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Effect of Nanoparticles on Morphological and Biochemical Parameters of Black Gram Seeds (*Vigna Mungo*)

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Abstract : ***Plants are essential fundamental components of all ecosystems, and the interaction between NPs and plants is an indispensable aspect of risk assessment. Plants need to be included to develop a comprehensive toxicity profile for nanoparticles. Effects of three types of nanoparticles (silver, sulphur, and iron oxide) on seed germination and root growth of Vigna mungo plant were investigated on two concentration i.e 100ppm and 500ppm. Originally, this paper focuses on NP phytotoxicity, which is an important precondition to promote the application of nanotechnology and to avoid the potential ecological risks. NPs phytotoxicity is tested on both morphological (plant height, germination %, seedling vigour & plant biomass) and biochemical parameters (Total soluble sugar, starch and amylase activity). FeO NPs showed maximum stress in both the concentration followed by Ag NP's and S NP's.***

Keywords: Biochemical parameters, nanoparticles, phytotoxicity, Vigna mungo.

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Introduction :

Recent advances in nanotechnology have made a great impact on industries including manufacturing, biomedical applications, electronics/ telecommunications, agriculture and renewable energy, among others (Ma, White, et al. 2015). Nanoparticles (NPs) are broadly defined as particles having at least one dimension between 1 and 100 nm in diameter (Auffan et al. 2009). Because of their unique properties and novel features, NPs have been widely used in many aspects of daily life and energy production, including in catalysts, semiconductors, cosmetics, drug carriers and environmental energy (Nel et al. 2006). The large-scale and unrestricted use of NPs has led researchers to consider the problems, challenges and consequences of their environmental impact (Gottschalk et al. 2015). As primary producers, plants are key for any community to function as they are responsible for converting solar energy into organic matter that can be used by other trophic groups (McKee & Filser 2016) . Plants serve as a potential pathway for the transportation of NPs (Rico et al. 2011). Through the food chain, NPs can be accumulated in high trophic-level consumers (Zhu et al. 2008). Organisms in the ecosystem could suffer from oxidative stress induced by NPs (Hong et al. 2014). In recent years, research in this area has been focused on the interaction between plants and NPs, and the effects of NPs on ecology, the food chain and human health; evaluating the pros and cons of NPs requires interdisciplinary knowledge (Tolaymat et al. 2015).

The main plant physiological indices of the toxic effects of NPs are the germination percentage, root elongation, biomass and leaf number (Lee et al. 2010). NPs can have substantial negative effects, such as reduction in seed germination and suppression of plant elongation, and can even cause plant death. Several previous plant nanotoxicity studies have determined the inhibition of plant species such as soybean, maize, wheat (Triticum aestivum), ryegrass and barley by exposure to NPs (MWCNTs, single-wall carbon nanotubes, ZnO NPs, Ag NPs and Fe NPs); several aspects of plant growth were affected including seed germination, shoot length, biomass and gene expression (Dimkpa et al. 2012).

The aim of our research is to determine the effect of different nanoparticles (Ag, S, and FeO) on physiology and biochemistry of *Vigna mungo* (black gram).

Materials and Methods :

Collection of samples : Seeds of Black gram(*Vigna mungo*) was collected from the local market in Patna, Bihar. It is an erect, sub-erect or trailing, densely hairy, annual herb. The tap root produces a branched root system with smooth, rounded nodules. The plant grows 30-100cm with large hairy leaves and 4-6 cm seed pods. The urad bean along with the mung bean, originally placed in phaseolus, has since been transferred to *Vigna*.

Synthesis and characterization of nanoparticles: Slphur, silver and iron oxide nanoparticles were synthesized by chemical reduction method and characterization was done by using UV-VIS spectrophotometer.

Method of treatment: Phytotoxicity tests will be carried out in two stages of plant development:

(1) During germination, when the germination percentage is measured, where the seeds must be exposed to the test solution for the duration of germination, and

(2) During seedling growth, in which root/shoot elongation and dry weight are frequently used variables to assess the effects of plant exposure to harmful substances.

Biochemical parameters:

Estimation of starch : Starch is extracted from seeds of both treated and non-treated seedlings of *Vigna mungo*. Alcoholic extracts were prepared by the method of AOAC as described by Horwitz (1965). The residue obtained was used for starch determination. Starch was extracted and determined according to the method of Clegg (1956) by using perchloric acid and the absorbance was taken at 630nm.

Estimation of total soluble sugar : The soluble sugar is estimated by the method of Dey (1990) by using phenol and sulphuric acid and the absorbance was taken at 490nm of both treated and non-treated seeds of *Vigna mungo*. The amount of sugar was expressed as mg g-1 fw-1.

Estimation of amylase : Amylase was extracted from seedlings of treated and non-treated *Vigna mungo* by extracting 1 g of sample material with 5-10 volumes of ice-cold 10 mM calcium chloride solution for 3 h at room temperature and then centrifuging it at 5000rpm for 10min, the residue obtained was used as source of crude enzyme and the absorbance was taken at 56onm.

Result and Discussion :

Effect of different nanoparticles on growth and development of *Vigna mungo*:

Table 1. Morphological parameters of *Vigna mungo* after treatment with nanoparticles *Vigna mungo*

S. No.	Physical Parameters	Non-Treated	Treated					
		Control	S NP's		Ag NP's		Fe NP's	
1.	Concentration	100 ppm	100 ppm	500 ppm	100 ppm	500 ppm	100 ppm	500 ppm
1.	% of germination	100%	70%	50%	80%	60%	70%	80%
2.	Seedling vigour index	1220	875	430	824	576	574	1080
3.	Day of emergence of first leaf	3	6	8	4	7	4	8
4.	No. of leaves	16	4	2	6	4	4	6
5.	Colour of leaf	Light green	Dark green	Light green	Light green	Light green	Dark green	Light green
6.	Plant height (cm)	12.8	10.5	8.6	10.3	9.6	8.2	11.4
7.	Shoot length (cm)	10.2	8.2	7	8.2	7.8	6.8	9.5
8.	Root length (cm)	2.6	2.3	1.6	2.1	1.8	2.4	1.9
9.	Plant fresh weight (mg)	1148	1028	960	750	680	824	1038
10.	Plant dry weight (mg)	432	325	264	165	196	202	345

The present study (Table 1) revealed that the roots were sensitive to the increase in AgNPs , and S NP's concentrations. Morphologically, radicals of the germinated seeds were stunted with brown tips when they treated with high concentration of AgNPs. Correspondingly, Geisler-Lee et al. (2013) have demonstrated that AgNPs-treated seedlings had shorter roots and exhibited visible brown root tips as compared with control plants. As observed in this study, the higher sensitivity of roots to AgNPs might be due to the fact that the roots comes in direct contact with AgNPs in the exposure medium and also, the radicle was not adapted to the treatments (Yin et al.,2012).

FeO NP's shows more stress to plants at low concentration (100ppm) than at high concentration (500ppm). This may be due to the high accumulation of FeO NP's is at low concentration than in high concentration. A similar experiment reveals that nano iron oxide at the concentration of 0.75g/L (750ppm) increased leaf and pod dry weight.The highest grain yield was observed by using 0.5g/L (500ppm) nano ironoxide that showed 48% increase in grain yield in comparison with control (Sheykhbaglou et al., 2010).

Effect of nanoparticle on different biochemical parameters of *Vigna mungo*:

The extract of the seeds (***Vigna mungo***) was evaluated fort various parameters.

Estimation of total soluble sugar: The result obtained signifies that FeO NP's shows more stress to plant at both 100ppm and 500ppm followed by Ag NP's and then S Np's which shows least stress. This may be due to the accumulation of iron oxide nanoparticles with enzymes and hormones by inhibiting their functions. (Fig 1)

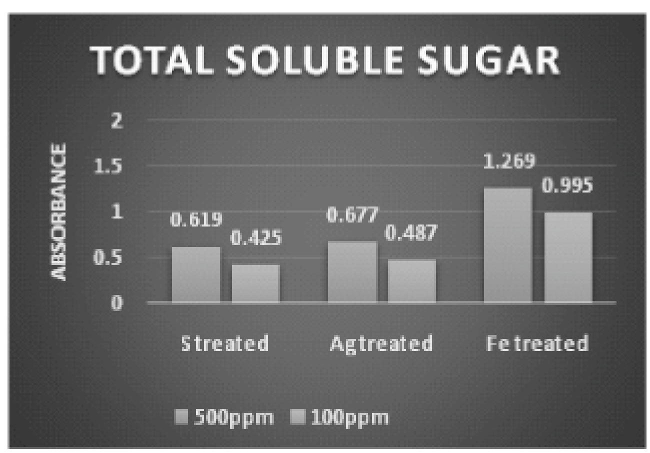


Fig. 1. Absorbance of TSS at 490nm of treated seeds

Our result obtained is not in agreement with Alidoust D et.al., 2013, who states that photosynthetic potential is enhanced by treatment with iron oxide nanoparticles. And in our result iron oxide nanoparticles shows more stress to the seeds of *Vigna mungo*.

Estimation of starch: This result (Fig2) reveals the stress caused by Ag, S and FeO nanoparticles at two different concentration 100ppm and 500ppm on starch content in plant. The absorbance was taken at 630nm and the result obtained shows that silver has the minimum absorbance at both 100ppm and 500ppm i.e 0.128 & 0.226; followed by sulphur with 0.286 &0.345 and iron which has the maximum absorbance i.e 0.302 & 0.371 respectively. (Fig 2)

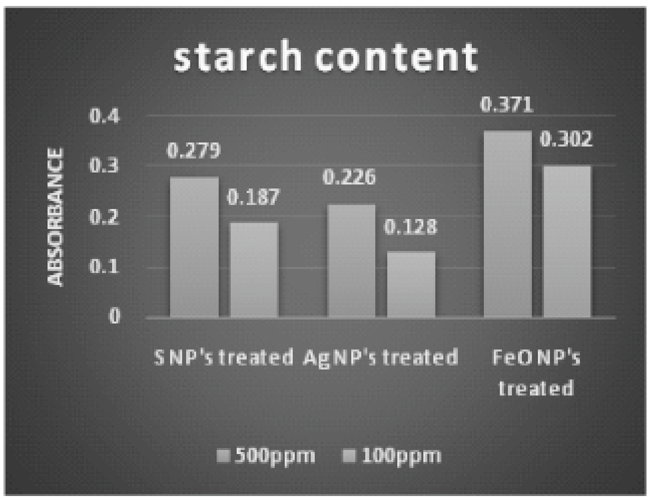


Fig. 2. Absorbance of Starch at 560nm of treated seeds

The result shows that FeO NP's caused more stress to plant as compared to S NP's and Ag NP's. Trujillo-Reyes et al. (2014) found that iron nanoparticles (Fe/Fe3O4) reduced root size and changed root architecture, affecting as well to the root water content and the chlorophylls accumulation in the leaves of *Lactuca sativa*, concequently reducing the starch content.

Estimation of Amylase: The result (fig 3) reveals the amylase activity of *Vigna mungo* treated with sulphur, silver and iron oxide nanoparticle solution at 100ppm and 500ppm. We observed that the absorbance taken at 510 nm. The stress induced by sulphur is minimum followed by silver and iron oxide which caused maximum stress to plant. (Fig 3)

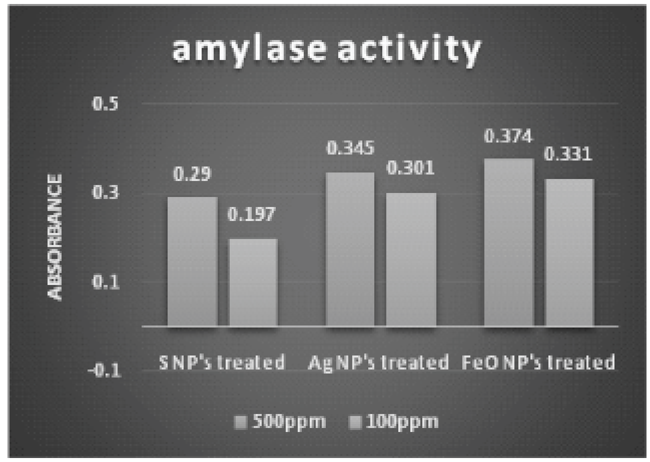
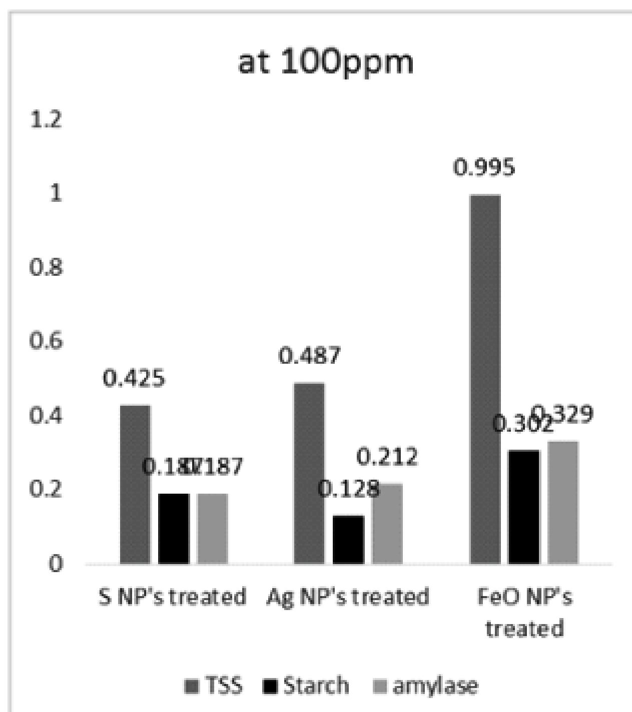


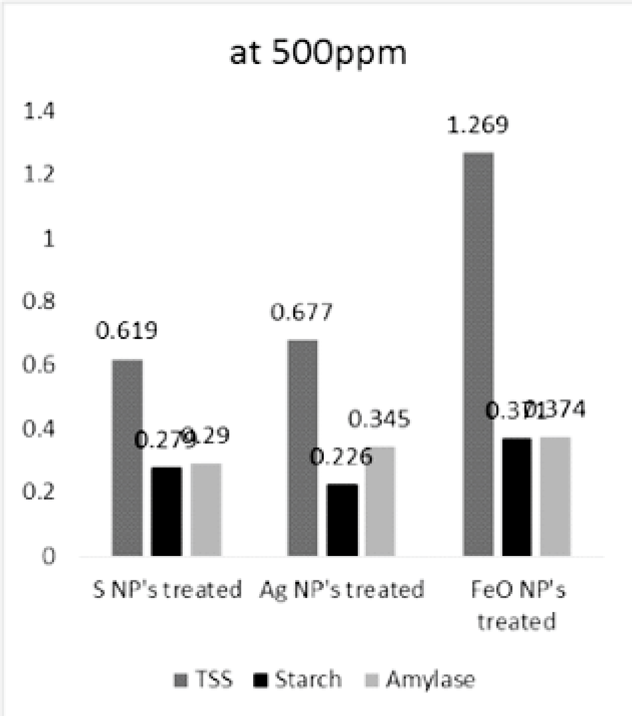
Fig. 3. Absorbance of Amylase activity at 630 nm of treated seeds

The reason may be the harsh effect of FeO NP's in amylase activity due to which the breakdown of starch and soluble sugar also a effected *Vigna mungo*.

All the three biochemical parameters on comparison shows that FeO NP's produced the maximum stress on seeds of *Vigna mungo* at both 100 ppm and 500ppm (Fig4). All the three parameters (starch, total soluble sugar and amylase) are interrelated to each other as - amylase, an endohydrolase that is able to rapidly degrade the starch into soluble substrates for other enzymes to attack (Beck & Ziegler, 1989) (Fig 4a and 4b)



(a) At 100ppm



(b) at 500ppm

Fig 4. Quantitative estimation of phytoconstituents of *Vigna mungo* at 100ppm and 500ppm (a and b).

The clogging efects of Fe NPs in apoplast under the high concentration treatment may lead to extremely low levels of iron content in the central cylinder cells, and may fnally severely block the nutrients transport (e.g. iron). This view point was supported by numerous studies that the potential adherence to the root causes adverse efects in plants when the concentration is relatively high (Keller, A. A., et. al.,2012). Also, the iron defciency in the central cylinder cells may lead to structural damages in the plants.

However, it should be pointed out that the reactive oxygen species (ROS) which is produced by Fe NPs oxidation may be another factor which is harmful to plants (Libralato, G. et al.2016). Therefore, improvements in the understanding of the toxicological efects of Fe NPs will also be required in further studies.

Conclusion :

In this study, we observed the toxicity of three different nanoparticles S NP's, Ag NP's and FeO NP's at two different concentration 100ppm and 500ppm with control. The highest toxicity was exhibited by FeO NP's followed by Ag NP's and then S NP's. So, our observations indicate that nanoparticles are harmful for plants and if used for cultivation practices and accumulated in seedling will be phytotoxic.

Pulses were known to form the important source of protein and other dietry constituents in indian diet. Black gram (***Vigna mungo***) has occupied an important place in human nutrition as rich source of protein in the diet of consumers of India and western diets. So if nanoparticles will be used to grow this plant then it will be harmful for human health as well as animals and through food chain other living organisms will also be affected. More investigations are needed to determine the negative impact of nanoparticles on plants used in day to day life.

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References :

Alidoust D, Isoda A (2013). Effect of Fe2O3 nanoparticles on photosynthetic characteristic of soybean (Glycine max (L.) Merr.): foliar spray versus soil amendment. Acta Physiol Plant. 35: 3365.

Auffan M, Rose J, Bottero JY, Lowry JP, Weisner MR. (2009) Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective. Nat Nanotechnol. 4:634-641

Beck, E. and Ziegler, P. (1989). Biosynthesis and degradation of starch in higher plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 40: 95-117

Geisler-Lee, J., Wang, Q., Yao, Y., Zhang, W., Geisler, M., Li, K., Huang, Y., Chen, Y. Kolmakov, A., Ma, X. (2013). Phytotoxicity, accumulation and transport of silver nanoparticles by Arabidopsis thaliana. Nanotoxicology 7, 323–337

Gottschalk F, Lassen C, Kjoelholt J, Christensen F, Nowack B. (2015). Modeling flows and concentrations of nine engineered nanomaterials in the Danish environment. Int J Environ Res Public Health. 12:5581–5602

Hong J, Peralta-Videa JR, Rico C, Sahi S, Viveros MN, Bartonjo J, Zhao L, Gardea-Torresdey JL. (2014). Evidence of translocation and physiological impacts of foliar applied CeO2 nanoparticles on cucumber (Cucumis sativus) plants. Environ Sci Technol. 48:4376–4385

Keller, A. A., Garner, K., Miller, R. J. & Lenihan, H. S. (2012). Toxicity of nano-zero valent iron to freshwater and marine organisms. Plos One 7, e43983.

Lee CW, Mahendra S, Zodrow K, Li D, Tsai YC, Braam J, Alvarez PJ. (2010). Developmental phytotoxicity of metal oxide nanoparticles to Arabidopsis thaliana. Environ Toxicol Chem. 29:669–675.

Libralato, G. et al. (2016). Phytotoxicity of ionic, micro- and nano-sized iron in three plant species. Ecotoxicol. Environ. Safety 123, 81–88.

Ma C., White J.C., Dhankher O.P., Xing B. (2015). Metal-based nanotoxicity and detoxification pathways in higher plants. Environ Sci Technol. 49:7109–7122.

McKee MS, Filser J. (2016). Impacts of metal-based engineered nanomaterials on soil communities. Environ Sci Nano. 3:506–533.

Nel A, Xia T, Mädler L, Li N. (2006). Toxic potential of materials at the nanolevel. Science. 311:622–627.

Rico CM, Majumdar S, Duarte-Gardea M, Peralta-Videa JR, GardeaTorresdey JL. (2011). Interaction of nanoparticles with edible plants and their possible implications in the food chain. J Agric Food Chem. 59:3485–3498.

Tolaymat T, El Badawy A, Sequeira R, Genaidy A. (2015). A system-of-systems approach as a broad and integrated paradigm for sustainable engineered nanomaterials. Sci Total Environ. 511:595–607

Trujillo-Reyes, J., Majumdar, S., Botez, C. E. (2014). Peralta-Videa, J. R. & Gardea-Torresdey, J. L. Exposure studies of coreshell Fe/Fe3O4 and Cu/CuO NPs to lettuce (Lactuca sativa) plants: are they a potential physiological and nutritional hazard? J. Hazard. Mater. 267, 255-263.

Yin L., Colman B. P., McGill B. M., Wright J. P., Bernhardt E. S. (2012). Effects of silver nanoparticle exposure on germination and early growth of eleven wetland plants. PLoS ONE

Zhu H, Han J, Xiao JQ, Jin Y. (2008). Uptake, translocation, and accumulation of manufactured iron oxide nanoparticles by pumpkin plants. J Environ Monitor. 10:713–717