Nanoparticles and their Role in Restoration of Stem Girth and Chlorophyll Index in *Oryza sativa* L. grown Under Aluminium Toxic Soil

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Abstract

Nanoparticles (NP) are one of the engineered nanomaterials with brilliant physiochemical properties, moreover, they are bioactive. These can be utilized for the restoration of morphology and physiology of plants grown under hazardous waste sites. In my case, rice was the targeted plants. The average stem girth was significantly enhanced by about 31.90% with respect to T1 when treated with Fibroin NPs upon Aluminium stress whereas only sole Fibroin NPs were applied (T6). KNO₃ Nanoparticles when applied upon Aluminium stress (T6). The average chlorophyll index was significantly reduced by about 28.81% with respect to T1 when treated with Fibroin NPs upon Aluminium stress whereas only sole Fibroin NPs were applied (T6). KNO₃ Nanoparticles when applied upon Aluminium stress (T6).

Keywords: Agriculture, Biotic, Crops, Density, Energy, Forage, Gap, Rice, Silk

Introduction

Nanotechnology plays various roles in agricultural research namely, Agricultural diagnostics, Manipulation of agricultural crops, drug delivery, nanobiosensors, nanobiofarming, nano-pesticides, Nanoherbicides and controlled release of nano-fertilizers and nanocomplexes [1, 2, 3, 4 and 5]. To cope with Aluminium toxicity plants have developed certain adaptive mechanisms to survive in even toxic conditions which are as follows. (1) Exclusion or Resistance to Aluminium to exclude the entry of metal into the cell by secreting certain Organic acids or phenolic compounds which further bind Al³⁺ and prevents its uptake into the cytosol. (2) Exhibiting Internal tolerance by compartmentalizing Al in vacuoles, the formation of Al complexes with organic substances in the cytosol and improved scavenging via ROS, to avoid toxicity [6, 7, 8, 9, 10 and 11]. Ample of detoxification methodologies have been adopted by the plants in order to fight back with the metal toxicity and their accumulation such as a cellular antioxidant system that constitutes Superoxide dismutase (SOD), Ascorbate peroxidase(APX), Glutathione reductase (GR) and Catalase (CAT). They help in the detoxification of oxyradical which further inhibits the oxidation of biomolecules [12, 13, 14, 15, 16 and 17].

Many plant species have developed certain plant species for the alleviation of Al internally and/or externally such as secretion of various organic acids anions (citrate, malate, and oxalate) from roots which further chelate Al ions in the rhizosphere [18,19, 20, 21, 22, 23 and 24]. Furthermore, a number of Al tolerance genes have been
explored in plants especially rice [25, 26, 27, 28, 29 and 30]. It was found that NH₄⁺ ions reduced aluminum accumulations in the roots by altering the cell wall properties which took place due to a decrease in ph by the NH₄ uptake [31, 32, 33, 34 and 35]. Al-induced Oxidative stress leads to the splitting of membrane integrity and stability [36, 37, 38, 39, and 40]. Plants such as Vigna radiate (green gram), Oryza sativa (rice) and Lolium penne (ryegrass)[41, 42, 43, 44, 45, 46 and 47] exhibited enhanced Lipid peroxidation onto Al exposure. Even Brassia juices genotypes verified enhanced oxidative stress upon Al exposure. Al enhanced the content of (ASA), Ascorbate, dehydroascorbate (DHA) and total Ascorbate (ASA+DHA) in B. juncea species. [48, 49, 50, 51 and 52]. When plants are brought under Al exposure they are seen to be involved in free radical scavenging activities such as DPPH and HRSA in two genotypes of mustard [31, 36, 38, 39 and 50]. The same findings were shown by (Chutipaijit 2016) which exaggerates on better the DPPH activity, more shall the rice genotypes be adaptive to osmotic stress based on antioxidant activities. Aluminum at very low concentration induces growth in native crops which have developed adaptive mechanisms [23, 24, 26, 27, and 29]. Low level of Al-induced root biomass synthesis in Tabebuia chrysantha tree, whereas the effect was opposite when high levels of exposure were made [30, 34, 37, 39, 41, 45, and 47]. An increased root growth Al can induce or even have no effect on the essential nutrient uptake especially in hyperaccumulator crops [23, 27, 28 and 29]. Al induces alkaline Phosphatase activity and organic phosphorus activity in the marine diatom Thalassiosira weissflogii [30, 31, 32, and 33] Aluminium induces color changes in some hyperaccumulator plants.

Methodology

The experiment was conducted at Natural Ventilated Poly house, School of Agriculture, Lovely Professional University (LPU), Phagwara, Punjab. The farm situated at attitude 232 meters above sea level, latitude 31.244604 N and longitude 75.701022 E as per Google map (Figure 1).

Figure 1. Google map of the experimental site

(Source: Google Earth, 2019)
Punjab Trans-Gangetic Plains Region Phagwara falls in the Central Plain Zone of Punjab. Generally, in June the hottest month of the year with a maximum temperature of 45°C and a minimum of 27°C, the annual average temperature is 24°C. In January during winters the temperature falls down up to 4°C to 6°C. Monsoon starts in the last of June / early of July having a normal annual rainfall of 686mm.

TREATMENTS DETAILS

The pot experiment was conducted on the farm of the School of Agriculture, Lovely Professional University, Jalandhar Punjab with one genotype Pusa Basmati 1121 of Rice. Genotype took from Punjab Agriculture University, Punjab. The pot size for the experiment will be diameter: 30 cm and height 25 cm. Heavy metal stress was created by foliar application of aluminium (100ppm) at the flowering stage. KNO3 protein nanoparticle (1%) and Fibroin Nanoparticle (1%) were applied through a foliar application at the flowering stage. The various measurements were taken at 90 DAT (Table 1 and 2).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Details of the treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-0</td>
<td>Control</td>
</tr>
<tr>
<td>T-1</td>
<td>Al (100ppm)</td>
</tr>
<tr>
<td>T-2</td>
<td>Fibroin nanoparticle (1%)</td>
</tr>
<tr>
<td>T-3</td>
<td>KNO3 protein nanoparticle (1%)</td>
</tr>
<tr>
<td>T-4</td>
<td>Al (100ppm) + Fibroin nanoparticle (1%)</td>
</tr>
<tr>
<td>T-5</td>
<td>Al (100ppm) + KNO3 protein nanoparticle (1%)</td>
</tr>
<tr>
<td>T-6</td>
<td>Al (100ppm) + KNO3 protein nanoparticle (1%) + Fibroin Nanoparticle (1%)</td>
</tr>
</tbody>
</table>

Table 2: Layout Details

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Particulars</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Layout</td>
<td>CRD</td>
</tr>
<tr>
<td>2.</td>
<td>Treatment</td>
<td>7</td>
</tr>
<tr>
<td>3.</td>
<td>Replications</td>
<td>3</td>
</tr>
<tr>
<td>4.</td>
<td>Total Number of pots</td>
<td>7*3=21</td>
</tr>
<tr>
<td>5.</td>
<td>Soil per pot</td>
<td>7 kg</td>
</tr>
<tr>
<td>6.</td>
<td>Genotype</td>
<td>Pusa Basmati 1121</td>
</tr>
</tbody>
</table>

OBSERVATION TO BE RECORDED

The observations were recorded at 60 and 90 DAT. The recorded observations of morphological and the standard procedure adopted during the course of study are given below:

MORPHOLOGICAL PARAMETER

Stem girth
The stem girth was recorded from the node present on the stem of the plant at 90 days of time interval by using a digital Vernier caliper. Mean stem girth was calculated and expressed in the cm.

**BIOCHEMICAL PARAMETERS**

Chlorophyll Index was measured by SPAD 502 Plus Chlorophyll Meter for the rapid, accurate and non-destructive measurement of leaf chlorophyll concentrations.

**RESULTS and DISCUSSION**

**Stem girth (mm)**

Effect of Silk Fibroin Nanoparticle (NP) and Potassium Nitrate (KNO₃) and their combination on stem girth (mm) was studied in rice variety Pusa Basmati 1121 under the Aluminium toxicity stress. Data were recorded at 90 days after transplanting (DAT) (Table 3 and Fig. 2). It is evident that the average stem girth was significantly decreased by 29.02% when exposed to heavy metal stress (T1) as compared to control (T0) at 90 DAT of interval. Exogenous application of KNO₃ particles on the leaves (T3) enhanced the number of stem girth by 26.04% as compared to (T1) at 90 DAT. In comparison to T1, the exogenous application of Fibroin Nanoparticle (T2) showed enhancement in the number of stem girth by 29.33%, on proposed DAT. The treatments T4, when compared with T1, showed that Fibroin NPs enhanced the stem girth by only 22.43% whereas KNO₃ NPS in T5 enhanced the same by 12.72% when applied along with Aluminium stress. The average stem girth was significantly enhanced by about 31.90% with respect to T1 when treated with Fibroin NPs upon Aluminium stress whereas only sole Fibroin NPs were applied (T6). KNO₃ Nanoparticles when applied upon Aluminium stress (T6). Tammam et al. (2018) studied the response of an Aluminium tolerant wheat cultivar (Sakha 93) to different doses of Aluminium (100, 200, 400, 500) micro. Seedlings were pretreated with boron and grown in a hydroponic solution. The cultivar showed have higher adaptive mechanism against the Al stress. Aluminium (200,400) reduced the fresh weight of roots whereas the low concentration (100) did not have much significant effect. Ahmad et al. (2018) examined the damage as well as the response of Mustard (Brassica juncea) to the Aluminium stress. Eleven genotypes of mustard were studied for growth under Aluminium stress out of which two genotypes (Pusa Tarak and Pusa Vijay) were subjected to Aluminium stress for 24 and 72 hours. Enzymatic activity was seen too much in Pusa Tarak in comparison to control and Pusa Vijay [52]
Table 3. Stem Girth (mm) of rice during *Kharif*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Stem girth of rice at 90 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>0.7467±0.0318</td>
</tr>
<tr>
<td>T1</td>
<td>0.53b±0.03512</td>
</tr>
<tr>
<td>T2</td>
<td>0.75a±0.0866</td>
</tr>
<tr>
<td>T3</td>
<td>0.7167ab±0.04055</td>
</tr>
<tr>
<td>T4</td>
<td>0.6833ab±0.05457</td>
</tr>
<tr>
<td>T5</td>
<td>0.6073ab±0.0442</td>
</tr>
<tr>
<td>T6</td>
<td>0.7783a±0.09011</td>
</tr>
</tbody>
</table>

where, Data are in the form mean± SEM. Significance at P≤0.05 using SPSS ver. 22. T0= Control; T1: Aluminium chloride (100ppm); T2: Fibroin nanoparticle (1%); T3: KNO₃ nanoparticle (1%); T4: Aluminium chloride (100ppm) + Fibroin nanoparticle (1%); T5: Aluminium chloride (100ppm) + KNO₃ Nanoparticle (1%); T6: Aluminium chloride (100ppm) + Fibroin nanoparticle (1%) + KNO₃ Nanoparticle (1%).
where, Data are in the form mean± SEM. Significance at P≤0.05 using SPSS ver. 22. T0= Control; T1:
Aluminium chloride (100ppm); T2: Fibroin nanoparticle (1%); T3: KNO₃ nanoparticle (1%); T4: Aluminium chloride (100ppm) + Fibroin nanoparticle (1%); T5: Aluminium chloride (100ppm) + KNO₃ Nanoparticle (1%);
T6: Aluminium chloride (100ppm) + Fibroin nanoparticle (1%) + KNO₃ Nanoparticle (1%).

Chlorophyll Index

Effect of Silk Fibroin Nanoparticle (NP) and Potassium Nitrate (KNO₃) and their combination on chlorophyll index was studied in rice variety Pusa Basmati 1121 under the Aluminium toxicity stress. Data were recorded at 90 days after transplanting (DAT) (Table 4 and Fig. 3). It is evident that the average chlorophyll index was significantly increased by 19.14% when exposed to heavy metal stress (T1) as compared to control (T0) at 90 DAT of interval. Exogenous application of KNO₃ particles on the leaves (T3) enhanced the chlorophyll index by
17.28% as compared to (T1) at 90 DAT. In comparison to T1, the exogenous application of Fibroin Nanoparticle (T2) showed enhancement in the chlorophyll index by 5.9%, on proposed DAT. The treatments T4, when compared with T1, showed that Fibroin NPs reduced the chlorophyll index by only 0.6 % whereas KNO₃ NPS in T5 reduced the same by 8.25% when applied along with Aluminium stress. The average chlorophyll index was significantly reduced by about 28.81% with respect to T1 when treated with Fibroin NPs upon Aluminium stress whereas only sole Fibroin NPs were applied (T6). KNO₃ Nanoparticles when applied upon Aluminium stress (T6). Bera et al. (2017) discussed the effect of different concentrations of aluminum on the rice crop. There was significant linear growth in the root and the root hairs [53]. Zhang et al. (2017) discussed the role of melatonin in the roots of soybean which acts as an ameliorator to aluminum toxicity [54]. It was found that the exposure of less concentration of melatonin to aluminum affected plants showed an upsurge in the growth of roots and reduced the production of H₂O₂. Yu et al. (2016) discussed the role of putrescine in ameliorating aluminum (Al) toxicity in wheat seedlings. Al inhibited the root growth but putrescine exposure, on the other hand, mitigated the reduction in root growth. Here two wheat cultivars were tested i.e Al-tolerant and Al-sensitive varieties [55]. Where Al-sensitive cultivar showed root inhibition and ethylene production at the root apices, all such symptoms were altered in the Al-tolerant cultivar by the presence of ethylene biosynthesis inhibitors. Tanik et al. (2017) have discussed the several aspects of Aluminium (Al) uptake and translocation within the plants. Myriad of industrialization in addition to fertilizer doze applications made the soil ph dip below 5, hence acidic soils lead to solubilization of the trivalent form of Al that is the most toxic form of Al, which further causes inhibition of root growth and other metabolic functions [56].

**Table 4. Chlorophyll Index of rice during Kharif**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Chlorophyll Index of rice at 90 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>24.5ᵇ ± 4.71487</td>
</tr>
<tr>
<td>T1</td>
<td>30.3ᵃᵇ ± 1.594783</td>
</tr>
<tr>
<td>T2</td>
<td>32.2ᵃᵇ ± 3.317127</td>
</tr>
<tr>
<td>T3</td>
<td>36.6³ᵃ ± 2.58865</td>
</tr>
<tr>
<td>T4</td>
<td>30.1ᵃᵇ ± 3.370954</td>
</tr>
<tr>
<td>T5</td>
<td>29.7ᵃᵇ ± 3.732292</td>
</tr>
</tbody>
</table>
where, Data are in the form mean± SEM. Significance at P≤0.05 using SPSS ver. 22. T0: Control; T1: Aluminium chloride (100ppm); T2: Fibroin nanoparticle (1%); T3: KNO$_3$ nanoparticle (1%); T4: Aluminium chloride (100ppm) + Fibroin nanoparticle (1%); T5: Aluminium chloride (100ppm) + KNO$_3$ Nanoparticle (1%); T6: Aluminium chloride (100ppm) + Fibroin nanoparticle (1%) + KNO$_3$ Nanoparticle (1%).

### Table

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Chlorophyll Index (SPAD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>20.5±2.1ab</td>
</tr>
<tr>
<td>T1</td>
<td>24.6±2.5ab</td>
</tr>
<tr>
<td>T2</td>
<td>30.2±2.7ab</td>
</tr>
<tr>
<td>T3</td>
<td>35.8±3.0a</td>
</tr>
<tr>
<td>T4</td>
<td>29.7±3.2ab</td>
</tr>
<tr>
<td>T5</td>
<td>25.3±2.9ab</td>
</tr>
<tr>
<td>T6</td>
<td>27.9±1.64208ab</td>
</tr>
</tbody>
</table>

**Figure 3. Chlorophyll Index of rice during Kharif**
where, Data are in the form mean± SEM. Significance at P≤0.05 using SPSS ver. 22. T0= Control; T1: Aluminium chloride (100ppm); T2: Fibroin nanoparticle (1%); T3: KNO₃ nanoparticle (1%); T4: Aluminium chloride (100ppm) + Fibroin nanoparticle (1%); T5: Aluminium chloride (100ppm) + KNO₃ Nanoparticle (1%); T6: Aluminium chloride (100ppm) + Fibroin nanoparticle (1%) + KNO₃ Nanoparticle (1%).

Conclusion

The effects of nanoparticles were both positive and negative depending upon the crop cultivar, treatment, and several growth conditions. It is clear that nano fertilizers possess certain specific features such as enhanced production, ultra-high absorption, enhanced photosynthesis and increased surface area in leaves. It was observed that wheat crop when treated with bulk material and chitosan NPK fertilizer where enhanced the polysaccharides content also lowered the total soluble sugars and protein content in the wheat grain of plants grown in clay, clay-sandy and sandy soils and increased the fat content in wheat crop. Based on the above study it is clear that, the influence of metal and metal oxide nanoparticles on various crops at several diagnostic levels. Magnetic nanoparticle exposure, on the other hand, showed positive results in case of growth and also ensured that plant operates mechanisms to protect itself from oxidation stress. The number of Non-Effective tillers was reduced by 11.87% in T4. N/K ratio was enhanced by 37.06% and 20.21% in T5 in Leaf and Root samples.

Acknowledgments

P.K and Purnima gratefully acknowledge the support provided by Lovely Professional University.

Author Contributions

The study was designed by P.K. and Purnima, the morphological protocolizations were established, experiments were carried out and the data analyzed and interpreted were collected. The paper has been written by P.K.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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