Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

p-ISSN: 0972-6268
e-ISSN: 2395-3454

Vol. 18 | No. 2 | pp. 525-530 | 2019

Original Research Paper | Open Access

Cadmium Induced Changes in Total Starch, Total Amylose and Amylopectin Content in Putrescine and Mycorrhiza Treated Sorghum Crop

Prasann Kumar*(**)(***)†, Anaytullah Siddique*, Satyajyoti Thongbam*, Prafful Chopra* and Sunil Kumar*
***Department of Agronomy, School of Agriculture, Lovely Professional University, Jalandhar, Punjab, 144 411, India
****Division of Research and Development, Lovely Professional University, Jalandhar, Punjab, 144 411, India
*****Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, 221005, U.P., India
†Corresponding author: Prasann Kumar

Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 04-11-2018
Accepted: 09-12-2018

Key Words:
Cadmium
Sorghum
Polyamines
Mycorrhiza

ABSTRACT

The current investigation was carried out to evaluate the ameliorative effect of polyamines and mycorrhiza in the induced toxic effect of cadmium at 30, 60 and 90 DAS old of sorghum variety CSV15. The significant hazardous effects and oxidative damage of cadmium nitrate (70 ppm and 150ppm) were evidenced by decreased content of total starch, total amylose and amylopectin content ($mg.g^{-1}$ fresh weight). The reverse responses were observed by the application of putrescine and mycorrhiza dose on the plants.

INTRODUCTION

Sorghum is popular among the people residing under unsecured semi-arid tropics (Kumar et al. 2016a). Heavy metal contamination threats the critical limit of alarm in most of the cultivated and periurban areas around us. That is why it is considered as the major concern in India and abroad. Polyamine like putrescine content is altered in response to the exposure of heavy metals (Kumar et al. 2011a, b and Kumar et al. 2016a, b). Polyamines level in stressed plants have adjustive importance, thanks to their involvement in the significant balance of aiming the pigment loss, membrane integrity and the function of all organelles within the cells (Kumar & Dwivedi 2018a,b,c and Kumar et al. 2018b). The phospholipid part of plasma membrane is integrated with the externally applied polyamines and plays a significant role in the stability of the same. Polyamines like putrescine also protect the membrane from oxidative damage as they act as free radical scavengers (Kumar et al. 2018a, Pathak et al. 2017). Response to abiotic injury and mineral nutrient deficiency is associated with the production of conjugated PAs in plants. We have tested many plant species for their capability of scavenging significant metals from soil and sludge and eventually we tend to reached on the conclusion that among the tested plants, *Sorghum vulgare* L. is quite custom-made to grow on contaminated places with relation to alternative plant and ready to mitigate the significant metal toxicity from venturous waste site or cultivated site (Kumar & Dwivedi 2018, Kumar et al. 2012, Kumar et al. 2013, Siddique et al. 2018). The metallic element (Cd) may be an extremely deadly element, which has been hierarchial seventh among the highest twenty toxins (Kumar & Dwivedi 2018a). The metallic element may be a doubtless deadly metal and so its transfer from plants to humans is of major concern.

MATERIALS AND METHODS

The experimental work was carried out under a pot experiment with one genotype of sorghum CSV 15 in the polyhouse of the Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. Seeds were collected from the Directorate of Sorghum Research for the experiment. The pot size for the experiment was of the diameter of 30 cm and 25 cm tall with the capacity of 10 kg soil, having a tiny low hole at the underside. The soils of the pots were in the combination of soil + FYM in 3:1, and inoculated with seeds of *Sorghum vulgare* L. Targeted pots were inoculated with endomycorrhiza *Glomus* sp. and at that time significant metal stress was created in the plants by the exogenous application of cadmium nitrate in the soil. Based on the initial screening, the two significant concentrations of Cd were selected, i.e., 0.07 % per 10 kg and 0.15 % per 10 kg of soil.

With the rate of 2.5 and 5.0 mM, the growth regulator putrescine was applied in the form of foliar way. The CRD design was used for the arrangement of pots with recommended spacing. In the experiment, 18 treatments were applied. Five times replication was done with each and every treatment. The data were analysed by the application of Origin 6.1-advance scientific graphing. One way multivariate analysis was performed.

Estimation of total starch (mg.g^{-1} fresh weight): The total starch in the plant sample was estimated by the following method proposed by Sadasuvam & Manickam (1992). In order to remove the sugars, the sample was treated with 80% alcohol and then starch was extracted with perchloric acid. Starch is hydrolysed to glucose in a hot acidic medium and dehydrated to furfural hydroxymethyl. This compound forms anthrone-coloured green products. The amount of glucose content in the sample was measured with the help of a standard. The value obtained was multiplied by a factor 0.9 to arrive at the total starch content in the sample.

Estimation of total amylose (mg.g^{-1} fresh weight): The total amylose content in the plant sample was estimated following the method proposed by Sadasuvam & Manickam (1992). Amylose is a linear polymer of D-glucose units which are linked by α-1, 4 glycosidic bonds. Amylose gives purple colour with iodine. It exists in coiled form and each coil contains six glucose residues. The iodine is absorbed within the helical coils of amylose to produce a blue-coloured complex which is measured colorimetrically at 590 nm. 100 mg of dry and powdered sample was mixed with 1 mL of 80% ethanol and 10 mL of NaOH. This mixture was mixed well and kept overnight at room temperature. After that 2.5 mL of extract was taken and it was added with 20 mL of distilled water and three drops of 0.1% phenolphthalein indicator. A pink colour was developed. The 0.1 N HCl was added in the mixture until the pink colour disappeared. The iodine reagent of 1 mL was added and the final volume was made to 50 mL with the help of distilled water. The absorbance was taken at 590 nm with the help of a spectrophotometer. The amount of amylose in the sample was measured with the help of a standard curve prepared from amylose (range 0.2-1.0 mg) against a blank for which diluted 1 mL of iodine reagent was added to 50 mL of distilled water. 100 mg of amylose was added in 10 mL of 1N NaOH and the mixture was finally diluted to 100 mL with the help of distilled water (1 mg.mL^{-1}). From this stock solution, different concentrations of the amylose solution were prepared by taking 0.2, 0.4, 0.6, 0.8 and 1.0 mL of the stock solution in a separate test tube. The final volume of these test tubes was made to be 1 mL by adding distilled water. The standard curve was prepared by plotting the absorbance value at 590 nm.

Estimation of amylopectin (mg.g^{-1} fresh weight): The amount of amylopectin was calculated by subtracting the amylose concentration from the concentration of starch.

Amount of amylopectin (mg) = Amount of starch (mg) – Amount of amylose (mg)

RESULTS AND DISCUSSION

During the two subsequent years under cadmium stress, the effect of polyamine (putrescine), mycorrhiza and their combinations on total starch (mg.g^{-1} fresh weight) were studied in sorghum variety CSV15. At 30, 60 and 90 days after sowing (DAS) data were recorded (Fig. 1a & 1b). During the first year, it is evident that the average total starch content was significantly reduced by 56.70%, 23.2%, and 8.07% when exposed to heavy metal stress (T6) as compared to control (T0) at 30, 60 and 90 DAS of interval. When sorghum was exposed to a higher dose of heavy metal (T12), the total starch content was significantly reduced by 67.07%, 42.61% and 32.2% compared to control (T0) at the proposed interval. Endomycorrhiza in the soil (T7) showed the amelioration effect of cadmium in terms of total starch content enhancement with the rate of 6.92%, 0.74% and 0.56% with respect to T6. The total increased starch content was 4.76%, 1.11% and 1.79% in the T13 with respect to T12. Compared to T6, the putrescine (T8) exogenous application showed a mitigating effect by increasing the total starch content of proposed DAS by 12.90%, 10.80% and 2.01 percent. The average value of the starch was increased significantly by 22.94%, 13.40% and 1.23% compared to T6 when exposed with a higher dose of putrescine (T9) compared to T8. The total starch content increased significantly with 6.92%, 3.53% and 3.58% at proposed rate of interval, when T14 was compared with T12. The total starch increased significantly when treated with a higher dose of putrescine (T15) as compared to T12 by 9.22%, 4.09%, and 3.47 percent with respect to T14. The best mitigation was shown by a combination of putrescine and mycorrhiza in T10 treatment by increasing the total starch content by 24.67%, 16.38% and 1.34%, respectively. The comparison results of the T11 and T6 showed the enhancement of total starch content by 30.30%, 16.01% and 2.35%, respectively. In treatment T16, the increased starch content was 14.28%, 4.36% and 4.26%, respectively. The treatment T17 showed better results; a significant increase in total starch content by 12.12%, 1.48% and 5.26% with respect to T12 was observed. So, the combination of putrescine and mycorrhiza showed the best combination for the mitigation of cadmium toxicity for the total starch content. The similar trends were found during the study made in the second year of the experiment. Kumar et al.

2018 a, b, c, reported that, total starch, total amylose and amylopectin were decreased by 100 µM Cd, but reduced by 200 µM Cd in mature maize leaves and reduced by both concentrations in aging leaves (Kumar 2018 Kumar & Dwivedi 2014, 2018a,b,c,d; Kumar et al. 2011a,b 2012, 2013, 2016a,b, Pathak 2017). Low concentration of cadmium (0.05 and 0.1 µM) also has a negative effect on the synthesis of starch, amylose and amylopectin (Siddique et al. 2018, Kumar et al. 2018).

In concern to the total amylose content in sorghum, the proposed protocol was followed (Fig. 2a & 2b). It is evident that the average total amylose content was significantly reduced by 28.579%, 5.76% and 3.89% when exposed to heavy metal stress (T6) as compared to control (T0). In treatments T12, sorghum was exposed to a higher dose of Cd that leads to reduction in total amylose by 63.49%, 12.5% and 8.65% as compared to control (T0). Endomycorrhiza application in the soil (T7) showed the mitigation effect by increasing the total amylose content by 3.17%, 0.64% and 0.43% as compared to T6. When treatment T13 was compared to T12, the total amylose content increased significantly by 12.69%, 2.56% and 1.73%. In comparison to T6, the exogenous application of putrescine (T8) showed a mitigating effect by increasing total amylose content by 1.58%, 0.32% and 0.21%, respectively. The average total amylose content was significantly enhanced as compared to T6 by 3.17%, 1.60% and 1.08% when treated with a higher dose of putrescine (T9) with respect to T8. In the treatment T14, the total amylose content increased significantly by 25.39%, 3.52% and 3.46% with respect to T12. The average total amylose was significantly enhanced as compared to T12 by 15.87%, 3.20% and 2.16% when treated with a higher dose of putrescine (T15) with respect to T14. The combination of putrescine and mycorrhiza showed the best mitigation effect in treatment T10 by increasing the total amylose content by 4.76%, 0.96% and 0.64% with respect to treatment T6 at proposed DAS. When treatment T11 was compared with treatment T6, a significant total amylose content was increased by 1.58%, 0.32% and 0.21%, respectively. A similar effect was seen in the treatment (T16) with respect to treatment T12. In this treatment (T16), the total amylose content increased significantly by 22.22%, 4.48% and 3.03%, respectively at proposed DAS. The treatment T17 was found to show better results; a significant increase in total amylose content by 22.22%, 4.84% and 3.67% with respect to T12 was observed. So, the combination of putrescine and mycorrhiza showed the best combination for the mitigation of cadmium toxicity for the total amylose content. The similar trends were found during the study made in the second year of the experiment. A three Cd^{2+} treatment field experiment was conducted with two different Cd^{2+} tolerance wheat cultivars to investigate the effects of cadmium (Pathak et al. 2017 and Siddique et al. 2018). The results indicated that during the grain filling stage, the change of the ratio of amylose and amylopectin content in two cultivars in different Cd^{2+} concentrations varied as "up-down-up", except the change of the ratio of amylose and amylopectin content in Xinmai 21 showed "up-down" in high Cd^{2+} concentration. Compared to control, the average ratios of amylose and amylopectin content in Xinmai 21 treated by low and high Cd^{2+} concentrations were increased, but on the contrary, in aikang 58 were reduced (Kumar & Dwivedi 2016a, b; Kumar 2018; Kumar et al. 2012, 2013, 2018 a, b, c).

Amylopectin (mg.g^{-1} fresh weight) was studied in sorghum variety CSV15 during the two subsequent years under the cadmium stress (Fig. 3a & 3b). During the first year, it is evident that the average amylopectin content was significantly reduced by 40.0%, 26.12% and 21.08% when exposed to heavy metal stress (T6) as compared to control (T0). Exposure with a higher dose of heavy metal (T12) leads to amylopectin reduction by 60.92%, 41.78% and 32.51% as compared to control (T0). Exogenous application of endomycorrhiza in the soil (T7) showed the mitigation effect by increasing the amylopectin content by 11.76%, 0.806% and 6.27% as compared to T6. When treatment T13 was compared to T12, the amylopectin content increased significantly by 0.84%, 0.57% and 0.44% at proposed DAS. With respect to T6, the exogenous application of putrescine (T8) showed a mitigating effect by increasing amylopectin content by 9.66%, 6.62% and 5.15%. The average amylopectin content was significantly enhanced as compared to T6 by 9.24%, 6.34% and 4.93% when treated with a higher dose of putrescine (T9) with respect to T8. T14 compared to T12, the total amylopectin content increased significantly by 1.68%, 1.15% and 0.89%. The average total amylopectin was significantly enhanced as compared to T12 by 4.20%, 2.88% and 2.24% when treated with a higher dose of putrescine (T15) with respect to T14. The combination of putrescine and mycorrhiza showed the best mitigation effect in treatment T10 by increasing the total amylopectin content by 12.18%, 8.35% and 6.50% with respect to treatment T6. When treatment T11 was compared with treatment T6 then significant total amylopectin content was increased by 13.00%, 9.22% and 7.17%, respectively. In this treatment (T16), the total amylopectin content was found to increase significantly by 34.87%, 23.91% and 18.60%, respectively. The treatment T17 was found to show better results; a significant increase in total amylopectin content by 14.70%, 10.08% and 7.84% with respect to T12 was observed. So, the combination of

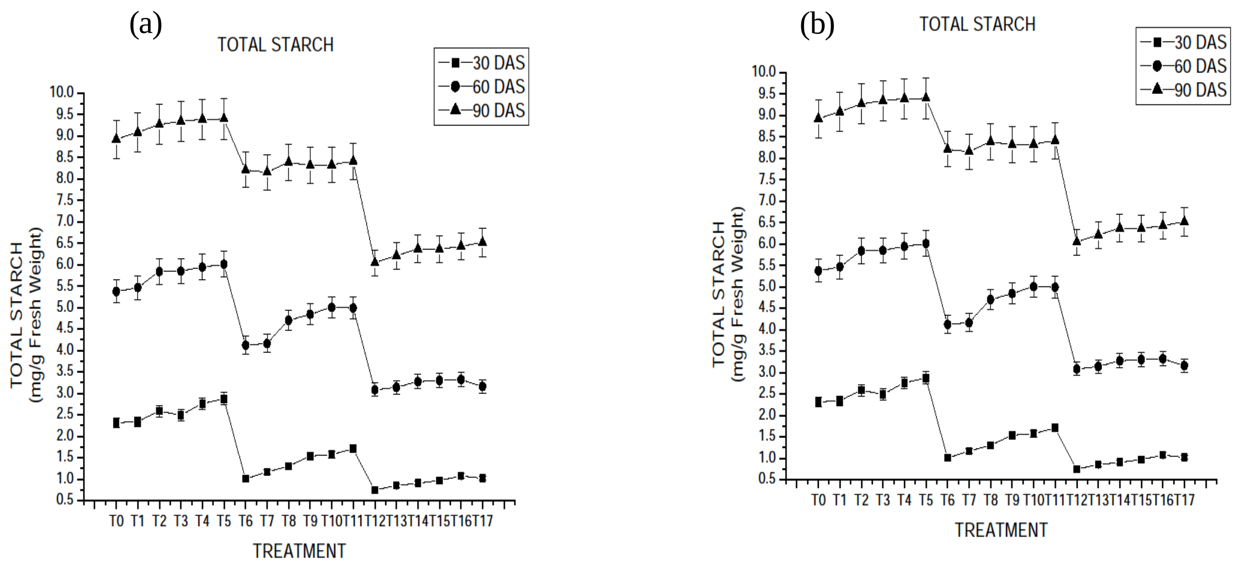

Fig. 1a & 1b: Total starch (mg.g[-1] fresh weight) of sorghum during Kharif season of first and second year subsequently.

Note: DAS=Days after sowing. Data are in the form of Mean±SEM. S=Significance at P≤0.05 and P≤0.01, NS=Non Significant at P≤0.05 and P≤0.01 using Origin 6.1. T0=Control, T1=Control + Mycorrhiza, T2=Control + 2.5mM Putrescine, T3=Control + 5mM Putrescine,T4=Control + 2.5mM Putrescine + Mycorrhiza, T5=Control + 5mM Putrescine + Mycorrhiza, T6=0.07% $Cd(NO_3)_2$, T7=0.07% $Cd(NO_3)_2$ + Mycorrhiza, T8=0.07% $Cd(NO_3)_2$ + 2.5mM Putrescine, T9=0.07% $Cd(NO_3)_2$ + 5mM Putrescine, T10=0.07% $Cd(NO_3)_2$ + 2.5mM Putrescine + Mycorrhiza, T11=0.07% $Cd(NO_3)_2$ + 5mM Putrescine + Mycorrhiza, T12=0.15% $Cd(NO_3)_2$, T13=0.15% $Cd(NO_3)_2$ + Mycorrhiza, T14=0.15% $Cd(NO_3)_2$ + 2.5mM Putrescine, T15=0.15% $Cd(NO_3)_2$ + 5mM Putrescine, T16=0.15% $Cd(NO_3)_2$ + 2.5mM Putrescine + Mycorrhiza, T17=0.15% $Cd(NO_3)_2$ + 5mM Putrescine + Mycorrhiza)

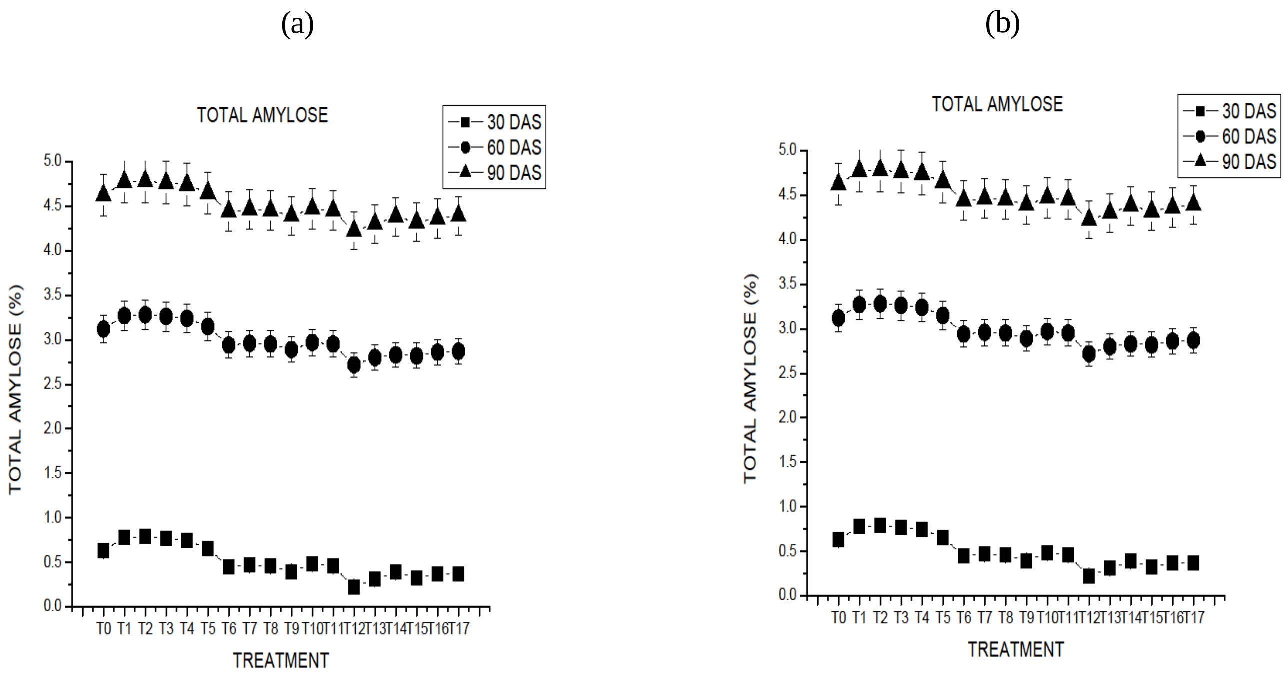

Fig 2a & 2b: Total amylose (%) of sorghum during Kharif season of two subsequent years of research.
(Note as per Fig. 1)

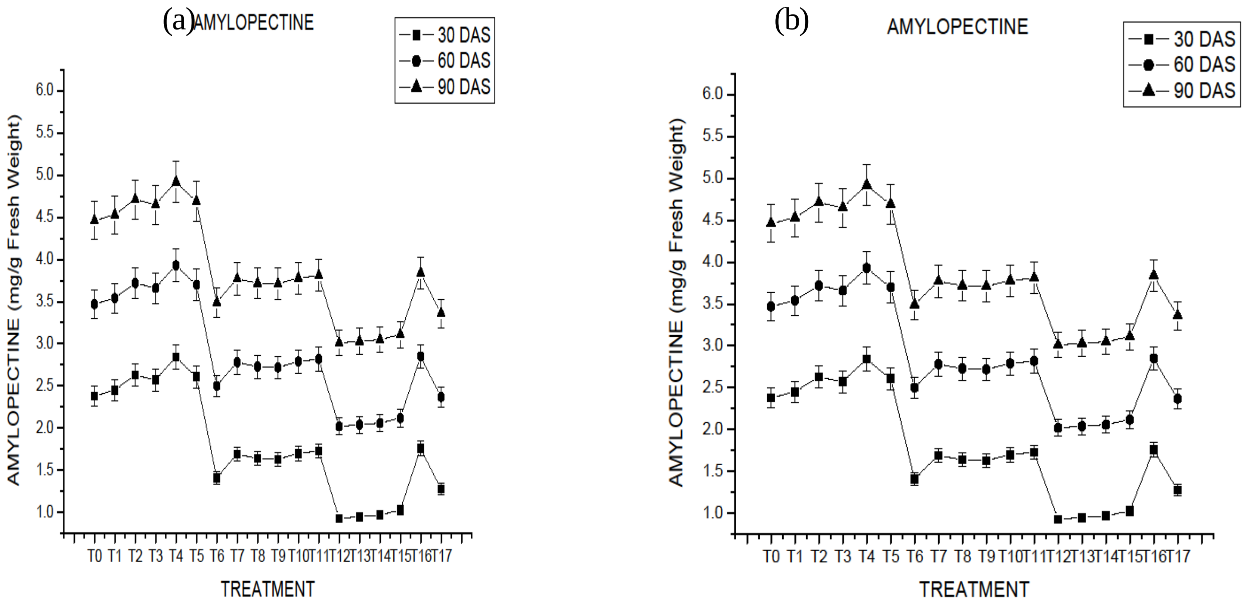

Fig. 3a & 3b: Amylopectin (mg.g^{-1} fresh weight) of sorghum during Kharif during the two subsequent years (left to right). (Note as per Fig. 1)

putrescine and mycorrhiza showed the best combination for the mitigation of cadmium toxicity for the total amylopectin content. The similar trends were found during the study made in the second year. Pathak et al. (2017) reported that carotenoids which protect the chlorophyll from photo-oxidative destruction and a reduction in carotenoids could have serious consequences on chlorophyll pigments in presence of Cd (Kumar & Dwivedi 2016a, b; Kumar 2018, Kumar et al. 2012, 2013, 2018 a, b, c). In maize, Cd inhibits the growth promoting factors of leaves like starch, amylose, and amylopectin (Pathak et al. 2017). It reduces both the total starch and the amylose, though the later decrease to a lower extent, thus leading to a drop in the amylopectin (Siddique et al. 2018).

CONCLUSIONS

Polyamines like putrescine and mycorrhiza *Glomus* impart significant mitigation of cadmium-induced toxicity in sorghum mediated through their defensive role in plants by increasing the total starch, total amylose and amylopectin in the sorghum leaves.

REFERENCES

Kumar, Prasann 2018. Signal Transduction in Plant with Respect to Heavy Metal Toxicity: An Overview. CRC Press, Taylor & Francis Group, pp. 394.

Kumar, Prasann, Dwivedi, Padmanabh and Singh, Pallavi 2012. Role of polyamine in combating heavy metal stress in *Stevia rebaudiana* Bertoni under in vitro conditions. International Journal of Agriculture, Environment and Biotechnology, 5(3): 193-198.

Kumar, Prasann and Dwivedi, Padmanabh 2014. Phytoremediation of Cadmium Through Sorghum. Daya Publishing House, pp. 311-342.

Kumar, Prasann, Dwivedi, Padmanabh and Hemantaranjan, Akhori 2016a. Short term responses of crops under mercury contamination at hazardous waste sites. In: Plant Stress Tolerance Physiological and Molecular Strategies, Scientific Publishers, pp. 149.

Kumar, Prasann, Dwivedi, Padmanabh and Hemantaranjan, Akhori 2016b. Physiological and biochemical properties of *Gliricidia*: Its cultivation a scope for remunerative venture for farmers. Plant Stress Tolerance Physiological and Molecular Strategies, pp. 359.

Kumar, Prasann and Dwivedi, Padmanabh 2018a. Putrescine and *Glomus* mycorrhiza moderate cadmium actuated stress reactions in *Zea mays* L. by means of extraordinary reference to sugar and protein. Vegetos, 31(3): 74-77.

Kumar, Prasann and Dwivedi, Padmanabh 2018b. 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.

Kumar, Prasann and Dwivedi, Padmanabh 2018c. Putrescine and *Glomus* mycorrhiza moderate cadmium actuated stress reactions in *Zea mays* L. by means of extraordinary reference to sugar and protein. Vegetos, 31(3): 74-77.

Kumar, Prasann, Kumar, Sunil and Naik, Mohit 2018a. Glomus and putrescine based mitigation of cadmium induced toxicity in maize. Journal of Pharmacognosy and Phytochemistry, 7(5): 2384-2386.

Kumar, Prasann, Mandal, Biswapati and Dwivedi, Padmanabh 2011a. Heavy metal scavenging capacity of *Mentha spicata* and *Allium cepa*. Medicinal Plants-International Journal of Phytomedicines and Related Industries, 3(4): 315-318.

Kumar, Prasann, Mandal, Biswapati and Dwivedi, Padmanabh 2011b. Heavy metals scavenging of soils and sludges by ornamental plants. Journal of Applied Horticulture, 13(2): 144-146.

Kumar, Prasann, Mandal, Biswapati and Dwivedi, Padmanabh 2013. Phytoremediation for defending heavy metal stress in weed flora. International Journal of Agriculture, Environment and

Biotechnology, 6(4): 647.
Kumar, Prasann, Misao, L. and Jyoti, Nada 2018b. Polyamines and mycorrhiza based mitigation of cadmium induced toxicity for plant height and leaf number in maize. International Journal of Chemical Studies, 6(5): 2491-2494.
Kumar, Prasann, Pathak, Shweta, Kumar, Mukul and Dwivedi, Padmanabh 2018c. Role of secondary metabolites for the mitigation of cadmium toxicity in sorghum grown under mycorrhizal inoculated hazardous waste site. In: Biotechnological Approaches for Medicinal and Aromatic Plants. Springer, Singapore, pp. 199-212.
Pathak, Shweta, Kumar, Prasann, Mishra, Pankaj Kumar and Kumar, Mukul 2017. Mycorrhiza assisted approach for bioremediation with special reference to biosorption. Pollution Research, 36(2): 330-333.
Siddique, Anaytullah, Dubey, Anand Prakash and Kumar, Prasann 2018. Cadmium induced physico-chemical changes in roots of wheat. Vegetos, 31(3): 113-118.