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COMPARATIVE EVALUATION OF NON TARGET TOXIC EFFECT OF FREE AND POLYMER COATED CHEMOGENIC METALLIC NANOPARTICLES AGAINST *VIGNA MUNGO*

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ABSTRACT

In the present study,an attempt has been made to evaluate phytotoxicity of free and chitosan coated silver and copper nanoparticles against Vigna mungo under microplot condition and the toxic effect on soil microbial status and microbial population has been studied.Respective nanoparticles were synthesized by chemical reduction of respective metal salt precursor with reducing agents and polymer coated nanoparticles were by chemical reduction of respective metal salt precursor with reducing agent and further coating with chitosan.Synthesized nanoparticles were characterized by UV visible spectrophotometry,scanning electron microscopy,fourier transform infrared spectroscopy and energy dispersive x ray spectroscopy. Phyototoxicity of respective nanoparticles against Vigna mungo was studied under microplot condition. Effect of respective nanoparticles on the plant growth parameters such as shoot length, total foliage density, total number of new branches emerged and foliage surface area were studied. Among the nanoparticles treatment, drastic reduction on the all tested plant growth parameters including soil microbial population was recorded in free copper and silver nanoparticles treatment. But, soil enzyme activity was not affected in all the nanoparticles treatment.

KEY WORDS: **phytotoxicity, nanoparticles, Vigna mungo, chitosan, plant growth parameters**

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INTRODUCTION

The application of nanoscale materials and structures, usually ranging from 1 to 100 nanometers (nm), is an emerging area of nanoscience and nanotechnology. Nanomaterials may provide solutions to technological and environmental challenges in the areas of solar energy conversion, catalysis, medicine, and water treatment[1]. Nanotechnology is a new, fast-developing industry, posing substantial impacts on economy, society and environment that likely will produce a huge number of new materials during the coming decades. Nanotechnology is estimated to far exceed the impact of the industrial revolution and is projected to become a $1 trillion market by 2015 and employ about 2 million workers[2] and Currently, more than 475 nanotechnology products, including tennis rackets, pants, and precision instruments, are available in the U.S. market. Thus, it generates both positive and negative responses from governments, scientists and social media throughout the world[3]. Particles in such a size (<100 nm) fall in the transitional zone between individual atoms or molecules and the corresponding bulk material, which can modify the physicochemical properties of the material (e.g., performing exceptional feats of conductivity, reactivity, and optical sensitivity) Therefore, such materials can generate adverse biological effects in living cells[4.] Increasing numbers of commercial products, from cosmetics to medicine, incorporate manufactured nanomaterials (MNMs) that can be accidentally or incidentally released to the environment[5]. The term “nano (eco-) toxicology” has been developed as a separate scientific discipline with the purpose of generating data and knowledge about nanomaterials effects on humans and the environment[6.] Introduction of nanoparticles into the environment might have significant impacts as they may be extremely resistant to degradation and have the potential to accumulate in bodies of water or in soil. However, nanoparticles can act on living cells at the nano level resulting in biologically desirable effects. Recently, nanomaterials such as nanotubes, nanowires, fullerene derivatives and quantum dots have received enormous attention in the creation of new types of analytical tools for biotechnology and the life sciences[7]. Synthesis of noble metal nanoparticles for applications such as catalysis, electronics, optics, environmental, and biotechnology is an area of constant interest. Gold, silver, and copper have been used mostly for the synthesis of stable dispersions of nanoparticles, which are useful in areas such as photography, catalysis, biological labeling, photonics, optoelectronics and surface-enhanced Raman scattering (SERS) detection[8, 9]. There is now a wider debate about the risks and benefits of the many manufactured NMs and consumer products and this includes consideration of risks to the environment[10]. The unique properties of ENPs, such as high specific surface area, abundant reactive sites on the surface as a consequence of a large fraction of atoms located on the exterior rather than in the interior of nanoparticles as well as their mobility, could potentially lead to unexpected health or environmental hazards[11, 12]. Therefore, organisms, and especially those that interact strongly with their immediate environment such as algae, plants, and fungi, are expected to be affected as a result of their exposure to ENPs. Developmental phytotoxicity of nanoparticles is a critical knowledge gap because nanoparticles entering wastewater streams may predominantly be incorporated into sewage sludge and applied to agricultural fields[13]. As compared with algae and fungi, plants might also be exposed to NPs in atmospheric and terrestrial environments[14]. Airborne NPs will be attached to leaves and other aerial parts of plants whereas roots will interact with waterborne or soil-material-associated NPs. Therefore, one can expect that plant communities with higher leaf area indexes (LAI) will also have a higher interception potential for airborne ENPs, thus increasing their entrance into trophic webs. For example, typical LAIs of spruce (Picea abies) forests in Southern Germany ranged between 5.3 and 7.9, and the total leaf area of single trees reached up to 750m^2 [15]. In the present study, comparative phytotoxic effect of free and polymer coated silver and copper nanoparticles against *Vigna mungo* was studied under microplot condition.

MATERIALS AND METHODS

Synthesis of nanoparticles

Synthesis and **c**haracterization of both free and chitosan coated silver and copper nanoparticles were carried out by the reduction of respective metal precursor with reducing agent followed by chitosan[16]. Synthesized nanoparticles were purified by successive centrifugation by 10,000 rpm and the collected pellets were washed thrice with deionized water, the washed suspension thus obtained was freeze dried.

Phytotoxicity studies

Collection of seeds and nanoparticles treatment

Healthy seeds of *Vigna mungo* were obtained from Agriculture Department, Thiruvallur in sterile polythene bags and the collected seeds were surface sterilized with 0.1% mercuric chloride solution followed by three successive washings in sterile distilled water. Washed seeds were immersed in the respective reconstituted nanoparticle suspension for 1 hour. Reconstitution was carried out by dissolving the respective lyophilized nanoparticles in deionized water with the final concentration of 0.1mg/ml. Treated seeds were allowed to shade dry and used for microplot assay.

Microplot assay

The trail was carried out with five treatments such as control, free silver nanoparticles, (T1) , free copper nanoparticles ,(T2) , chitosan coated silver (T3) and chitosan coated copper nanoparticles (T4) having three replication each. The plot size of 5 x 3 m leaving a gangway of one meter all around the plot has been maintained for this experiment. Respective nanoparticles treated seeds was sown at a spacing of 30 x 10 cm. All the crop management practice was followed except the pesticide application. Water was sprinkled regularly.Seedlings emergence was recorded regularly. After the 20 days after seedlings emergence (DASE), the plant growth parameters mainly shoot length, total new branches emerged, leaf surface area, total foliage density, chlorophyll content and leaf surface microflora was determined at 20 ,40,60 and 80 DASE. Effect of nanoparticles on soil microbial status and soil enzyme activity was also studied from the soil samples collected from the microplot

Effect of nanoparticles on soil parameters

Effect on soil heterotrophic microbial population

Soil dilution method was used to determine the microbial status[17]. One gram aliquots of soil (sample from 10 different sites were pooled) in each nanoparticles treated plot collected from different sites were suspended for 1 hour at 100 rpm in 250 ml of conical flask containing 100 ml of sterile distilled water. The resulting suspension was serially diluted. 0.1ml aliquot of the sample was spread plated on nutrient agar and potato dextrose agar. The seeded plates were incubated at 37ºC (bacteria) for 24 hrs, fungi 28ºC for 5 days. The colony count was recorded after the incubation period.

Effect on soil enzyme activity

Preparation of soil extract

1 gram fresh weight of soil collected from respective treatment was homogenized in 50 ml of 50mM sodium acetate buffer, pH 5.0 and the homogenate was used as the source of the enzyme [18].

Enzyme assay

Extracellular enzyme activity was measured using assay techniques[19]. Soil α-amylase activity was measured by 3, 5-dinitro salicylic acid colorimetry using soluble starch as the substrate. The amount of maltose released over 24 hrs was assayed colorimetrically at 508 nm and expressed as µmol maltose g^{-1} dry sample. Soil saccharase (EC 3.2.1.26; SAC) activity was measured by 3, 5-dinitro salicylic acid colorimetry with sucrose as the substrate. The amount of 3-amino-5-nitro-salicylic-acid released over 24 hrs was assayed colorimetrically at 508 nm and expressed as µmol glucose g^{-1} dry sample. Soil cellulase activity was measured by nitrosalicylic acid colorimetry.The amount of glucose released over 72 hrs was assayed colorimetrically. Soil alkaline phosphatise activity was measured by disodium phenyl phosphate colorimetry. The amount of phenol released over 24 hrs was assayed colorimetrically at 660 nm and expressed as µmol phenol g^{-1} dry sample. All

determinations of enzymatic activities were performed in triplicates.

Table 1
Effect of nanoparticles on seedling emergence of V.mungo

S.No	Treatment	Seedlings emergence (%)
1	Control	100.0
2	Free silver nanoparticles (T1)	51.0
3	Free copper nanoparticles (T2)	57.0
4	Chitosan coated silver nanoparticles (T3)	76.0
5	Chitosan coated copper nanoparticles(T4)	81.0

Treatment	Plant growth parameters	Days after treatment				
		0	20	40	60	80
Control	Shoot length(cm)	12.0	16.5	21.4	26.0	30.1
F-AgNps		12.0	13.5	14.0	17.0	20.0
F-CuNps		12.0	15.0	16.0	18.0	21.0
CS-AgNps		12.0	17.0	21.0	26.0	30.0
CS-CuNps		12.0	17.0	20.7	26.0	30.0
Control	New branches emerged/plant	1.0	2.5	3.0	3.0	3.0
F-AgNps		1.0	1.0	1.0	1.5	1.7
F-CuNps		1.0	0.7	1.0	1.0	1.3
CS-AgNps		1.0	2.0	2.0	2.3	2.3
CS-CuNps		1.0	2.0	2.0	2.0	2.0
Control	Foliage density/plant	13.0	26.0	36.0	41.0	49.0
F-AgNps		13.0	17.5	23.0	30.0	36.0
F-CuNps		13.0	16.6	21.6	31.2	37.4
CS-AgNps		13.0	25.0	35.0	41.5	50.0
CS-CuNps		13.0	24.7	34.2	40.3	49.2
Control	leaf surface area (cm)	5.0	6.0	6.4	6.7	7.1
F-AgNps		5.0	5.3	5.7	6.0	6.1
F-CuNps		5.0	5.2	5.5	6.0	6.0
CS-AgNps		5.0	6.3	6.4	6.7	7.0
CS-CuNps		5.0	6.0	6.4	6.5	6.9

Table 3
Effect of nanoparticles on soil heterotrophic microbial population (CFU/g)

S.No	Microorganism	Treatment	Colony count (CFU/g) 0th day	80 Th Day
1	Bacteria	Control	$14.4X10^6$	$10.5X\ 10^7$
2		F-Ag Nps	$12.5X\ 10^6$	$10.0X10^3$
3		F-CuNps	$13.0\ X\ 10^6$	$10.3X\ 10^5$
4		CS-AgNps	$11X10^6$	$75X10^6$
5		CS-CuNps	$27X10^6$	$3X10^6$
6	Mold	Control	$46X10^4$	$12X10^6$
7		F-AgNps	$6.9X10^4$	$11.0X10^3$
8		F-CuNp	$7.0X\ 10^4$	$10.9X\ 10^2$
9		CS-AgNps	$54X10^4$	$35X10^4$
10		CS-CuNps	$42X10^4$	$31X10^4$
11	Yeast	Control	$7.5X\ 10^3$	$10.7X\ 10^5$
12		F-AgNps	$6.9X\ 10^3$	$11.0X\ 10^3$
13		F-CuNp	$7.0X\ 10^3$	$10.9X\ 10^2$
14		CS-AgNps	$52X10^3$	$32X10^3$
15		CS-CuNps	$41X10^3$	$31X10^3$

Table 4
Effect of nanoparticles on soil enzyme activity (µ/ml)

S.No	Treatment	Enzyme activity (µ /ml) 0th Day		80th Day	
		AP	U	AP	U
1	Control	5.1	2.5	7.7	3.3
2	F-Ag Nps	5.7	2.8	6.0	3.5
3	F-CuNps	5.2	2.4	6.7	3.4
4	CS-AgNps	5.4	2.4	6.0	3.0
5	CS-CuNps	5.5	2.5	6.0	3.6

AP- alkaline phosphatase, U- urease

RESULTS AND DISCUSSION

Both the free and chotosan coated silver and copper nanoparticles were synthesized and characterized as described earlier[16]. Synthesized nanoparticles were the phytotoxicity of NPs was evaluated by the seed germination technique. The germination index has been extensively used as an indicator of phytotoxicity in soils[20]. In the present study, drastic reduction in seed germination was recorded in both the free nanoparticles treatment. 51.0 and 57.0 % of seed germination was recorded in free silver and copper nanoparticles treatment respectively. Polymer coated silver and copper nanoparticles revealed 76.0 and 81.0 % of seed germination (Table 1). Similar report of biogenic silver nanoparticles effect on seedling emergence and growth parameters of economic important plants [21]. As in seed germination, plant growth parameters of V.mungo were affected in free nanoparticles treatment. The shoot length, leaf surface area , number of new branches emerged of the test plant were measured at periodic time intervals.It was observed that all the tested plant growth parameters of *V.mungo* was reduced continuously from the day of seedling emergence (Table 2). But there was no effect on growth parameters was observed in polymer coated silver and copper

nanoparticles treatment.As in control, all the growth parameters was increased at the periodic time intervals in polymer coated both the nanoparticles treatment and also studied phytotoxicity of biogenic silver nnaoparticles on economic important plants and the nanoparticles mediated phytotoxicity was not observed in all the tested plants21. Soil microbial population in free nanoparticles treatment showed distinct effect (Table 3). Free copper nnaoparticles treatment showed maximum effect on fungi. Bacterial population was reduced in silver nanoparticles treatment.However,soil enzyme activity and soil macronutrients was not reduced in both free and polymer coated nanoparticles (Table 3) Similar observation could be observed in soil macronutrients.(Table 4). Eco toxic effect of various nanoparticles and nanodevices such as photoactive ZnO or TiO2 [22], bactericide Ag [23], hydrophobic Carbon nanotubes [24] and fullerenes [25] or Cadmium oxide particles [26, 27] Concern over the potentially harmful effects of nanoparticles has stimulated the advent of nanotoxicology as a unique and significant research discipline. However, the majority of the nanotoxicology research has focused on mammalian cytotoxicity or impacts to animals and bacteria, and only a few studies have considered the toxicity of nanoparticles to plants. Developmental phytotoxicity of nanomaterials is a critical knowledge gap because nanoparticles entering wastewater streams may predominantly be incorporated intosewage sludge and applied to agricultural fields. In the present study, the effect of free and chitosan coated silver,copper nanoparticles on the plant growth parameters of *V.mungo,* soil microbiota and soil enzymes has been carried out.The present study reveals both the free nanoparticles recorded distinct phytotoxicity,soil microbial status.Further study under field trail will useful to assess the toxicity of metallic nanoparticles to the environment and would suggest the possible utilization of nanoprinciples for human welfare without affecting the environment.

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