Distribution Of Free Proline In Rice Stalk And Roots Grown Under Aluminium Toxic Soil

Prasann Kumar[1,] Purnima[1, 2,] Premasis Sukul[3]

[1]Department of Agronomy, School of Agriculture
Lovely Professional University, Jalandhar, Punjab, 144411, India
[2]Divisions of Research and Development
Lovely Professional University, Jalandhar, Punjab, 144411, India
[3]Department of Soil Science, School of Agriculture
Lovely Professional University, 144411, India

Email: premasis.20644@lpu.co.in

Abstract: The harmful amounts of metals in soils may be related to natural vegetation due to planting, manufacturing, mining, and waste management activities. Many of the acidic soils below pH 5.0 are essential growth restricting factors in plants, pH value as large as 5.5, however, may be easily reached. The issue is especially bad in extremely acidic subsoils that face trouble during liming and are aggravated by strongly acidic nitrogen engravings. The strong acidity in the subsoil (AI toxicity) decreases height of the plants, increases drought susceptibility and decreases the use of subsoil nutrients. The high temperature exacerbates aluminum toxicity d in cotton and wheat. The treatments T4, when compared with T1, showed that Fibroin NPs decreased the total free proline in rice stalk by only 14.19% whereas KNO_3 NPS in T5 enhanced the same by 39.92% when applied along with Aluminium stress. The total free proline in rice stalk was significantly enhanced by about 9.88% concerning T1 when treated with Fibroin NPs upon Aluminium stress whereas only sole Fibroin NPs were applied (T6). KNO_3 Nanoparticles when applied upon Aluminium stress (T6). The total free proline in rice roots was significantly decreased by about 17.39% concerning T1 when treated with Fibroin NPs upon Aluminium stress whereas only sole Fibroin NPs were applied (T6). KNO_3 Nanoparticles when applied upon Aluminium stress (T6).

Keywords: Acidity, Biology, Crop, Density, Energy, Forage

1. INTRODUCTION

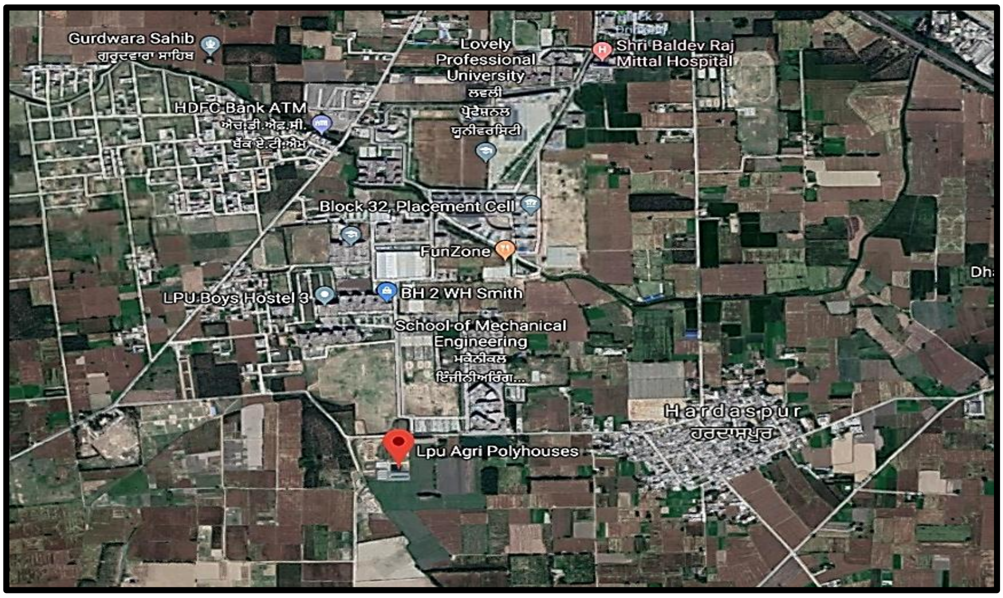
Many plant species have developed mechanisms for the alleviation of Al internally and/or externally such as secretion of various organic acids anions (citrate, malate, and oxalate) from roots which further chelate Al ions in the rhizosphere (Kumar, P., Dwivedi, P. (2018a), Kumar, P., Kumar S. et al. (2018b), Kumar, P., Misao, L., et al., 2018c, Kumar P, Dwivedi, P. 2018d, Kumar, P. and Purnima et al., 2018e, Kumar, P. Pathak, S. 2019f, Kumar, P. Siddique, A. et al., 2019g) Furthermore, several Al tolerance genes have been explored in plants especially rice. It was found that NH^{+}_{4} ions reduced aluminum accumulations in the roots by altering the cell wall properties which took place due to a decrease in pH by the NH_4^+ uptake. Ample of detoxification methodologies have been adopted by the plants 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 (Kumar, P., 2014r, Kumar, P., Dwivedi, P., Singh, P., 2012s, Mishra, P.K., Maurya, B.R., Kumar, Pp. 2012t, Kumar, P., Mandal, B., Dwivedi, P. 2011u. Kumar, P., Mandal, B., Dwivedi, P. 2011v, Kumar, P., Pathak, S. 2016w, Pathak, S., Kumar, P., Mishra, P.K., Kumar, M. 2016x). In India, it has been mainly grown in the Gangetic plains and coastal areas (Kumar, P., Pandey, A.K., et al., 2018aa, Kumar, P., Kumar, S. et al., 2018bb, Kumar, P., Krishna, V., et al., 2018cc, Kumar, P. and Dwivedi, P. 2018gg. Kumar P., Siddique A., et al., 2018ff, Kumar, P, Pathak, S, Kumar, M and Dwivedi, P. 2018cd, Kumar P. and Pathak S. 2018kk, Kumar P and Pathak S. 2018pq). Aluminum (Al) is the third-largest metallic element in the Earth's crust following oxygen and silicon. A large quantity of aluminosilicate minerals are found in soil. However, very small amounts are found in soluble form, able to influence biotechnology systems. The bioavailability is restricted mainly due to the acid environment and consequently leading to the toxicity. The most significant limitations of agriculture production are acidic soils (with a pH of 5.5 or lower). Acidic soils harm the production of staple food crops, in particular grain crops (Kumar, P., Harsavardhn, M. et al., 2018y. Kumar, P., Yumnam, J. et al., 2018z). Furthermore, acidification of agricultural soils is the result of certain agricultural practices such as the removal of products, the liquidation of nitrogen below the root zone, injudicious use of nitrogen fertilizers and organic build-up (Singh et al 2020a., Singh et al., 2020b., Sood, et al., 2020., Bhadrecha et al 2020, Singh et al., 2020c, Sharma et al., 2020, Singh et al., 2020d, Bhati et al., 2020, Singh et al., 2019, Sharma et al., 2019). Many plant species have developed certain mechanisms 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. Furthermore, several Al tolerance genes have been explored in plants especially rice. It was found that NH^{+}_{4} 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. Al-induced oxidative stress leads to the splitting of membrane integrity and stability. Plants such as Vignita radiate (green gram), Oryza sativa (rice) and Lolium penne (ryegrass) exhibited enhanced lipid peroxidation onto Al exposure. Even Brassia juncea genotypes verified enhanced oxidative stress upon Al exposure. Al enhanced the content of Ascorbate, dehydroascorbate (DHA) and total Ascorbate (ASA+DHA) in B.juncea species.When plants are brought under Al exposure they are involved in free radical scavenging activities such as DPPH and HRSA in two genotypes of mustard. 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 of native crops which have developed adaptive mechanisms (ChitraMani & Kumar, P. (2020); Sharma, M., & Kumar, P. (2020); Chand, J., & Kumar, P. (2020); Naik, M., & Kumar, P. (2020); Kumar, P., & Naik, M. (2020); Kumar, P., & Dwivedi, P. (2020); Devi, P., & Kumar, P. (2020); Kumari, P., & Kumar, P. (2020); Kaur, S., & Kumar, P. (2020); Devi, P., & Kumar, P. (2020); Sharma, K., & Kumar, P. (2020); Kumar, S. B. P. (2020); Devi, P., & Kumar, P. (2020); Chand, J., & Kumar, P. (2020).

2. MATERIALS AND METHODS

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)

Climate Condition

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 was procured from Punjab Agriculture University, Punjab. The pot size for the experiment was 30 cm jn diameter and 25 cm in height. Heavy metal stress was created by foliar application of aluminum (100 ppm) 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. The treatment and experimental lay out details are presented in Table 1 and 2, respectively.

Table 1: Treatments Detail

Treatments	Details of the treatments
T-0	Control
T-1	Al (100ppm)
T-2	Fibroin nanoparticle (1%)
T-3	KNO_3 protein nanoparticle (1%)
T-4	Al (100ppm) + Fibroin nanoparticle (1%)
T-5	Al (100ppm) + KNO_3 protein nanoparticle (1%)
T-6	Al (100ppm) + KNO_3 protein nanoparticle (1%) + Fibroin Nanoparticle (1%)

Table 2: Layout Details

S. No.	Particulars	Details
1.	Layout	CRD

2.	Treatment	7
3.	Replications	3
4.	Total Number of pots	7*3=21
5.	Soil per pot	7 kg
6.	Genotype	Pusa Basmati 1121

The total free proline was estimated by the method described by Bates et al., 1973.

3. RESULTS AND DISCUSSION

Total free Proline in rice stalk (µm g^{-1} fresh weight)

Effect of Silk Fibroin Nanoparticle (NP) and Potassium Nitrate (KNO_3) and their combination on total free proline in rice stalk 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). The average total free proline in rice stalk was significantly decreased by 32.01% when exposed to heavy metal stress (T1) as compared to control (T0) at 90 DAT of interval. Exogenous application of KNO_3 particles on the leaves (T3) decreased the total free proline in rice stalk by 1.93% as compared to (T1). In comparison to T1, the exogenous application of Fibroin Nanoparticle (T2) showed an increment in the total free proline in rice stalk by 25.48%. The treatments T4, when compared with T1, showed that Fibroin NPs decreased the total free proline in rice stalk by only 14.19% whereas KNO_3 NPS in T5 enhanced the same by 39.92% when applied along with Aluminium stress. The total free proline in rice stalk was significantly enhanced by about 9.88% concerning T1 when treated with Fibroin NPs upon Al stress whereas only sole Fibroin NPs were applied (T6). KNO_3 Nanoparticles when applied upon Al stress (T6). The effects of Al on the sugars (Sucrose, Glucose, Fructose) and phytohormones in the roots of Quercus Serrata Thumb seedlings were studied previously. It was found that when the ten-week-old plant was hydroponically brought in contact with Al, the concentration of starch and sucrose was reduced but the concentration of glucose was enhanced in the roots. Even abscisic acid (ABA) was seen to increase at a gradual rate during the experiment. Al was seen to promote root growth by a signal pathway for which glucose served as an energy source (Kumar P. 2018i., Kumar P. 2018ii., Kumar P. 2018iii, Kumar P.2018iv, Kumar P. 2018v. , Kumar P. 2018vi, Kumar P. 2018vii, Kumar P. 2018viii, Kumar P., Pathak S. 2018ix, Kumar P., Pathak S. 2018x, Kumar P., Pathak S. 2018xi, Kumar P., Pathak S, Kumar P., Pathak S. 2018xiii, Kumar P., Pathak S. 2018xiv, Kumar P., Pathak S. 2018xv, Kumar P., Pathak S. 2018xvi, Kumar P., Pathak S. 2018xvii, Kumar P., Pathak S. 2018xviii). Al on exposure to two different wheat cultivars having different Al resistance was found to reduce the Ca^{+2} and Mg^{+2} content of the leaves as well as a gradual increase in the lipid peroxidation. Further Al-resistant cultivar was seen to assemble more concentration of Ca^{+2} and Mg^{+2} in the leaves. However, Al stress on plants immediately suppresses the respiration process and produces Reactive Oxygen Species (ROS). Mitochondrial Alternative Oxidase (AOX) was found to suppress Al stress by inhibiting ROS accumulation and thereby, reducing mitochondrial oxidative stress and enhancement in the growth capability of tobacco cells.

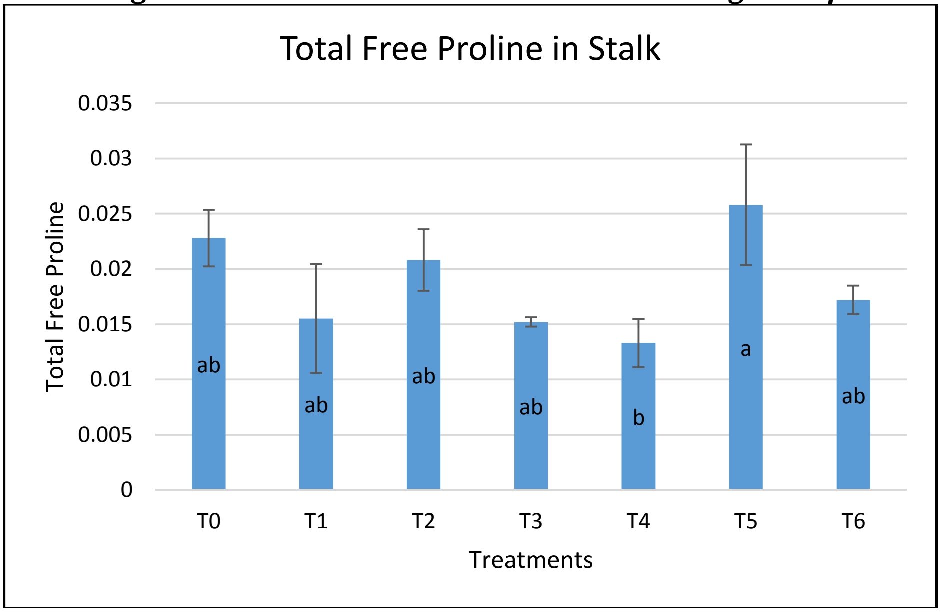
Table 3. Total free Proline (ppm) in rice stalk during *Kharif*

Treatments	Total free proline in rice stalk at 90 DAT
T0	$0.0228^{ab} \pm 0.00256$
T1	$0.0155^{ab} \pm 0.00492$

T2	$0.0208^{ab} \pm 0.00278$
T3	$0.0152^{ab} \pm 0.00041$
T4	$0.0133^{b} \pm 0.00219$
T5	$0.0258^{a} \pm 0.00546$
T6	$0.0172^{ab} \pm 0.00128$

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%).

Figure 2. Total free Proline in rice stalk 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: 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%).

Total free Proline in rice roots (μm g^{-1} fresh weight)

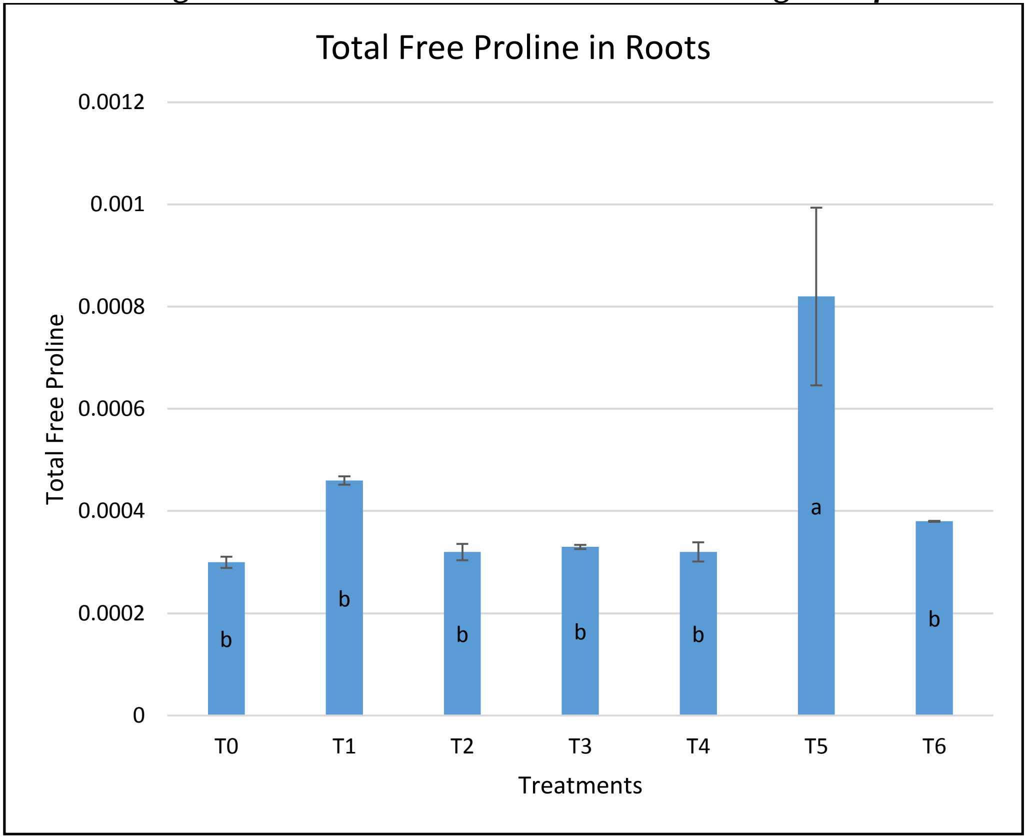
Effects of Silk Fibroin Nanoparticle (NP) and Potassium Nitrate (KNO_3) and their combination on total free proline in rice roots were studied in rice variety Pusa Basmati 1121 under the Aluminium toxicity stress (Kumar, P. (2019); Kumar, D., Rameshwar, S. D., & Kumar, P. (2019); Dey, S. R., & Kumar, P. (2019); Kumar et al. (2019); Dey, S. R., & Kumar, P. (2019); Kumar, P., & Pathak, S. (2018); Kumar, P., & Dwivedi, P. (2018); Kumar, P., & Pathak, S. (2018); Kumar et al.,2018; Kumar, P., & Hemantaranjan, A. (2017); Dwivedi, P., & Prasann, K. (2016). Kumar, P. (2014); Kumar, P. (2013); Kumar et al. (2013); Prasann, K. (2012); Kumar et al. (2011); Kumar et al. (2014). Data were recorded at 90 days after transplanting (DAT) (Table 4 and Fig. 3). The average total free proline in rice roots was significantly increased by 34.78% when exposed to heavy metal stress (T1) as compared to control (T0) at 90 DAT of interval. Exogenous application of KNO_3 particles on the leaves (T3) decreased the total free proline in rice roots by 28.26% as compared to (T1) at 90 DAT. In comparison to T1, the exogenous application of Fibroin Nanoparticle (T2) showed a decrease in the total free proline in rice roots by 30.43%, on proposed DAT. The treatments T4, when compared with T1, showed that Fibroin NPs decreased the total free proline in rice roots by only 30.43% whereas KNO_3 NPS in T5 enhanced the same by 43.90% when applied along with Aluminium stress. The total free proline in rice roots was significantly decreased by about 17.39% concerning T1 when treated with Fibroin NPs upon Aluminium stress. The effect of Al on tea *Camellia sinensis* L. was found to induce certain biochemical changes in the cell wall. It also reduced the amount of xyloglucan in the root apices and thereby, reduced the Al binding sites by the activity of loosening agents and further enhanced the root length. The responses of two species (Al-tolerant and Al-sensitive) of *Cicer arietinum* (Chickpea) upon exposure to Al were studied earlier. Al-tolerant plants were found to exhibit less oxidative stress and reduced damage to root growth because of the accumulation of H_2O_2 and Lipid Peroxidation. In the wild species and flowering species of *Camellia japonica* L., wild species possess red colour whereas the flowering species possess purple colour which may revert to the red colour of the wild species the next year. This purple coloration in the plants was said to be the outcome of Al chelation with the anthocyanin. To study the role of NO^-_3 and NH^+_4 in Al binding capacity in the roots, it was found that on exposure to NH^+_4, Al binding capacity of rice roots reduced as compared to NO^-_3 exposure on roots. The reason may be attribute to the fact that NH^+_4 uptake by the roots led to pH changes which further gave rise to change in cell wall properties and reduction in not only the Al binding groups such as –OH and COO^- but also pectin and hemicellulose ((Siddique, A. Kumar, P. 2018h, Siddique, A., Kandpal, G., Kumar P. 2018i, Pathak, S., Kumar, P., P.K Mishra, M. Kumar, M. 2017j, Prakash, A., P. Kumar, 2017k., Kumar, P., Mandal, B., 2014L, Kumar, P., Mandal, B., Dwivedi P., 2014m., Kumar, P., Kumar, P.K., Singh, S. 2014n, Kumar, P. 2013o., Kumar, P., Dwivedi, P. 2015p, Gogia, N., Kumar, P., Singh, J., Rani, A. Sirohi, Kumar, P. 2014q).

Table 4. Total free Proline in rice roots during *Kharif*

Treatments	Total free proline in rice roots at 90 DAT
T0	$0.0003^b \pm 0.000011$
T1	$0.00046^b \pm 0.000008$
T2	$0.00032^b \pm 0.000016$
T3	$0.00033^b \pm 0.000004$
T4	$0.00032^b \pm 0.000019$
T5	$0.00082^a \pm 0.000174$
T6	$0.00038^b \pm 0.000001$

where, Data are in the form mean± SEM. Significance at $P \leq 0.05$ 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%).

Figure 3. Total free Proline in rice roots 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: 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%).

4. CONCLUSION

Based on the previous report, it is evident that there are many diagnostic degrees of impact of nanoparticles of metal and metal oxide on specific crops. Based on the above experiment conducted, it is clear that nanoparticles influenced the vital physiological and metabolic process. Among them, the total free proline was targeted to correlate the changes along with applied nanoparticles.

Acknowledgments

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

Author Contributions

The study was designed by P.K. and Purnima along with experimentation, data analysis and interpretation.The paper has been written by P.K and P.S.

Conflict of Interest Statement

The authors have no conflict of interest,

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