ISSN: 2347-971X (Print)
ISSN: 2347-9728(Online)

International Journal of Innovations in Scientific and Engineering Research (IJISER)

PROTECTIVE APPRAISAL OF ZINC OXIDE NANOFERTILIZERS TOWARDS SOIL MICROORGANISMS

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Abstract-The prominent zinc oxide nanoparticles are significantly very important material, due to its miscellaneous properties and its substantial application in agriculture. The zinc oxide nanoparticles (ZnO NPs) enhance the ability to spear in wide variety of plant root system. This will be a most available form to the different plants with different ratio. However, it still deficiency of awareness and information about their toxicity effects. By this preliminary evidence the work intends to evaluation and comparison the effect of urea uncoated and coated ZnO NPs and its toxicity effects. Our preliminary investigation revealed that, the uncoated ZnO NPs has negative impact on fenugreek plant by its different growth and development parameters. Growth retardation of the plant was observed by increasing the concentration of urea uncoated ZnO NPs. From the results, when compared to coated and uncoated ZnO NPs, the use of uncoated ZnO NPs, with extended experimental time considerably inhibits the growth of the plants stem, root and leaves. In the other hand, the standard plate count assay exhibited the viability of bacteria in soil sample decrease with its respective dosage.

Keywords- Nanofertilizers: ZnO Nanoparticles: Urea: Nanotoxicity: Fenugreek.

1. INTRODUCTION

The application of different nanoparticles has become progressively increasing in industrial, consumer and medicinal products [1-4]. In advance the researchers focused on agriculture practice for its improved development, soil fertility and yield [4-6]. It reflects, divergent types of nanoparticles were introduced in agriculture sector for different manner and propose which has great impact on the environment [6-8]. The nanoparticle undergoes different physical, chemical and biological changes in the agricultural soil, which make it challenging to measure their prevalence and assess their ecotoxicity levels. These specialised improvements are entirely depending on the nature of different nanoparticles and its culture medium [1]. In aqueous systems, the behaviour of nanoparticles differs from its hardness, pH, biochemical oxygen demand, alkalinity and organic matters so on.

Number of researchers has been striving to quantify the minute amounts of nanoparticles in the environment with précised methods or protocols. Nevertheless, the different scientific communities involved to accept the finest methodologies for accompanying such different studies. This evidence contributes the developing concerns about the facts and effects of different materials in the environment. It makes a great impact to get effective solutions to examine the real impact in usage of different nanomaterial and ecotoxicity. This impressive information on nanoparticles led to proper care towards different nanomaterials in order to avoid human and environmental issues [4].

One method is to prescribe the great impact in which nanoparticles come into the environmental and human through agricultural products. Numerous nanoparticles enter into extensive use and massive and unexpected quantities of nanoparticles are settled down in wastewater treatment plants. Almost, the sludge is used as a fertilizer in the agricultural areas. Nano-enabled agricultural method is predominantly very smart because of its favourable enhancements of farm mechanization and green revolution. Nanoformulations are enhance the efficacy of agricultural chemicals, improved delivery systems in plants to uptake the nutrients and produce good amount of yield with good nutritive values and also reduce the environmental impact. Nanopesticides and nanofertilizers have been easily available for numerous decades, and verities of new products are expected to inundate the market by this nano agricultural method. Unluckily, the potential and harmed nano-enabled products are quite attractive and efficient knowledge gap exists on the effect of nanoparticles on crop productivity, quality and quantity of the food with respect of human health [5,6]. Furthermore, it is remarkable to indicating the different nanoparticles progress structure, function and increasing solubility of the pesticides to enhance the resistance against photodecomposition and hydrolysis [7-9].

Zinc is one of the indispensable mineral elements for number of plants to prevent from sterility and growth arrest. If the concentration of zinc is elevated it becomes toxic to the plants. In the zinc fertilizers zinc sulfate plays a key component, but there is great attention towards zinc oxide nanoparticles in agricultural usage. It has a property to enhance the crop yield and growth [9-11]. But the, toxicological impact of these zinc oxide nanoparticles has to take into consideration [12-13].

The work intends to evaluate and compare the consequence of urea uncoated and coated zinc oxide nanoparticles (ZnO NPs) and evaluate its positive and negative impact on microorganisms exist in the soil.

2. MATERIALS AND METHODS

2.1 Chemicals

Zinc nitrate and all other required chemicals were used first class analytical grade and purchased from Sigma -Aldrich Chemical Co., USA.

2.2 Synthesis of Zinc Oxide Nanoparticles

Zinc oxide nanoparticles were synthesized by taking 500 ml of *Citrullus lanatus* flesh extract was taken in a clean conical flask and 0.2 M $Zn(NO_3)_2.6H_2O$ was added to the solution and mixed thoroughly and kept in shaker incubator for 2 hours at 60^0C as per our earlier studies. After incubation that mixture permitted to cool at 37 ^{0}C. Further the solution was allowed to centrifugation for 20minutes at 400 rpm. After centrifugation supernatant was discarded and obtain pellet were separated and kept in Hot air oven for 6-7 hours at 80^0C and denoted as ZnO NPs [14-18]. The prepared ZnO nanoparticles was coated with 1% urea powder and denoted as C- ZnO NPs.

2.3 Experimental Plant

We used Fenugreek (*Trigonella foenum graecum)* seeds as an experimental plant. The seeds were purchased from local market and authenticated by institutional botanist.

2.4 Plant Cultivation and Experimental Design

Fenugreek seeds were treated for a month with urea coated and uncoated zinc oxide nanoparticles concentration 0 (Control), 10, and 100mg/L. The experimental samples (6 plants) were cultivated for every week and it was allowed to examine different parameters like plant dry weight, length and its fresh weight. Finally, toxicity studies of nanoparticles against soil microorganisms were performed as per the modified available literature [15-19].

2.5 Cytotoxicity Assessment

Dominant bacterial specie *E. coli* were used all the experiments. The isolates are obtained from the study area soil and 3×100 CFU mL^{-1} was maintained as a initial cell count for throughout the investigation. Bacterial strains were allowed to interact with ZnO NPs with the concentration range of 0, 10 and 100 mg/ respectively.

2.6 Cell Viability Assessment

Standard plate count assay (SPCA) was used to determine the percentage of reduction at different interaction period like 3, 6 and 24 h. The bacterial viability in test and control samples was observed. By using SPCA, the cell cultivability was identified in bacterial culture medium [19].

2.7 Oxidative Stress Assessment

2'-7'-Dichlorodihydrofluorescein diacetate fluorescence probe was effectively used to measure the origin of reactive oxygen species (ROS). In the presence of ROS and cellular esterase 2'-7'-Dichlorodihydrofluorescein diacetate act as a membrane permeable compound and oxidized to produce green fluorescence. Intracellular reactive oxygen species formation was evaluated in NP interacted bacterial cells at 3 h, 6 h and 24 h and control. The experiment was performed as per the modified available literature [20-26]. The fluorescence was observed by using spectrofluorometer at 485 nm and 530 nm. Nanoparticles without cells were used as a negative control which may interfere with the 2'-7'-Dichlorodihydrofluorescein diacetate dye.

2.8 Statistical Analysis

SPSS/10 student software was used to analyze all the collected samples data. One-way ANOVA followed by LSD was used for detecting exact hypothesis of the studies. The results were considerably different if $p < 0.05$, these values is expressed by using mean ± S.D.

3. RESULTS AND DISCUSSION

At present investigation revealed that, there is no much different and difficult process was observed between plants cultivated using zinc oxide nanoparticles. The dynamic effect of different nanoparticles towards plant growth and development was expressed by number of studies. As per the available literature [14], the interaction between cerium oxide nanoparticles towards wheat and barley were observed and the result expressed that, the cerium oxide shows significant effect on barley to enhance the shoot development, early maturity, more productivity and there is no much difference were observed in wheat. In addition, numerous studies revealed that, strong negative impact upon nanoparticles by administration into the soil by altering its fertility, microbial consortium, enzymes, number of nutrient cycles and bacteria-plant symbiotic functions. The nanoparticles will have benefited to agricultural, available and demanding substantiation to support effective and efficient agricultural practices [27-31].

In our experiment we cultivated the fenugreek seeds in presence of urea coated and uncoated zinc oxide nanoparticles separately. The investigation planned to evaluate last three weeks. Totally six plants were taken to evaluate the impact of zinc ions towards plant growth and development. The obtained data were depicted in Table 1. C-ZnO NPs has been found that the minor stimulation was observed in the experimented plants and it was compared with control plant. In the first week, the growth and development of plant was observed around 28 % and second and third week it was determined in the range of 8 and 4 percentage. The results were compared with control plants. The administration of ZnO NPs in the plants expressed opposite trend and its extended investigation time interval exhibits to found optimum concentration for amassed growth depression was determined after the 1^{st} and 2^{nd} week in the range of 7 and 16 percentage.

In addition, the zinc ions were applied into the plant biomass to determine the attraction between Zn ion and plant. The extended experiment time reveled that, the applying of zinc ion in the form of C-Zn NPs were showed significantly increasing fresh plant matter around 43.5 % in the first week and it was compared with control and dry matter. When the time period extended, the growth was depressed around 3.3 % in last week. The experiment revealed both fresh and dry weight growth parameters with respective of nanoceria and there is no statistically significant increasing in plant dry matter in the last week when compared with control.

ZnO NPs were allowed to interact with the selected plant and the biomass of the plant was significantly decreasing up to 19.4 % in the first week and similar results were obtained in second week. In last week the decreased biomass was determined around 20.2 % compared with control.

The effect of zinc oxide nanoparticle (ZnO NPs) in the dry matter was found to be slightly decreased around 27.9% over the specified time period.

Table: 1 Overview of plant treatments with control (% difference)

Growth parameter	Sample ID	Week 1	Week 2	Week 3
Plant Length	C-ZnO NPs(both concentrations)	28% ± 2.1%	8% ± 0.5%	4% ± 0.8%
	ZnO NPs (both concentrations)	-7% ± 1.3%	13 % ± 1.65%	16% ± 1.5%
Fresh weight	C-ZnO NPs(both concentrations)	44.5% ± 9.4%	39..7% ± 1.78%	33.4% ±2.3%
	ZnO NPs (both concentrations)	-19.4% ± 3.78%	-20.2% ± 2.5%	-18.2% ± 5.43%
Dry Matter	C-ZnO NPs(both concentrations)	40.2% ± 6.79%	36.7% ± 4.34%	30% ± 1.3%
	ZnO NPs (both concentrations)	-21% ± 1.1%	-22% ± 1.28%	-25% ± 1.48%

For every interval the values are represented by X ± S.D. Within a line, values without a common letter are significant at $p < 0.05$ as evaluated by ANOVA.

From the above table 1 and preliminary investigation upon zinc oxide nanoparticle (ZnO NPs) towards fenugreek shows negative impact on the growth parameters.

3.1 Cell Viability Assessment

The reduction of cell viability *E. coli* is depending upon the concentration of zinc oxide nanoparticle (ZnO NPs) by SPCA. It expressed drastic decline in the viability of cells which is interacted with nanoparticles was observed 12.4 ±0.6 % & 1.4 ± 0.2% for ZnO NPs & C-ZnO NPs dark respectively for 3 hours as compared with control, at 1 mg/L was depicted in the figure-1&2.

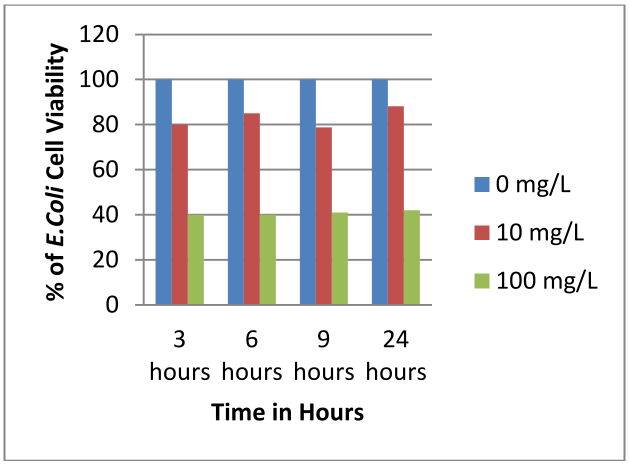

Figure 1: Cell viability assessment treated with ZnO NPs

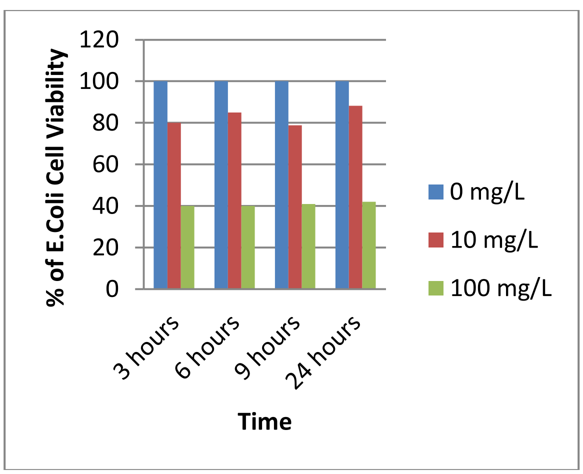

Figure 2: Cell viability assessment treated with C-ZnO NPs

The cytotoxicity and cell viability of ZnO NPs was found to be reduced with different time interval. The toxicity of two different test samples (ZnO NPs and C-ZnO NPs) were determined at three different concentrations (0 (Control), 10 & 100mg/l). cell viability was observed under dark and light condition at low concentration of two different test samples and it shows non-significant results.

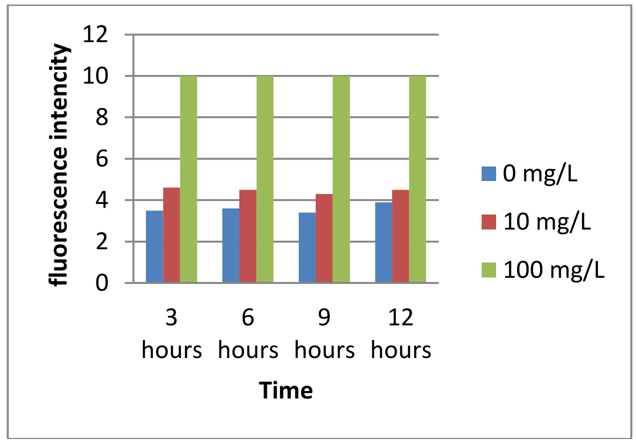

Figure 3: ROS generation in light condition treated with ZnO NPs

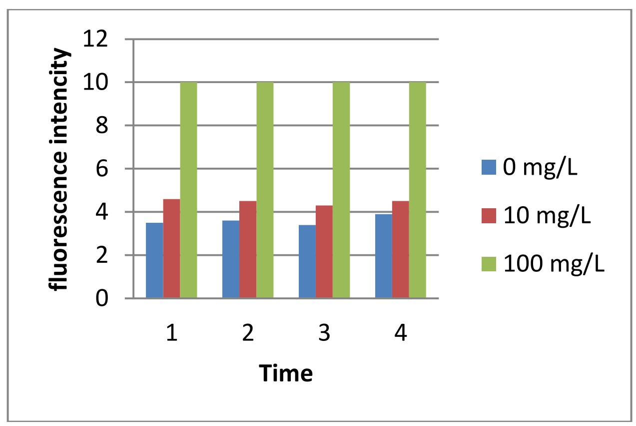

Figure 4: ROS generation in light condition treated with C-ZnO NPs

The effective dosage for intracellular reactive oxygen species formation was determined by comparing with control. The concentration of reactive oxygen species was observed after 24 hours of interaction was significantly increased (figure-3 &4)

for ZnO NPs (10.5 ± 0.2 %) as compared to C-ZnO NPs (0.62 ± 0.2%).

4. CONCLUSION

Conventional fertilizers including Zn possess great impact of its availability, accessibility and solubility in the soil. This study helps to enhance the knowledge about nanofertilizers and nanopesticides up-take by different plants and plants nutrition. The present investigation generalized that the zinc and zinc oxide nanoparticle (ZnO NPs) toward the impact upon environmental risk. These effective nanoceria gave increasing usage as fertilizers in the agricultural sector. In this study, understanding the basic chemistry of the ZnO NPs with bacterial cell interaction will be improves future studies.

ACKNOWLEDGMENT

The authors would like to thank the management of Sacred Heart College (Autonomous), Tirupattur for providing all the facilities to do the research activities in the form of Don Bosco Research Grant with the sanction number SHC/DB Grant /2017/05.

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