

Mitigation of Aluminium Contamination in *Oryza sativa* L. after a Treatment with Silk Fibroin Nanoparticle and KNO_3^+ Protein Nanoparticle

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Abstract

Natural soil properties or farming, manufacturing, mining, and waste disposal practices can be the reason for toxic levels of metals in soils. For plants, many acid-related soils below pH 5.0 but may have photographic levels as high as 5.5 are important growth-limiting factors. In highly acidic subsoils that are hard to lime, the problem is particularly serious and is being exacerbated by heavy applications of nitrogen fertilizer that form acids. The strong acidity in the subsoil (Al toxicity) decreases depths of the plants, increases drought susceptibility and decreases the use of subsoil nutrients. The high temperature of aluminium toxicity is exacerbated in cotton and wheat. The present study was carried out to evaluate the compatibility of Silk fibroin nanoparticle and Potassium nitrate nanoparticle in the mitigation of the induced toxic effect of aluminium 90 DAT of rice variety Pusa Basmati 1121. At this time, we are talking the mitigation with special reference to total effective tiller count in rice plant. 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.

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

Introduction

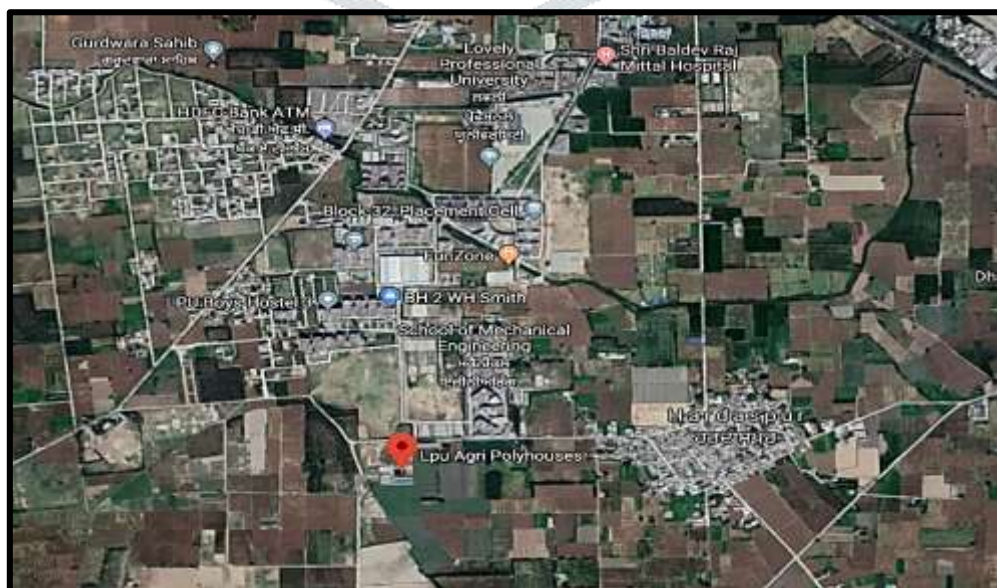
Many plant species have developed certain plant species for the alleviation of Al internally and/or externally such as secretion of various organic acids anions (citrate, malate, and oxalate) from roots which further chelate Al ions in the rhizosphere [1, 2, 3 and 4]. Furthermore, a number of Al tolerance genes have been explored in plants especially rice [5, 6, 7, 8 and 9]. 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 (Wang, et al 2015). 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 [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]. 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]. The word rice is derived from the French word *ris* 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).

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 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 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

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 PARAMETERS

Total Non-Effective Tillers number

Total non- effective tiller numbers were counted at 90 DAT.

RESULTS and DISCUSSION

Total Non-Effective Tiller Number

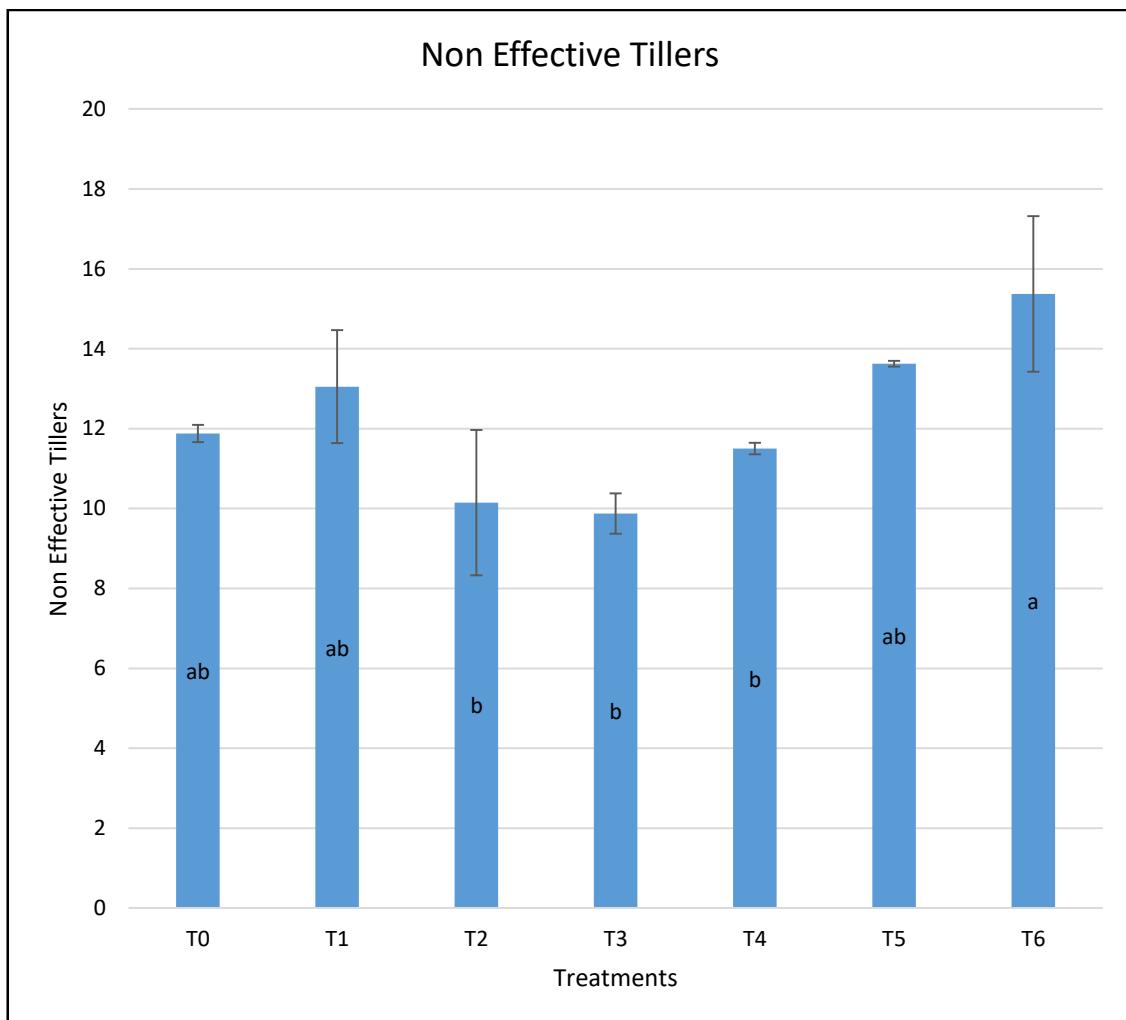
Effect of Silk Fibroin Nanoparticle (NP) and Potassium Nitrate (KNO_3) and their combination on total non-effective tiller number was studied in rice variety Pusa Basmati 1121 under the Aluminium toxicity stress. Data were recorded at 90 days after transplanting (DAT) (Table 3 and Fig. 2). It is evident that the average number of non-effective tillers were significantly increased by 13.05 % 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) reduced the number of non-effective tillers by 24.32% as compared to (T1) at 90 DAT. In comparison to T1, the exogenous application of Fibroin Nanoparticle (T2) showed a reduction in the number of non-effective tillers by 22.22%, on proposed DAT. The treatments T4, when compared with T1, showed that Fibroin NPs reduced the non-effective tillers number by only 11.87 % whereas KNO_3 NPS in T5 enhanced the same by 4.22% when applied along with Aluminium stress [41, 42, 43 and 44]. The average non-effective tiller number was significantly enhanced by about 9.0% 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 (T6). Scientists 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. W Workers examined the damage as well as the response of Mustard (*Brassica juncea*) to the Aluminium stress [45, 46, 47, 48 and 49]. 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 [50 and 51]. Enzymatic activity was seen too much in Pusa Tarak in comparison to control and Pusa Vijay. Sade *et al.* (2016) discussed Al as one of the major limiting factors affecting the plant productivity especially in the acidic soils and is said to pose around 25-80% of yield losses depending upon the plant's cultivar. Many Aluminium tolerance factors were discussed here such as the exclusion of Al from the roots and the tolerance ability of symplast for Al. Though no Al tolerant cultivar has been brought to light yet, still formation of transgenics has been discussed here

Table 3. Total Non-Effective Tiller Number of rice during *Kharif*

Treatments	Non-effective tiller number at 90 DAT
T0	11.875 ^{ab} ± 0.216506
T1	13.05 ^{ab} ± 1.414508
T2	10.15 ^b ± 1.818653
T3	9.875 ^b ± 0.505181
T4	11.5 ^b ± 0.144338
T5	13.625 ^{ab} ± 0.072169
T6	15.375 ^a ± 1.948557

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: 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. Total Non-Effective Tiller Number of rice during *Kharif*



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

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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.

References

1. P. Kumar, P. Dwivedi, "Cadmium-induced alteration in leaf length, leaf width and their ratio of glomus treated sorghum seed" *Journal of Pharmacognosy and Phytochemistry*, vol.7 (6), pp. 138-141, 2018.
2. P. Kumar, S. Kumar et al. "Glomus and putrescine based mitigation of cadmium-induced toxicity in maize" *Journal of Pharmacognosy and Phytochemistry*. vol.7 (5), pp.2384—2386, 2018.
3. P. Kumar, L. Misao, et al., "Polyamines and Mycorrhiza based mitigation of cadmium-induced toxicity for plant height and leaf number in maize" *International Journal of Chemical Studies*, vol.6 (5), pp.2491-2494, 2018.
4. P. Kumar, P. Dwivedi, "Putrescine and Glomus Mycorrhiza moderate cadmium actuated stress reaction in *Zea mays* L. by means of extraordinary reference to sugar and protein" *Vegetos*. vol.31 (3), pp.74-77, 2018.
5. P. Kumar, Purnima et al., "Impact of Polyamines and Mycorrhiza on Chlorophyll Substance of Maize Grown under Cadmium Toxicity" *International Journal of Current Microbiology and Applied Sciences*, vol. 7(10), pp. 1635-1639, 2018.
6. P. Kumar, S. Pathak, "Responsiveness index of sorghum (*Sorghum bicolor* (L.) Moench) grown under cadmium contaminated soil treated with putrescine and mycorrhiza" *Bangladesh J. Bot.* vol.48 (1), 2019.
7. P. Kumar, A. Siddique, et al., "Role of Polyamines and Endo-mycorrhiza on Leaf Morphology of Sorghum Grown under Cadmium Toxicity" *Biological Forum – An International Journal*. vol.11 (1) pp. 01-05, 2019.
8. A. Siddique, P. Kumar, "Physiological and Biochemical basis of Pre-sowing soaking seed treatments-An overview" *Plant Archive*, vol.18(2), pp. 1933-1937, 2018.
9. A. Siddique, G. Kandpal, P. Kumar "Proline accumulation and its defensive role under Diverse Stress condition in Plants: An Overview" *Journal of Pure and Applied Microbiology*, vol.12 (3) pp.1655-1659, 2018.

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
11. A. Prakash, P. Kumar, "Evaluation of heavy metal scavenging competence by in-vivo grown *Ricinus communis* L. using atomic absorption spectrophotometer" *Pollution Research*, vol.37(2), pp.148-151, 2017.
12. P. Kumar, B. Mandal, P. Dwivedi, "Combating heavy metals toxicity from hazardous waste sites by harnessing scavenging activity of some vegetable plants" *vegetos*, vol.26(2), pp. 416-425, 2014.
13. P. Kumar, B. Mandal, P. Dwivedi, "Phytoremediation for defending heavy metal stress in weed flora" *International Journal of Agriculture, Environment & Biotechnology*, vol.6(4), pp. 587-595, 2014]
14. P. Kumar, P.K. Kumar, S. Singh, "Heavy metal analysis in the root, shoot and leaf of *psidium guajava* l. by using atomic absorption spectrophotometer" *Pollution Research*, vol.33 (4) pp.135-138, 2014.
15. P. Kumar, "Cultivation of traditional crops: an overlooked answer. *Agriculture Update*, vol.8 (3), pp.504-508, 2013.
16. P. Kumar, P. Dwivedi, "Role of polyamines for mitigation of cadmium toxicity in sorghum crop" *Journal of Scientific Research, B.H.U.*, vol.59, pp.121-148, 2015.
17. N. Gogia, P. Kumar, J. Singh, A. Rani, A. Sirohi, P. Kumar, "Cloning and molecular characterization of lactine gene from garlic (*Allium sativum* L.)" *International Journal of Agriculture, Environment and Biotechnology*, vol.7 (1), pp.1-10, 2014.
18. P. Kumar, "Studies on cadmium, lead, chromium and nickel scavenging capacity by in-vivo grown *Musa paradisiacal* using atomic absorption spectroscopy" *Journal of Functional and Environmental Botany*, vol.4(1), pp.22-25, 2014.
19. P. Kumar, P. Dwivedi, P. Singh, "Role of polyamine in combating heavy metal stress in *stevia Rebaudiana* Bertoni plants under in vitro condition" *International Journal of Agriculture, Environment and Biotechnology*, vol.5(3) pp.185-187, 2012.
20. P.K. Mishra, B.R. Maurya, P. Kumar, "Studies on the biochemical composition of *Parthenium hysterophorus* L. in different season" *Journal of Functional and Environmental Botany*, vol. 2(2) pp.1-6, 2012
21. P. Kumar, B. Mandal, P. Dwivedi, "Heavy metal scavenging capacity of *Mentha spicata* and *Allium cepa*" *Medicinal Plant-International Journal of Phytomedicines and Related Industries* vol. 3(4) pp. 315-318, 2011.
22. P. Kumar, B. Mandal, P. Dwivedi, "Screening plant species for their capacity of scavenging heavy metals from soils and sludges. *Journal of Applied Horticulture* vol.13 (2) pp.144-146, 2011.
23. P. Kumar, S. Pathak, "Heavy metal contagion in seed: its delivery, distribution, and uptake" *Journal of the Kalash Sciences, An International Journal*, vol. 4(2), pp. 65-66, 2016.
24. S. Pathak, P. Kumar, P.K. Mishra, M. Kumar, "Plant-based remediation of arsenic-contaminated soil with special reference to sorghum- a sustainable approach for the cure". *Journal of the Kalash Sciences, An International Journal*, 4(2): 61-65, 2016.

25. P. Kumar, P. Dwivedi P, "Role of polyamines for mitigation of cadmium toxicity in sorghum crop" Journal of Scientific Research, B.H.U., vol. 59, pp.121-148, 2015.
26. P. Kumar, "Signal transduction in the plant with respect to heavy metal toxicity: An overview" CRC Press, Taylor & Francis Group, Pp. 394, 2018.
27. P. Kumar, P. Dwivedi, "Phytoremediation of cadmium through Sorghum" Daya Publishing House. Pp. 311-342, 2014.
28. P. Kumar, A.K. Pandey, V. Krishna, "Phytoextraction of lead, chromium, cadmium, and nickel by tagetes plant grown at the hazardous waste site" Annals of biology, vol.34(3), pp. 287-289, 2018.
29. P. Kumar, S. R. Dey, Komal, et al., "Effect of Polyamines and endo-mycorrhiza on chlorophyll a, b ratio and total carotenoids in leaves of Sorghum grown under cadmium toxicity" International Journal of Chemical Studies. vol.7 (1), pp. 2402-2406, 2019.
30. P. Kumar, P. Dwivedi, "Ameliorative Effects of Polyamines for Combating Heavy Metal Toxicity in Plants Growing in Contaminated Sites with Special Reference to Cadmium" CRC Press, Taylor & Francis Group, UK. Pp. 404, 2018.
31. P. Kumar, A. Siddique, Thakur Vishal, Singh Manpreet, "Effect of putrescine and glomus on total reducing sugar in cadmium treated sorghum crop" Journal of Pharmacognosy and Phytochemistry, vol.8(2), pp.313-316, 2019.
32. P. Kumar, A. Siddique, Thongbam Satyajyoti, Chopra Prafful, Kumar Sunil, "Cadmium-induced changes in total starch, total amylose and amylopectin content in putrescine and mycorrhiza treated sorghum crop" Nature, Environment and Pollution Technology, vol.18(2), 2019.
33. P. Kumar, S. R. Dey, "Carbon Pools: Key for Global Climate Models" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
34. P. Kumar, S. R. Dey, "Three Dimensional Approach for the Mitigation of Cadmium and Lead Toxicity in Legumes" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
35. P. Kumar, S. R. Dey, "Apple of Tropics: Its Production and Quality Assurance with Special Reference to Nutrition" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
36. P. Kumar, S. R. Dey, "Wheat Cultivation: Strategic Cereal Crops for Majority" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
37. P. Kumar, S. R. Dey, "Sorghum [*Sorghum bicolor* (L.) Moench]: A Major Cereal of the World" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
38. P. Kumar, S. R. Dey, "Cadmium Induced Toxicity in Legumes with special reference to *Pisum sativum* L." Accepted in: EnV Book Series, Discovery Publication, 2020-21.
39. P. Kumar, S. R. Dey, "Aluminum Induced Oxidative Stress in Rice (*Oryza sativa* L.)" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
40. P. Kumar, S. R. Dey, "Isolation of Secondary Metabolites: An Overview" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
41. P. Kumar, S. R. Dey, "Leaf Area Index: Process Based Model" Accepted in: EnV Book Series, Discovery Publication, 2020-21.

42. P. Kumar, S. R. Dey, "Effect of Nitrogen and Phosphorus on Pearl Millet with Respect to Its Growth and Development" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
43. P. Kumar, S. R. Dey, "Fluorine and Its Effect on Crops" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
44. P. Kumar, S. R. Dey, "Polyamine and plant stress response: Potential mechanism of action" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
45. P. Kumar, S. R. Dey, "Physiological Attributes Of Sorghum" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
46. P. Kumar, S. R. Dey, "Plant Morphology: An Overview" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
47. P. Kumar, S. R. Dey, "Metal Extraction from Soil by Microorganism-Assisted Plant" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
48. P. Kumar, S. R. Dey, "Photosynthesis it's Significance in Plant Growth, Development and Bio productivity Gaseous Fluxes in Atmosphere" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
49. P. Kumar, S. R. Dey, "Plant Hormones, Senescence and Abscission In Crops" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
50. P. Kumar, S. R. Dey, "Degradation of Natural Resources: Perhaps the Gravest Lapses of Mankind" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
51. P. Kumar, S. R. Dey, "Cultivation of Black Rice: Remunerative Advantage for Farmers" Accepted in: EnV Book Series, Discovery Publication, 2020-21.