Bioremediation of Arsenic by Arsenic Resistant *Bacillus nakamurai KDSA1* in Groundnut

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Abstract

Heavy metal pollution is one of the most serious dangers to human health and the environment. It is a major problem to deal with from an agricultural standpoint. In the present study, the effect of arsenic tolerant strain (Bacillus nakamurai KDSA1) was studied to evaluate its impact on the growth and yield of groundnut. The concentration of arsenic was determined by using Atomic Absorption Spectroscopy (AAS) and it is observed that B. nakamurai KDSA1 strain showed significant reduction in arsenic i.e. 19.48% when compared to control in groundnut kernel.

It was also observed that B. nakamurai KDSA1 also has some plant growth promoting effects on groundnut as the highest plant height, shoot weight, shoot dry weight, root length, nodule number, pod yield, kernel yield, shoot nitrogen accumulation and N-uptake in kernel were found significantly superior in seeds inoculated with B. nakamurai whereas the highest N-content in kernel was found in T_4 seeds inoculated in presence of arsenic and B. nakamurai KDSA1 when compared to all other treatments. Local Rhizobium biofertilizer was used to improve the nodulation.

Keywords: Groundnut, Arsenic resistant bacteria, Heavy metal, *Bacillus nakamurai*, Bioremediation.

Introduction

India's agricultural sector relies heavily on the oil seed crop. India is the 2nd largest groundnut producer in the world with 6,857,000 tonnes production volume per year. Groundnut (*Arachis hypogaea L.*) is known as the "King of Oil Seeds"[11]. In year 2020, the Indian Council of Medical Research (ICMR) recommends that each person consume 20 gm edible oil each day. Oil seed is grown on 24.7 million hectares around the world with a total production of 33 million tonnes.[20] Groundnut seeds are a good source of B vitamins and contain 40 to 50% oil and 20 to 30% protein[16]. Arsenic (As), copper (Cu), lead (Pb), zinc (Zn), cadmium (Cd) and nickel (Ni) are some of the hazardous elements found in sewage sludge from current sources including smelting, mining, metal forging, the use of phosphate fertilizer and the use of metals containing insecticides as the main sources of pollution[2,13].

Heavy metal pollution in Chhattisgarh has increased in recent years as a result of extensive industrialization and mining activities, as evidenced by various sources[5,6,17,18,21]. According to the CEPI index 83, Chhattisgarh is among India's top five most critically polluted industrial areas posing a threat to environment in the near future[19]. The irrigation of agricultural soil with heavy metals contaminated water can cause elevated concentrations of heavy metals in plants and soil. The illnesses itai-itai and minamata were brought to Japan by paddy rice farming on cadmium (Cd) and mercury (Hg) polluted soil[3].

Arsenic (As) in the water table under the earth's surface has been found in India. A large number of people on the planet are suffering from skin wounds, cancers and tumors. Arsenic (As) is accumulated in fresh water that is used for both consumption and plant harvesting. According to 28 years of data due to arsenic contamination, Bihar, Uttar Pradesh, Jharkhand, West Bengal, Chhattisgarh, Manipur and Assam are the seven worst impacted states in India[4]. Chakraborti et al[4] recorded the first case of arsenic contamination among all heavy metals in Koudikasa village of Rajnandgaon district in undivided Madhya Pradesh state (Now in Chhattisgarh). Since then, various workers across Chhattisgarh have recorded multiple instances of arsenic pollution in soil, water, air and on human bodies[1,8,14,15].

As a result of pollution from rapidly developing manufacturing areas, mining and the excess use of chemical fertilizers and pesticides, heavy metal contamination of agricultural soils have become common phenomenon. The present study was carried out with the objective to characterize the selected strains and evaluate the groundnut (*Arachis hypogea* L.) in presence of heavy metal resistant bacterial strains.

Material and Methods

Collection of *Bacillus nakamurai* KDSA-1 and local *Rhizobium*: Arsenic tolerant bacteria *Bacillus nakamurai* KDSA1 (NCBI accession number MH793476) strain and local *Rhizobium* biofertilizer were collected from the culture collection of Department of Agricultural Microbiology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya Raipur (Chhattisgarh). Brief biochemical properties and isolation history of *Bacillus nakamurai* KDSA1 are summarized in table 1 and table 2.

Table 1
Details of sample collection for isolation of *B. nakamurai KDSA1*.

Country	State	District	Villages/Cities	Sample Collected	Latitude	Longitude
India	Chhattisgarh	Korba	Dipka	Soil	22.35286	82.54522

Table 2
Biochemical characteristics of the isolate B. nakamurai KDSA1.

Name of Isolates	Gram stain	Shape	Coli form	Catalase	Starch Hydrolysis	Citrate Utilization	Gelatin Iron	Indole test	Methyl Red Test	Urease Test	Glucose	Lactose	Sucrose	H_2S	Gas production
B. nakamurai KDSA1	+	Rod	-	+	+	+	-	-	-	+	+	-	-	+	+

Table 3
Treatment details carried out in groundnut

Treatment	Treatment details
T_1	Groundnut seeds (Control)
T_2	Groundnut seeds + Arsenic
T_3	Groundnut seeds + *B. nakamurai* KDSA1
T_4	Groundnut seeds + Arsenic + *B. nakamurai* KDSA1

Revival of Bacterial strain: Arsenic tolerant bacterial strain *Bacillus nakamurai* KDSA-1 was streaked in nutrient agar medium with supplement of 1mM concentration of sodium arsenate ($Na_2HAsO_4.7H_2O$) and *Rhizobium* was streaked in yeast extract mannitol agar medium. The inoculated agar plates and YEMA medium plates were incubated at 37°C and 28°C for 24 h to 48 h (till colonies developed) in a BOD incubator. Individual colonies of inoculated bacterial strains with distinct shape and color were selected and inoculated in agar slant and YEMA slant and were kept in BOD incubator for a day at 37°C and 28°C. The agar slant and YEMA slant showing well desired growth were picked and stored at 6-8°C in a refrigerator for further use. Sub culturing was done at a regular interval period of 15 days.

Performance of groundnut with arsenic resistant bacterial strain: The experiment was conducted during the *Rabi* season in 2020-21. Groundnut was taken as a test crop variety TAG 24. The study was examined in polybags having 10 kg capacity. Details of the treatments are summarized in table 3. All polythene bags were filled with mixture of sand and soil in the ratio of 3:1. Uniform level of N: P: K @ 20:60:40 kg/ha was applied as basal dose through the urea, single super phosphate (SSP) and murate of potash (MOP) in all treatment and 1mM concentration of sodium arsenate solution was applied in T_2 and T_4 treatment respectively. Thus experiment was having four treatments and five replications. Local *Rhizobium* bio-fertilizer was used to improve the nodulation.

All observations recorded from this experimental study were tabulated systematically. The final observations were statistically analyzed by completely randomized design with four treatments and five replications as described by Gomez and Gomez[10]. Statistical analysis was done by taking the mean value of two plants from each treatment in each replication. For the study of plant biomass, following parameters were examined. Plant height and root length, fresh weight and dry weight of plant, number of nodules, pod yield and kernel yield, N content and N-uptake in shoot and kernel were recorded. The kernel of groundnut was dried, grinded into fine powder. This powder was used for the estimation of Arsenic (As) content absorbed by plant through AAS (Atomic Absorption Spectroscopy) analysis in the laboratory of National Center for Natural Resources Pt. Ravishankar Shukla University Raipur, Chhattisgarh, India.

Calculation of Arsenic concentration:

Total Arsenic (ppm) = Reading (Arsenic concentration in ppm) x Dilution factor

where Dilution factor =Volume of solution / weight of sample.

The percentage reduction in arsenic was calculated by the following formula:

$$\% \text{ reduction in arsenic} = \frac{B - S \times 100}{B}$$

where B = Blank reading value without any bacteria and S = Sample reading value with bacterial culture

Statistical Analysis: All the observations were recorded and tabulated in a systematic manner. All treatments were replicated to fulfill the minimum statistical requirements for conducting an experiment. The experiments were a complete randomized design (CRD). The obtained data was the mean of independent experiments with desired number of replicates. Student's t test was used to analyse the data and p values < 0.05 were considered as statistically significant.

Results and Discussion

Evaluation of groundnut in presence of arsenic resistant bacterial strain: Arsenic accumulates in humans from drinking water, agricultural crops and vegetables increasing the risk of arsenic poisoning in humans through the food chain[7]. As a result, arsenic removal from drinking water and agricultural land is critical. The features that have allowed legume crops to adapt to harsh environmental conditions have been found in legume crops cultivated under various agrochemical settings and cropping systems with distinct cultural practices. Kumar et al[12] have isolated and identified a number of microorganisms, including arsenic-tolerant bacteria[12].

Plant height was recorded in three different stages (fig 1). It has been observed that the maximum height of plant (25.6 cm at 95DAS) was recorded in treatment T_3 (*B. nakamurai*). Similarly, shoot fresh weight, root length (46 cm), shoot dry weight (4.04 g), number of nodule (112) and highest pod yield (4.60 g/plant) were also associated with treatment T_3 (*B. nakamurai*). In case of kernel yield, the observed data revealed that the kernel yield was recorded maximum 3.58 g/plant in treatment T_3. Similar results were also found by Dey et al.[9]

It has been observed that the maximum N-content in shoot 2.50% was also from treatment T_3 (*B. nakamurai*). In case of kernel, the observed data revealed that the highest N-content in kernel was recorded maximum 3.14% in treatment T_4 (arsenic + *B. nakamurai*). Furthermore, N-uptake in shoot 101 mg/plant was again recorded maximum in treatment T_3 whereas the minimum N-uptake in shoot 69.86 mg/plant was recorded in treatment T_2 (Arsenic). In case of kernel the observed data revealed that the N-uptake in kernel recorded highest 104.53 mg/plant in treatment T_3.

Arsenic concentration in kernel: Treatment T_2 and T_4 (as they have arsenic) checked for arsenic concentrations. The seed inoculated with heavy metal resistant *B. nakamurai* KDSA1 strain (fig. 3) has reduced the concentration of arsenic as compared to un-inoculated seed. The reading from the AAS revealed that the concentration of arsenic in kernel was observed maximum (13.051 ppm/g) in treatment T_2 (Arsenic) and the lesser concentration of arsenic (10.508 ppm/g) was observed in treatment T_4 (arsenic + *B. nakamurai* KDSA1). It was observed that a total 19.48% reduction in arsenic is being carried out by bacterial strain *B. nakamurai* KDSA1 (table 4 and fig. 2).

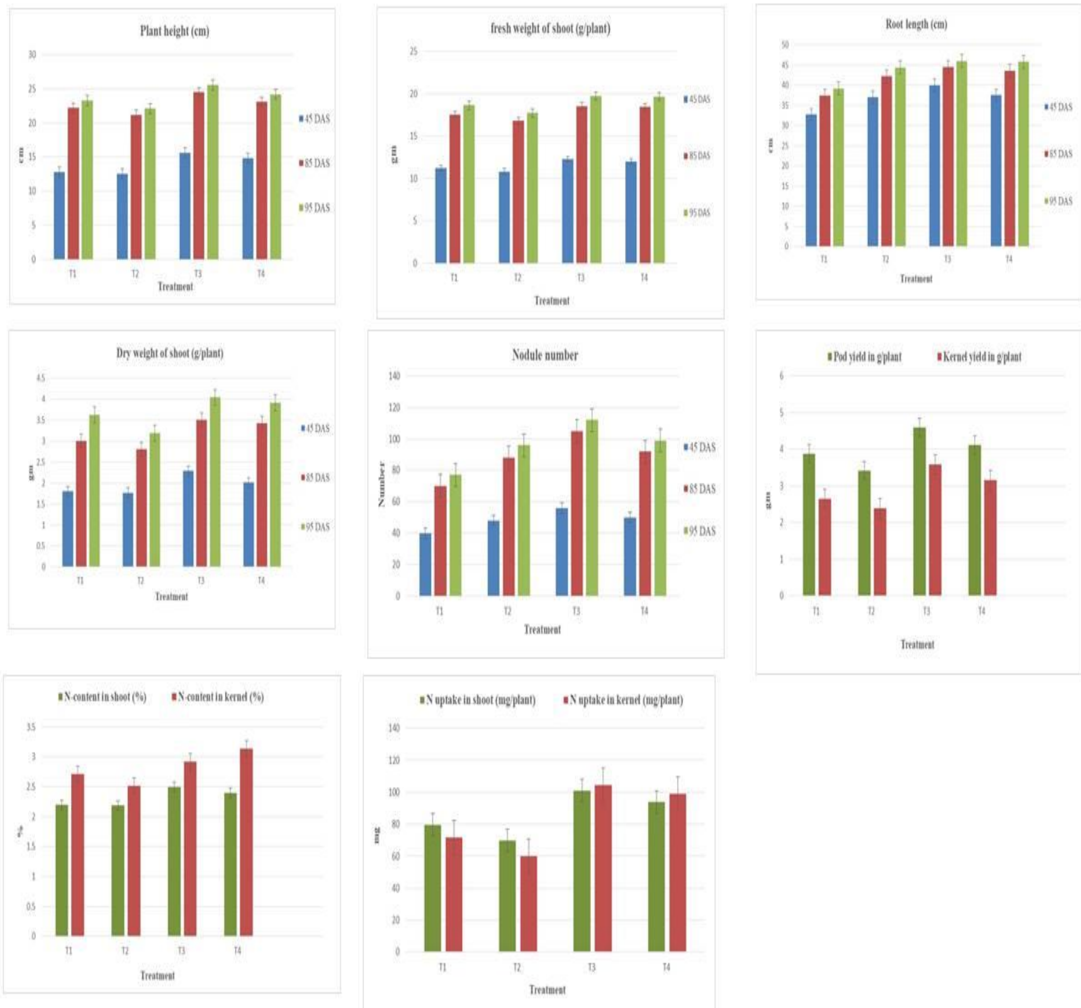

Figure 1: Plant growth parameters at different stage of groundnut influenced by treatments of arsenic utilizing *Bacillus nakamurai* KDSA-1. Each data represents the mean ± SE obtained from independent experiments with 5 replicates. Student's t test was used to analyze the data and *p < 0.05

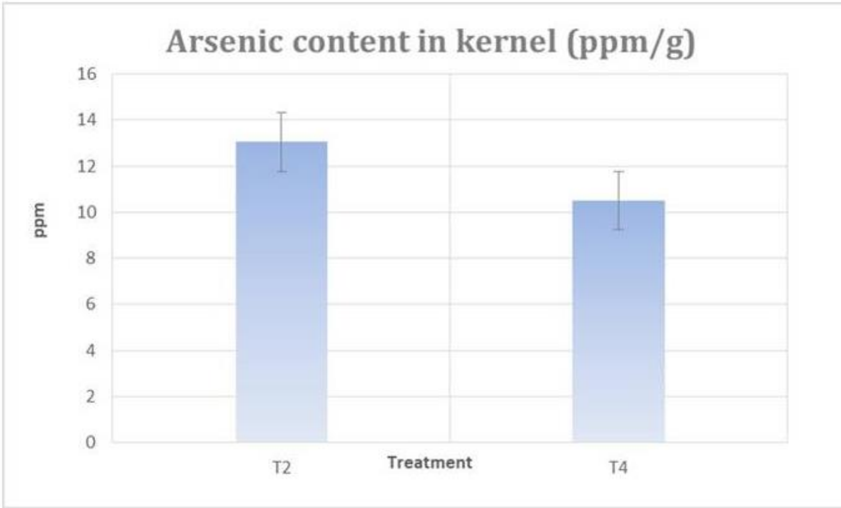

Figure 2: Bioremediation of Arsenic in kernel by application of *Bacillus nakamurai* KDSA-1 This bioremediation assay was conducted with four treatment i.e. T1: Uninoculated (Control); T2: inoculation with Arsenic; T3: inoculation with *B. nakamurai KDSA1 and* T4: inoculation with Arsenic and *B. nakamurai KDSA1*. Treatment T1 and T3 were not analysed through AAS as they didn't inoculated with arsenic. Each data represents the mean ± SE obtained from independent experiments with 5 replicates. Student's t test was used to analyze the data and *p < 0.05 compared with T2: inoculation with Arsenic.

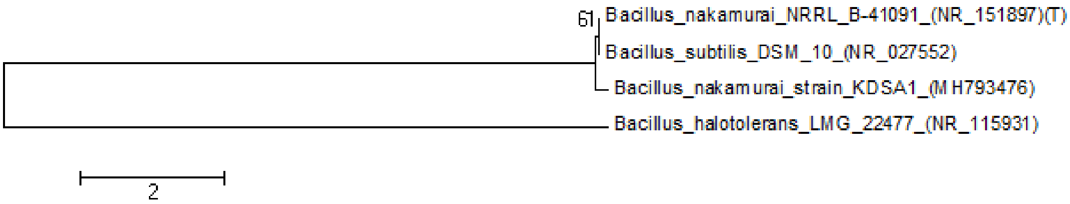

Figure 3: Phylogenetic trees based on 16S rRNA gene sequences of arsenic resistant bacteria *Bacillus nakamurai* KDSA-1 and other *Bacillus* strains constructed by Neighbour Joining method using MEGA6 software. Bootstrap values, expressed as a percentage of 1000 replications, are given at branching points when ≥ 50%. Bar, 0.1 substitutions per nucleotide position

Table 4
Response of *Bacillus nakamurai* strains on arsenic reduction in groundnut kernel.

TREATMENT	**Arsenic content in kernel (ppm/g)**
Arsenic	13.051
Arsenic + *Bacillus nakamurai* KDSA1	10.508
SEm±	**0.24**
CD (at 5%)	**0.79**

Conclusion

Keeping in view the findings related to parameters like highest plant height, shoot weight, shoot dry weight, root length, nodule number, pod yield, kernel yield, shoot nitrogen accumulation and N-uptake in kernel were found significantly superior in treatment T_3 (*B. nakamurai* + *Rhizobium*) whereas the highest N-content in kernel was found in treatment T_4 (Arsenic + *B. nakamurai*) when compared to all other treatments.

The biochemical characterization shows extra cellular enzymatic activity which would be helpful for the identification of bacteria species on the differences in the biochemical activity of different bacteria and the result of Atomic absorption spectroscopy (AAS) analysis suggested that the seed inoculation with arsenic + *B. nakamurai* was found in lesser concentration of arsenic as compared to seed with arsenic. Further, it can be concluded that *B. nakamurai* KDSA1 strain showed significant reduction in arsenic i.e. 19.48% when compared to treatment T_2. Inoculation of *B. nakamurai* strain is showcasing its aptness as a bioremediation agent.

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