Impact of Silk Fibroin Nanoparticle and KNO$_3^+$ Protein Nanoparticle on Total Tiller Number of Oryza sativa L. Grown under Aluminium Toxic Soil

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Abstract

The stress induced by Al Oxidative means that the membrane integrity and stability are split. Plants like *Vigna radiate, Oryza sativa*, and *Lolium penne* (ryegrass) showed improved lipid peroxidation to the exposition Al. Rice (*Oryza sativa* L) belongs to the grass family Poaceae. Genus *Oryza* consists of 22 wild species (Annual and Perennial) and two cultivated species i.e, sativa (Grown in Asia, America, and Europe) and glaberrima (grown in Africa). Aluminum is the most abundant element in the earth’s crust after Oxygen and silicon (atomic number: 13; atomic mass: 26.982; melting point: 660.3°C). Silk Fibroin Nanoparticles and KNO$_3^+$ Nano Particles were applied for the mitigation of Aluminium toxicity in rice. The average tiller number was significantly enhanced by about 2.62% with respect to T1 when treated with Fibroin NPs upon Aluminium stress whereas only sole Fibroin NPs were applied. KNO$_3$ Nanoparticles when applied upon Aluminium stress.

Keywords: Aluminium, Crop, Density, Economy, Nanoparticles, Rice, Silk

Introduction

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 Al$^{3+}$ and prevents its uptake into the cytosol. (2) Exhibiting of 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 [1, 2, 3 and 5]. 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 which 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 [1, 2, 3, 4 and 5]. In India, it has been mainly grown in the Gangetic plains and coastal areas [6, 7, 8, 9 and 10]. Amongst the wild species, seven are tetraploid (2n=48) and rest are all diploids (2n=24). It is a semi-aquatic crop which is grown in standing water with very high water requirement [11, 12, 13, 14 and 15]. The word rice is derived from the French word *riso* or the Italian word *riso* which came out to be a modification of the Sanskrit word *vrihi* [16, 17, 18 19 and 20]. World paddy production was 759.6 million tonnes in 2017 where China leads India by producing 210.3 million tonnes as against 166.5 million tonnes by the latter (www.fao.org). Paddy yielded 3.85 tonnes per hectare and was harvested
over 42.9 million hectares (ricestat.irri.org). Among the Indian states West Bengal bags first position (2015-2016) in rice production (15.10%) followed by Uttar Pradesh (11.99%), Punjab (11.33%), Tamil Nadu (7.65%), Andhra Pradesh (7.18%), Bihar (6.22%), Chattisgarh (5.84%), Orissa (5.64), Assam (4.93), Haryana (3.98) (www.apeda.com). Rice protein content differs from 6% to 14% based upon rice variety and culture environment [21, 22, 23, 24, 25 and 26]. It was thought that cultivated rice was originated in South India by De Candolle (1886) and Watt (1892). Vavilov, on the other hand, insisted on India and Burma to be the center of origin of cultivated rice [27, 28 29 and 30]. Aluminum is the most abundant element in the earth’s crust after Oxygen and silicon (atomic number: 13; atomic mass: 26.982; melting point: 660.3°C) [31, 32, 33, 34 and 35]. It was in the 19th century that a Danish physicist Christian Oersted first discovered Al by electrolytic reduction. Aluminum exposure on humans is said to have many deleterious effects. (1) Colon Inflammation and Inflammatory Bowel Disease (IBD) [36, 37, 38, 39 and 40].

**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).

![Google map of the experimental site](source: Google Earth, 2019)

**CLIMATE CONDITION**

Punjab Trans-Gangetic Plains Region Phagwara falls in Central Plain Zone of Punjab. Generally, June the hottest month of the year with a maximum temperature of 45°C and minimum 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 in 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. 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 flowering stage. KNO3 protein nanoparticle (1%) and Fibroin Nanoparticle (1%) were applied through a foliar application at flowering stage. The various measurements were taken at 90 DAT (Table 1 and 2).

Table 1: Treatments Detail

<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 adapted during the course of study are given below:

MORPHOLOGICAL PARAMETERS

Total Tillers number
Tiller numbers were counted at 60 and 90 DAT. Along with this number of Effective and Non Effective Tillers were also counted.

**RESULTS and DISCUSSION**

**Total Tiller Number**

Effect of Silk Fibroin Nanoparticle (NP) and Potassium Nitrate (KNO₃) and their combination on total tiller number 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). In the case of heavy-metal stress (T1) compared to control (T0) at an interval of 90 DAT it is evident that an average number of tillers has significantly increased by 16.83%. Exogenous application of KNO₃ particles on the leaves (T3) reduced the number of tillers by 27.74% as compared to (T1) at 90 DAT. In comparison with T1, Fibroin Nanoparticle (T2) exogenous application showed a 16.06 percent reduction in the number of tilers on the proposed DAT. T4, when compared with T1, showed that Fibroin NPs reduced the tillers number by only 2.62 % whereas KNO₃ NPS in T5 reduced the same by 20.15% when applied along with Aluminium stress. The average tiller number was significantly enhanced by about 2.62% with respect to T1 when treated with Fibroin NPs upon Aluminium stress whereas only sole Fibroin NPs were applied. KNO₃ Nanoparticles when applied upon Aluminium stress. Al stress on plants immediately suppresses the respiration process and produces Reactive Oxygen Species (ROS) [41, 42, 43, 44 and 45]. Mitochondrial Alternative Oxidase (AOX) was found to suppress Al stress by inhibiting ROS accumulation thereby reducing mitochondrial oxidative stress and enhancement in the growth capability of tobacco cells [46, 47, 48 49 and 50]. The Al hyperaccumulator plants especially the Symplocaceae family which includes many tropical and evergreen plant species [51, 52, 53, 54 and 55]. These species (seedlings and saplings) were grown in a hydroponic system with and without Al. It was seen that these seedlings were able to absorb the Al from the solution if provided with so and they showed a comparatively less mortality rate as compared to control treatment. The various mechanisms adopted by the plants against Aluminium stress such as Al tolerance and Al exclusion [56, 57, 58, 59 and 60]. The literature on Al exclusion was discussed how the Al-activated carboxylates are released on exposure to Al from the root tips. Carboxylate ligands (deprotonated Organic acids) helped in the internal detoxification of the Al which further led to the sequestration of Al-carboxylate complexes in the vacuole [61, 62, 63, 64 and 65]. Scientist discussed the peroxidase which is known for their variable structures and is present in plants, animals and even microorganisms [66, 67, 68, 69 and 70]. Researchers discussed on the threats that can be posed upon by the surging use of nano particles or nano-based products upon the various living and non-living forms on earth [71, 72, 73 74 and 74]. Mass research has been already performed on its construction and application but its contamination has still not been evaluated much [75, 76, 77, 78 and 79]. Agriculture and medicine have a lot of research going on upon nano products but they can be contaminative and can easily reach the soil or edible parts of the plant if applied in large quantities and could even be fatal in face of their ultra-fine size [80, 81 and 82]. Scientist discussed the loss of Nitrogen in the soil during the fertilization process. Nanotechnology plays a key role in increasing Nutrient Use Efficiency (NUE). Two nanocomposites based on Urea coated hydroxyapatite (UHA) displayed slow release behavior of Nitrogen in the soil [75, 76, and 80].

<table>
<thead>
<tr>
<th>Table 3. Tillers number in rice during Kharif</th>
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<tbody>
<tr>
<td>Treatments</td>
</tr>
<tr>
<td>------------</td>
</tr>
<tr>
<td>T0</td>
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<tr>
<td>T1</td>
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<td>T2</td>
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<td>T3</td>
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<td>T4</td>
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<td>T5</td>
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<td>T6</td>
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</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%).

Figure 2. Tiller number in 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**

Based on the above study it is clear that, the influence of metal and metal oxide nanoparticles on various crops at several diagnostic levels. Where growth and yield of crops were seen to be highly constrained, the concentration of nano particle was seen to rise in different plant parts such as grains, which could lead an easy entry to the food and humans. The effects of nano particles were both positive and negative depending upon the crop cultivar, treatment, and several growth conditions. Magnetic nano particles 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 average tiller number was significantly enhanced by about 2.62% with respect to T1 when treated with Fibroin NPs upon Aluminium stress whereas only sole Fibroin NPs were applied. KNO₃ Nanoparticles when applied upon Aluminium stress.

**Acknowledgments**

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Author Contributions

The study was designed by P.K. and Purnima, the morphological protocalizations were established, experiments were carried out and the data analysed 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.

References

10. S. Pathak, P. Kumar, P.K Mishra, M. Kumar, “Mycorrhiza assisted approach for bioremediation with special reference to biosorption”, Pollution Research, vol. 36(2), 2017


