Alleviation of arsenic stress from rice plant (*Oryza sativa* L.) and subsequent reduction in rice grain arsenic using different amendments

Synopsis submitted by

DEEPANJAN MRIDHA

Doctor of Philosophy

School of Environmental Studies

Faculty of Interdisciplinary Studies, Law and Management

Jadavpur University

Kolkata - 700032, India

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Rice grain arsenic (As) contamination is a worldwide problem nowadays, as it is the staple crop for 50% of the world population. Irrigation of agricultural fields with As-contaminated groundwater has led to build-up elevated level of arsenic in soil, with consequent elevation of arsenic in crops and vegetables grown up on this soil. Moreover, transportation of As through rice grain from As exposed to unexposed areas and consequent dietary intakes leads to great threats for the population residing in non-endemic areas. The arsenic species present in rice grain are mainly inorganic in nature, which are toxic and carcinogenic. This study includes the alleviation of arsenic toxicity from rice plants. Alleviation of As stress from rice seedlings were segregated into two parts i.e. i) alleviation of As stress from rice plant during the course of germination and seedling stage, and ii) alleviation of As stress and reduction of rice grain As. The rice or any plant is vulnerable to any kind of biotic/abiotic stress, which may cause yield loss, and growth retardation in plants. Thus, proper remediation strategies are badly required to address this burning issue nowadays. Again, rice grain is a primary source of nutrition for half of the world, so rice grain As contamination is in the priority list of researchers, which needs an immediate solution. For this remedial study, different amendments like potassium humate (K-humate, KH), iron oxide nanoparticles (FeO-NPs), selenium nanoparticles (SeNPs), inorganic sulfate compounds (S), biochars (iron modified mango leaf biochar, FeMBC) etc. were selected to reduce the As load in rice grain both in pot-scale and field level study. For improvement of rice seed germination under As stress, K-humate, iron oxide nanoparticles, and selenium nanoparticles were utilized. Whereas, for improvement of rice plant growth and reduction of rice grain As concentration, inorganic sulfate compounds, biochars etc. were used.

Rice seed germination with K-humate has been carried out in part, in first experiment we have used 100 mg/L of K-humate for seed priming. The germination percentage in K-humate primed seeds were $75 \pm 5.0\%$ and $68.3 \pm 2.9\%$ under AsV and AsIII stress, respectively. The vigour index I (VG I) and vigour index II (VG II) recorded on 12 DAS (days after seeding) were also increased by 1.47 and 1.51 fold, respectively with K-humate supplementation under As stress. Application of K-humate not only improved seed germination, seedling growth and nutrient uptake; however, also decreased the oxidative stress markers and antioxidant activities by minimizing As uptake and translocation in the seedlings. In second experiment, we have used varying dosage of K-humate

(25, 50, 75, 100 mg/L) to alleviate As toxicity (800 ppb AsIII) from rice seedlings. Application of KH significantly improved germination percentage, vigour indices and chlorophyll content by reducing the oxidative stress, antioxidant and antioxidant enzyme activities under As stress. In vivo detection of reactive oxygen species (ROS) using DCF-2DA fluorescent dye and scanning electron microscope (SEM) study of root further depicted that KH application effectively reduced ROS formation and improved root anatomical structure under As stress, respectively.

Experiment with green synthesized FeO-NPs (100 mg/L), seed germination under 50 µm AsIII and 50 µm AsV stress was significantly improved by 9.8% and 15.4%, respectively, as compared to control. The phytotoxic effect of AsIII on seed germination, seedling growth, and chlorophyll content was more severe than AsV. The uptake and translocation of As by seedlings were decreased with FeO-NPs fertigation under As stress.

In another experiment, biosynthesized SeNPs were used to assess the efficacy of SeNP in reducing As (25 µM and 50 µM AsIII) stress in rice seedlings. SeNP application improved the plant growth by increasing the chlorophyll content and reduced oxidative damage by increasing antioxidant activity. SeNP application did not alter the root uptake of As; however, it reduced the translocation of As by 34.3-30.2%. SeNP addition significantly upregulate OsPCS1, OsPCS2b and OsABCC1 gene expression in root which stimulates PC (Phytochelatin) content and subsequent vacuolar sequestration of As in roots.

Pot experiment with different levels of sulfate dosage (0, 20, 40, 60 and 80 mg/kg) was set up in this study to explore the influence of sulfate fertilizer on rice plant growth, yield, and As accumulation in rice grain. The sulfate application significantly ($p \leq 0.05$) enhanced the chlorophyll, tiller number, grains per panicle, grain and biomass yield under As stressed condition. The sulfate application also reduced the oxidative stress and antioxidant activity in rice plants. Sulfate fertigation improved the accumulation of total sulfur (S) and reduced the uptake and translocation of As in rice plants. Arsenic concentration in rice grain was reduced by 50.1% in S80 treatment (80 mg of sulfate/kg of soil) as compared to S0 set.

In our final pot study with mango (*Mangifera indica*) leaf-derived biochars (MBC) and Fe-modofied mango leaf biochar (FeMBC) to reduce As accumulation in rice grain. The results showed that 1% FeMBC enhanced the percentage of filled grains/panicle and biomass yield by 17% and 27%, respectively, compared to the control. The application of 0.5 and 1% FeMBC significantly ($p \leq 0.05$) reduced bioavailable soil As concentration by 33% and 48%, respectively, in comparison to the control. The concentration of As in rice grain~~s~~ was reduced by 6 and 31% in 1% MBC and 1% FeMBC, respectively, compared to the control. The reduction in As concentration in rice grain under 1% FeMBC was more pronounced due to reduced bioavailability of As and enhanced formation of Fe-plaque. The concentrations of micronutrients (such as Fe, Zn, Se, and Mn) in brown rice were also improved after the application of both MBC and FeMBC in comparison to the control.

In field study, all the amendments (KH, FeO-NPs, SeNPs, FeMBC and sulfate) were experimented for two years in As contaminated fields of Madhusudankathi village, located in highly arsenic endemic block Gaighata of North 24 Parganas district, West Bengal. The rice was cultivated in two seasons (monsoon and post-monsoon). It was observed that post-monsoon season showed better yield and higher rice grain As than monsoon season. Among all the treatments, KH showed the better yield. Both SeNPs and KH treatments showed similar rice grain As concentration, which was lower than the other treatments. However, long-term field trial with Selenium NPs are required. Thus, KH can be used as a reagent to improve rice yield and As-safe rice grain.