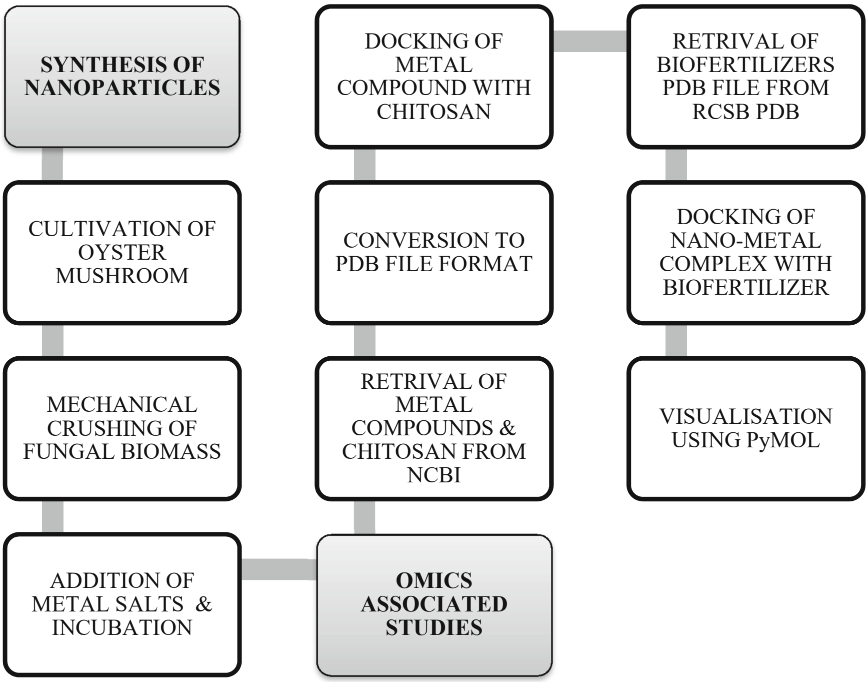


Fig. 2. Workflow of Nanoinformatics

- **PyMOL-** a cross-platform molecular graphic tool and has been widely used for 3D visualization of macromolecules.

We selected Oyster mushroom i.e. *Pleurotus ostreatus*, it was then cultivated using mycelium and grown in pre-fumigated dark room which took a growth period of 21days. The metals such as copper, silver, magnesium, iron, molybdenum, nitrogen and nickel were chosen as they were naturally present in the mushroom, these were further processed into metal solutions. The cultivated mushrooms were mechanically crushed to get a mushroom extract, which was then followed by a titration method, with each metal solution. The colour change was observed and then these solutions were kept for incubation for 15 days to observe intense change in colour. After incubation the solution was carried out was a centrifugation process, the pellet was collected and the dry weught was measured accordingly. Omics association studies were carried out using different computerized softwares. The SMILES of the metal compounds to be used as nanoparticles were retrieved from PubChem which was converted to PDB format using CACTUS file converter.

CANONICAL SMILES

- Magnesium [Mg]
- Nitrogen N#N
- Silver [Ag]
- Molybdenum [Mo]
- Copper [Cu]
- Nickel [Ni]

Chitosan FASTA sequence was obtained from NCBI, and PDB file was downloaded for the same using SWISS Model server. Chitosan - COC(=O)NC1C(C(C(OC1OC2C(OC(C(C2O)N)OC3C(OC(C(C3O)N)O)CO)CO)CO)OC4C(C(C(C(O4)CO)OC5C(C(C(C(O5)CO)OC6C(C(C(C(O6)CO)OC7C(C(C(C(O7)CO)OC8C(C(C(C(O8)CO)OC9C(C(C(C(O9)CO)O)O)N)O)N)O)N)O)N)O)N)O)N)O)N)O.

The Chitosan PDB was docked using Patchdock & HEX software consequently with each metal-PDBs, the docked nanocomplex was then docked with three different biofertilizers, i.e. 2RKO Frankia, 5KOH *Glucanoacetobacter diazotrophicus* & 1CP2 *Clostridium pasteururanium* which were retrieved from RCSB-PBB server. Docking score was observed for nanocomplex of Chitosan-metal with biofertilizer. The docked structures were then visualized using PyMOL software.

3 Result and Discussion

Synthesis of nanoparticles is confirmed by the colour change in each metal nano solutions, after the incubation at RT for 15 days the intense colour change is occurred for copper, magnesium and silver are pale green, pale white and pale brown respectively. The solution were carried out for centrifugation, pellet is measured of each metal copper, magnesium and silver was 2.81 g, 2.53 g and 0.20 g respectively. The difference in the weight is observed can be the aggregates of mushroom metal compounds.

The in-silico association studies of nanoinformatics has shown a good molecular docking scores of nanometal complex with 3 different biofertilizers, 2RKO, 5KOH and 1CP2 with nitrogen metal complex are 8016,10308 and 9530 respectively.

The future studies of antimicrobial activity of this nanoparticles can be performed by the *in-vitro* and *in-vivo* studies (Figs. 3, 4, 5, 6, 7, 8 and Tables 1, 2, 3, 4, 5, 6).

Table 1. Burette Reading - Before incubation:

METALS	COLOUR CHANGE	BURETTE READING
Cu(copper)	Pale light blue - intense sky blue	25 ml
Mg(magnesium)	Transparent - off-white	25 ml
Ag(Silver)	Pale dark blue – turbid pale white	9 ml

Table 2. Colour change after incubation of 48 h:

METAL	COLOUR CHANGE
Cu(copper)	Pale green
Mg(magnesium)	Pale white
Ag(silver)	Pale brown/golden
Diluted mushroom extract	Light yellow
Crude mushroom extract	Brown

Table 3. Dry weight of Metals

METALS	DRYWEIGHT
Copper	2.81 g
Magnesium	2.53 g
Silver	0.20 g

Fig. 3. Nanoparticle solution before the colour change

Fig. 4. Nanoparticle solutions after the colour change

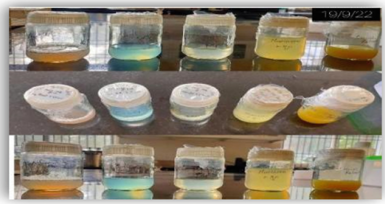


Fig. 5. Freshly prepared sample

Fig. 6. Samples of $CuSo_4$, $AgNO_3$ & $MgSo_4$ after incubation of 48h

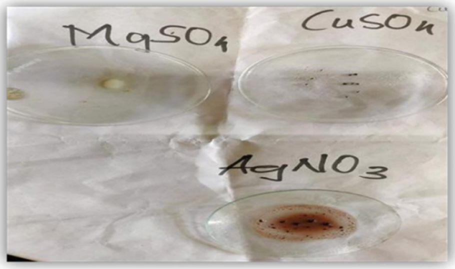


Fig. 7. Dry weight of $MgSO_4$, $CuSO_4$ & $AgNO_3$

Table 4. MolProbity Results

MolProbity Score	2.41	
Clash Score	12.97	(A220 HIS-A220 HIS), (A226 LEU-A231 ILE), (A242 ILE-A248 VAL)
Ramachandran Favoured	86.96%	
Ramachandran Outliers	3.86%	A220 HIS, A167 ASP, A171 PRO, A275 PRO, A274
		THR, A221 PRO, A210 GLY, A286 THR
Rotamer Outliers	1.64%	A231 ILE, A286 THR, A248 VAL
C-Beta Deviations	8	A217 THR, A285 SER, A296 HIS, A321 LYS, A170 SER, A167 ASP, A198 SER, A286 THR
Bad Bonds	1/1684	A283 ASP
Bad Angles	46/2304	A323 ASP, (A170 SERA171 PRO), A296 HIS, A166 ASP, A231 ILE, (A220 HIS-A221 PRO), A170 SER, (A282 THR- A283 ASP), (A353 GLY- A354 THR), (A168 GLYA169 PRO), A286 THR, A217 THR, A220 HIS, (A285 SER-A286 THR), A157 ASP, (A173 THR- A174 PRO), (A319 ALA- A320 PRO), (A274 THRA275 PRO), A283 ASP, (A216 HIS-A217 THR), A248 VAL, (A166 ASP- A167 ASP), (A330 GLUA331 HIS), A321 LYS, A285 SER, A331 HIS, (A286 THR-A287 THR), (A283 ASP-A284 GLY), (A343 GLY-A344 TYR), A226 LEU, A198 SER, (A346 LEU-A347 PRO),

(continued)

Table 4. (*continued*)

MolProbity Score	2.41	
		A214 SER, A171 PRO, A216 HIS
Cis Non-Proline	2 / 197	(A330 GLU-A331 HIS), (A353 GLY-A354 THR)

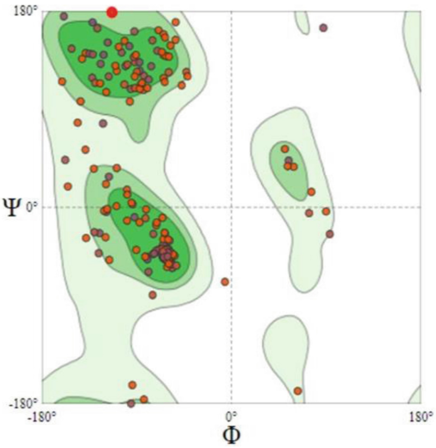


Figure 8. Ramachandran Plots

Table 5. Calculation of Molecular properties and Bioactivity Score

miLogP	TPSA	natoms	MW	nON	nOHNH	nrotb	volume	nviolations
[Mg]								
-0.71	0	1	24.30	0	0	0	40.59	0
[N]								
0.02	47.58	2	28.01	2	0	0	24.21	0
[Ni]								
-0.71	0	1	58.69	0	0	0	40.59	0
[Cu]								
-1.11	0	1	63.55	0	0	0	40.59	0
[Ag]								
-0.71	0	1	107.87	0	0	0	40.59	0
[Mo]								
-0.71	0	1	95.94	0	0	0	40.59	0

Table 6. Association Studies of Chitosan-Nanoparticle Complex with Biofertilizers -Magnesium, Nitrogen, Copper, Nickel, Silver and Molybdenum

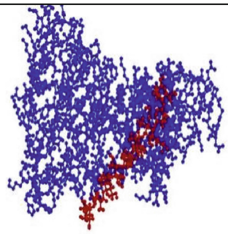
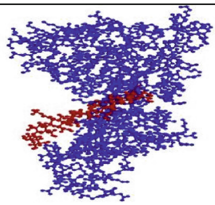
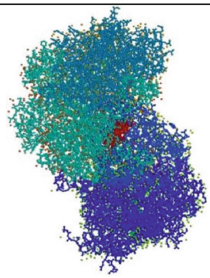
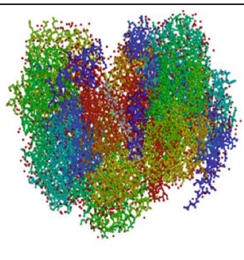
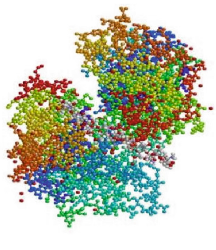
Sr. No	Protein(from RCSB-PDB)	Nano-complex	Docking score (in – kcal/mol)	Docked image
1.	2RKO	Chitosan-nitrogen	8016	
2.	2RKO	Chitosan-magnesium	7696	
3.	5KOH1	Chitosan-magnesium	10308	
4.	5KOH1	Chitosan-Nitrogen	10092	
5.	1CP21	Chitosan-magnesium	9352	

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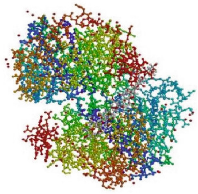
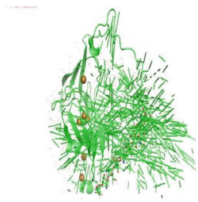
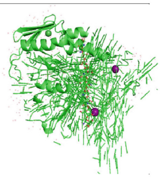
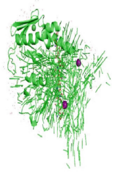
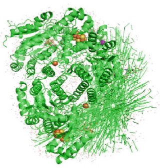
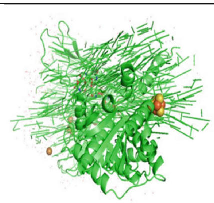
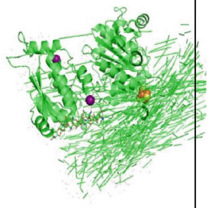
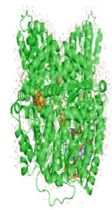
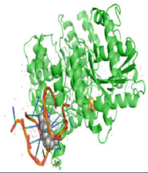
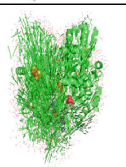
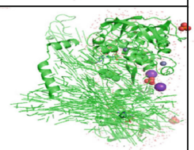
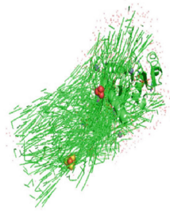
Table 6. (*continued*)

6.	1CP21	Chitosan-nitrogen	9530	
7.	2RKO (Frankia)	Chitosan-Copper	-8.058	
8.	2RKO	Chitosan-Nickel	-7.879	
9.	5KOH	Chitosan-Copper	0.000	
10.	5KOH	Chitosan-Nickel	0.000	
11.	1CP2	Chitosan-Copper	-7.771	

(*continued*)

Table 6. (*continued*)

12.	1CP2	Chitosan-Nickel	-7.986	
13.	5KOH	Chitosan-Silver	-6.436	
14.	2RKO	Chitosan-Silver	-6.777	
15.	1CP2	Chitosan-Silver	-6.093	
16.	5KOH	Chitosan-Molybdenum	0.000	
17.	2RKO	Chitosan-Molybdenum	-8.218	
18.	1CP2	Chitosan-Molybdenum	-7.785	

4 Conclusion

As per the invitro studied of metal nanoparticles by the means of Pleurotus ostreatus has bean synthesized, this nanoparticles can b used in agricultural fields to improve the efficiency of biofertilizers as per omics association studies it is seen that chitosan-nanoparticle complex has profound association with the biofertilizers with a good docking score. Hence this can be concluded that chitosan-nanoparticle improves the biofertilizers' efficiency.

In future aspects this nanoparticles can be used as a Nanobio-fertilizer in agricultural practises which can improve the plant growth and also helps in various antimicrobial property as well as soil fertility with less quantity of Nanobio-fertilizer in the field.

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