Ann. Bangladesh Agric. (2023) 27 (1) : 41-56
www.doi.org/10.3329/aba.v27i1.70896

ISSN 1025-482X (Print)
2521-5477 (Online)

REDUCTION OF ARSENIC UPTAKE IN LENTIL THROUGH ARBUSCULAR MYCORRHIZAL FUNGI

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Abstract

Arsenic (As) is a carcinogenic and hazardous substance that poses a serious risk to human health. The present research focused on the transport of As in seven lentil varieties developed by the Bangladesh Agricultural Research Institute (BARI) viz., BARI Mashur1, BARI Mashur 2, BARI Mashur 3, BARI Mashur 4, BARI Mashur 5, BARI Mashur 6 and BARI Mashur 7 and the role of arbuscular mycorrhizal fungi (AMFs) in mitigating As phyto-toxicity in them. The transportation of As from soil to root, shoot, and grain was lower after week 13 than in weeks 6 or 10 after lentil seedling emergence. The As accumulation in grains was higher in BARI Mashur 1 than in other test genotypes. Treatment with AMF significantly increased growth and biomass accumulation in lentil genotypes compared to non-AMF plants. Furthermore, AMF effectively reduced As concentrations in roots and shoots of lentil plants grown in 8 and 45 mg kg-1 As-contaminated soils. This study revealed a remarkable variation among the BARI-developed lentil genotypes in terms of As accumulation in plant parts. Treatment with AMF could effectively reduce plant uptake of As and mitigate As-induced phytotoxicity in lentils. The results reveal a great potential of AMF to mitigate As toxicity in lentils caused by the transport of As from soil to the shoot mass and reallocation to grains. This may enable farmers to expand lentil cultivation in As-affected areas throughout the world.

Keywords: Arsenic, AMF, lentil, mitigation, phytotoxicity.

Introduction

Arsenic (As) is a natural but hazardous element present in rocks, soils, water, air, and biological tissues (Hossain, 2006). In recent years, research on the occurrence, distribution, origin, and mobility of As in soils through natural, geochemical, and biological processes has intensified (Leung *et al.,* 2013). The U.S. Agency for Toxic Substances and Disease Registry (ATSDR) has, in its priority list of hazardous substances, designated As as the most hazardous substance in the United States (Leung *et al.,* 2013). Arsenic has been recognized as a carcinogenic substance, the cancer hazard depending on the concentration and duration of exposure (Singh *et al.,* 2015). Exposure to As may cause serious diseases, such as lung and skin cancers, and possible liver and kidney damage. Non-cancerous health effects of exposure to As include

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diabetes, chronic cough, and cardiac and nervous system collapse (SOS, 2019).

Furthermore, Arsenic contamination in water-soil-plant systems has been reported worldwide, particularly in Argentina, Australia, Bangladesh, Chile, China, Hungary, Mexico, Peru, Thailand, and Vietnam (Ahmed *et al.,* 2011). However, the most severe As contamination in surface soil, water and humans currently occurs in Asia, particularly in Bangladesh (Ahmed *et al.,* 2011), West Bengal, and India (Ahmed *et al.,* 2006). Accumulation of As-containing substances has led to unacceptable As levels in samples in soils and ultimately in crops.

Chemically, As exists as organic and inorganic species. The main sources of As in the environment are arsenic sulfide (As_2S_2), arsenic trisulfide (As_2S_3), and arsenopyrite or ferrous arsenic sulfide ($FeAsS_2$) (Hossain, 2006). Inorganic As has two main oxidation states, i.e., trivalent arsenite As(III) and pentavalent arsenate As(V). Inorganic forms of arsenate As(V) and arsenite As(III)) are usually dominant in As-contaminated soil (Cubadda *et al.,* 2010). Trivalent arsenite is 60 times more toxic than arsenate (Hossain, 2006). Arsenic has highly toxic effects on metabolic processes in plants causing reduced photosynthesis, DNA replication, disturbances in enzymatic activities, and growth inhibition (Alam *et al.,* 2019c). For example, the elongation of the roots and leaves of the mesquite plant *(Prosopis juliflora x P. velutina*) decreased significantly with increasing As (III) and As (V) concentrations in soils (Ntebogeng *et al.,* 2008). As contaminated water leads to accumulation of As in the soil (Heikens, 2006) which is then transported into edible parts of food crops (Cubadda *et al.,* 2010). The extensive use of pesticides, fertilizers, groundwater and industrial wastewater for irrigation purposes in crop fields has also resulted in elevated levels of As in soils and increased As uptake into edible parts of rice, lentils, and vegetables (Ahmed *et al.,* 2011). Consequently, many food crops have become hazardous, including lentil, a major leguminous crop worldwide. Leguminous crops are an excellent source of protein, minerals, and vitamins for human nutrition (Cui *et al.,* 2013), but in many parts of the world, high As levels in irrigation water and soil have contaminated these crops with this toxic element. Subsequently, a high average daily dose (ADD) from the environment and low excretion could cause As toxicity to humans from lentils and other food crop cultivation in As-contaminated soils (Cui *et al.,* 2013).

Mitigation of As in food crops in As-affected areas, especially in the very popular food item, lentil, in Bangladesh, has become essential. A possible solution includes arbuscular mycorrhizal fungi (AMF), which establishes a mutualistic symbiotic relationship with most terrestrial plants including lentil crops (Sharma *et al.,* 2017). AMF is actively involved in the reduction of As accumulation in root, shoot and grains (Orlowska *et al.,* 2012; Alam *et al.,* 2023; Alam *et al.,* 2022; Alam *et al.,* 2020a; Alam *et al.,* 2020b; Alam *et al.,* 2019a; Alam *et al.,* 2019b; Alam *et al.,* 2019c). In aerobic soils, the main form of As is arsenate As(V) which mimics phosphorus (P) and can be taken up by lentil plants and AMF by normal mechanisms of P uptake (Toulouze *et al.,* 2012). Mycorrhizal symbioses are important because they are formed by 90% of higher plants causing increased uptake of P compared to their non-mycorrhizal counterparts (NM) (Toulouze *et al.,* 2012). The association of AMF with food crops could reduce As uptake by various mechanisms (Li *et al.,* 2016). Being a high-protein food for humans' lentil is one of the major leguminous crops in the world, but the same lentil if contaminated with As may be a great cause for human health especially in As-contaminated regions. This calls for the development of suitable As mitigation techniques. The present research was focused on As uptake in the root, shoot and grain of lentil, the selection of lentil genotypes against As toxicity and its impact on biomass, and the reduction of arsenic accumulation in the biomass of lentil genotypes using arbuscular mycorrhizal fungi (AMF). It hypothesizes that this research will provide a solid foundation for the exploration of low As accumulator lentil genotypes that could supply As free grains for consumption by humans.

Materials and Methods

Experiment 1: Arsenic uptake in the roots and shoots of BARI lentil genotypes (pot experiment) Soil sampling area

Arsenic-contaminated soils were collected for this pot experiment from a farmer's field in the Faridpur regions at the Old Meghna Estuarine Floodplain agro-ecological zone (AEZ) of Bangladesh. The soil of the study areas was an Inceptisol according to the USDA (United States Department of Agriculture) soil classification with a silty loam texture.

Pot preparation

Soil samples collected from the 0-15 cm layer using a soil auger. Then soil samples were brought to the Department of Environmental Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU). The soil was air-dried at room temperature, ground, and sieved with a ≤250 µ mesh. One and a half killogram of soil mixed with 500g *Trichoderma* compost was placed in a 15cm x 15 cm clay pot. According to the recommendations of the Bangladesh Agricultural Research Institute (BARI), urea 225kgha^{-1}, TSP 450kgha^{-1} and MOP 175 kgha^{-1} were incorporated into the soil in each pot. Before sowing, lentil seeds were treated with the fungicide Vitavax 200 (1gL^{-1} water), and then 6 seeds were sown in each pot during the first week of November, 2018.

Chemical analysis of soil, water and compost

The pH of soil, irrigation water, and compost was determined by the glass electrode pH meter (Jackson, 1973). Total N in the soil and compost was determined by the Kjeldahl method (Jackson, 1973), available P by the Olsen method (Olsen and Sommers, 1982), exchangeable K by the neutral normal NH_4OAc method (Jackson, 1973), S by the barium sulfate turbidity method (Chesnin and Yien, 1951)

Treatments

The total As concentration of the background soil was 5 mgkg^{-1} and 15 mgkg^{-1}. Three soil As treatments (concentrations) were applied: T_1 = 5 mgkg^{-1} As, T_2 = 15 mgkg^{-1} As, and T_3 = 100 mgkg^{-1}As. The concentration of As in the background soils (5 mgkg^{-1}) was increased to 100 mgkg^{-1} by adding sodium arsenate ($Na_2HAsO_4.7H_2O$). A 0.395 g of sodium arsenate was used for each kg of soil in the pots to obtain the required concentration of arsenic. In this pot experiment, five replications with seven lentil varieties were used and the total number of pots was 105.

Lentil genotype

Seven lentil varieties were developed by the Bangladesh Agricultural Research Institute (BARI). BARI Mashur1, BARI Mashur 2, BARI Mashur 3, BARI Mashur 4, BARI Mashur 5, BARI Mashur 6 and BARI Mashur 7 were used in this experiment. The average yield of these varieties is 2.0-2.3 t/ha. The varieties varied in growth duration (seed to seed) from 100 to 105 days. These lentil varieties are grown from November to February in Bangladesh.

Data collected

At random, the shoot lengths were measured at week 3 in each of the treated pots. At this point, three lentil seedlings were thinned out of each arsenic-treated pot. Fresh weights were taken of each sample. Dry weights of the roots and shoots were measured separately after harvesting the three lentil seedlings from each As-treated pot during week nine. All samples were dried in an oven at 55^0 C for 72 hours and preserved to determine their As content.

Experiment 2: Arsenic uptake in lentil grains from As contaminated soils (field experiment)

Simultaneously with the pot experiment a field experiment was conducted in which the seven test lentil genotypes were sown on 12 November 2018 in plots 10 x 5 meters in size at BSMRAU research fields. The soil in the field plots had a total As concentration of 5 $mgkg^{-1}$. The BARC-recommended doses of urea, TSP and MOP were applied @ $225kgha^{-1}$, $450kgha^{-1}$ and 175 $kgha^{-1}$, respectively (Fertilizer Recommendation Guide [FRG], 2012). Lentil was harvested on 16 February 2019. The total duration was required to be 95 days from sowing to harvesting of lentil crops. During harvest, three samples of lentil grains were randomly collected from each plot, dried first at room temperature and then in an oven at 55^0 C for 72 hours and preserved for As analysis.

Experiment 3: Reduction of As uptake through AMF (pot experiment)

Lentil genotypes

Based on the results of the previous field experiment, BARI Mashur 1 and BARI Mashur 5 were selected as test varieties in a pot experiment to study the reduction of As uptake through AMF inoculation. The pot experiments were conducted in a BSMRAU net house in a controlled environment.

Arbuscular mycorrhizal fungi (AMF)

The AMF species were collected from the International Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM), West Virginia University (WV), USA. The AMF samples were mixed with soil and roots of the host plant of sorghum housed in the Department of Environmental Science, BSMRAU. The mixture of soil and roots was collected as a source of AMF. Based on the total biomass (soil) in the containers, soils with 4% AMF content were used for the experiment on the reduction of As uptake in lentils. Mycorrhiza spores in soil and root samples were observed following the wet sieving and decanting method (Gerdemann and Nicolson, 1963; Giovanetti and Mosse, 1980). About 70-100 spores were found per kg of soil. The colonization of AMF species was found to be 40-60% with the host plant of the sorghum root.

Culture of lentil genotypes in pot soils

The 8 $mgkg^{-1}$ As concentrated Bangladesh Jute Research Institute (BJRI) soils were collected from Faridpur to grow lentil plants. This background As content in the soil (8 $mgkg^{-1}$) was increased to 45 $mgkg^{-1}$ by adding sodium arsenate ($Na_2HAsO_4.7H_2O$) at the rate of 0.154 g/kg soil. Each pot was filled with 1.2 kg of soil. Recommended doses of fertilizers such as urea 225$kgha^{-1}$, TSP 450$kgha^{-1}$ and MOP 175 $kgha^{-1}$ were applied to each pot (FRG, 2012). Lentil seeds were sown in the pots in a greenhouse at BSMRAU where the temperature was maintained in the range of 18^0 to 20^0C for the growth of lentil genotypes in this pot experiment

Treatments

Two lentil varieties, BARI Mashur 1 and BARI Mashur 5 were used and the soil As treatments were T_1= 8 $mgkg^{-1}$ As and T_2= 45 $mgkg^{-1}$ As. The soil was inoculated with AMF by mixing 150 g of soil with the root mixture as the treatment to reduce As uptake by the root and shoot of lentil. The total number of pots was 40 for five replications with two lentil varieties and AMF and non-AMF soils.

Data collected

Lentil shoot length was measured at week 4, after which lentil plants were harvested. Fresh and dry weights of root and shoot were recorded. All root and shoot samples were dried in an oven at 55^0C for 72 hours and preserved for As analysis.

Determination of As in soil, compost and plant samples

Samples of soil and compost and lentil roots, shoots, and grains of lentil were digested separately following the heating block digestion procedure at BSMRAU (Rahman *et al.,* 2007). Of the soil/compost samples, 0.2 g was taken into clean, dry digestion tubes and 5 ml of HNO3 concentrate and 3 ml concentrate $HCLO_4$ added to it. The mixture was allowed to stand overnight under a fume hood. The next day, this vessel was put into a digestion block for 4 hours at 120^0 C. Similarly, 0.2 g of the ground root, shoot and grains samples were placed in a clean digestion vessel and 5 ml of HNO_3 concentrate was added. The mixture was allowed to stand overnight under the fume hood. On the following day, this vessel was placed in a digestion block for 1 hour at 120^0 C temperature. This content was cooled and 3 ml of $HCLO_4$ was added to it. Again, samples were put into the heating block for 3-4 hours at 140^0C. Generally, the heating stopped when a white dense fume of $HCLO_4$ was emitted into the air. The samples were cooled, diluted to 25 ml with de-ionized water, and filtered through Whatman No 42filter paper for soil and plant samples. Finally, the samples were stored in polyethylene bottles. Before digestion of the samples, all glassware was washed with 2% HNO3 followed by washing with de-ionized water and drying.

Analysis of total As

The sample digests were brought to the Bangladesh Council of Scientific and Industrial Research Laboratory (BCSIR) to determine As concentrations in lentil root, shoot, grain, soil and vermi-compost. Total As in the determined in root, shoot, grain, soil, vermicompost, and water samples were analyzed by flow injection hydride generation atomic absorption spectrophotometry (FI-HG-AAS, Perkin Elmer A Analyst 400, USA) using external calibration (Welsch *et al.,* 1990). Standard Reference Materials (SRMs) from the National Institute of Standards and

Technology (NIST), USA, were analyzed in the same procedure at the start, during, and at the end of the measurements to ensure the accuracy of analysis.

Experimental design and statistical analysis of data

The experimental pots/field plots were laid out in a completely randomized design (CRD). Analysis of variance (ANOVA), comparison of means of data, and treatment interactions were performed using the software R.

Results

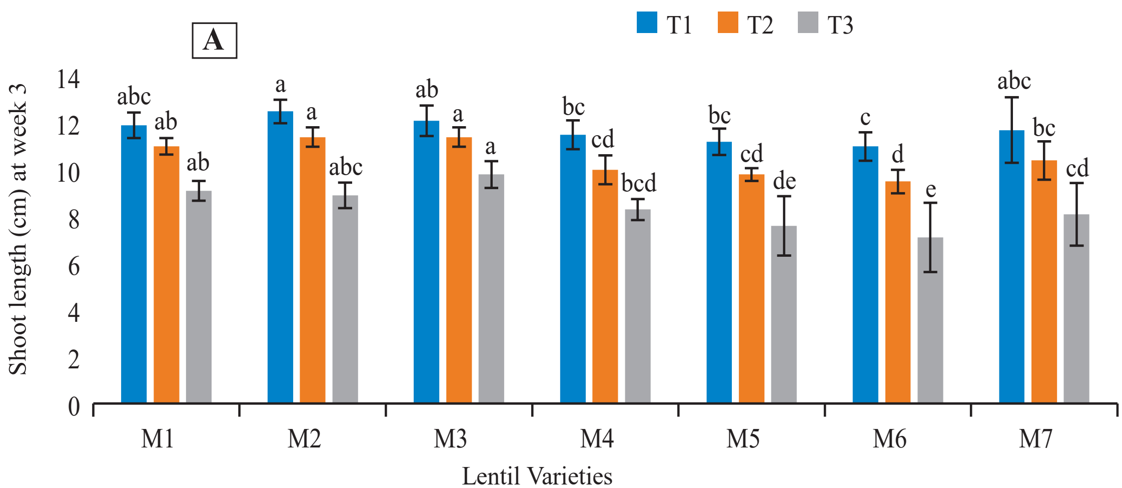
Experiment: 1

Growth of lentil varieties

Shoot lengths of BARI Mashur 2, BARI Mashur 2 and 3, and BARI Mashur 3 seedlings were 12.5, 11.4, and 9.8 cm in T_1 (soil As 5 $mgkg^{-1}$), T_2 (soil As 15 $mgkg^{-1}$) and T_3 (soil As 100 $mgkg^{-1}$) at week 3, respectively. The shoot length of T_3 treated BARI Mashur 6 was significantly lower (*p< 0.001)* than other lentil seedlings (Figure 1a). Fresh weight (0.182-0.20 g) was not significantly increased (*p< 0.001)* in T_3 treated lentil seedlings. The lowest fresh weight 0.189g was found in BARI Mashur 5 lentil seedlings at week 3 (Figure 1b). The dry weights of the roots and shoots were recorded comparatively lower in the T_3 treated lentil seedlings than in T_1 and T_2. The dry weight of the roots in the T_3 treated BARI Mashur 5 was found to be significantly different from other lentil genotypes. Meanwhile, the dry weight of shoot in T_3 treated BARI Mashur 7 was found significantly higher than BARI Mashur 1, 2, 4, 5 and 6 lentil genotypes at week 9 (Figure 1cd).

Arsenic concentrations in root and shoot

The mean comparison between treatment 1 and 2 ($0.001 \leq p < 0.01$), 1 & 3($p < 0.001$), and 2 and 3 ($p < 0.001$) for the uptake of As in roots were found significantly different. Furthermore, the mean comparison of treatment 1 and 3 and treatment 2 and 3 were found significantly identical ($p < 0.001$) on As uptake in the lentil shoot (Table 1). The interaction of BARI Mashur 1 and 3 ($0.01 \leq p < 0.0.05$),



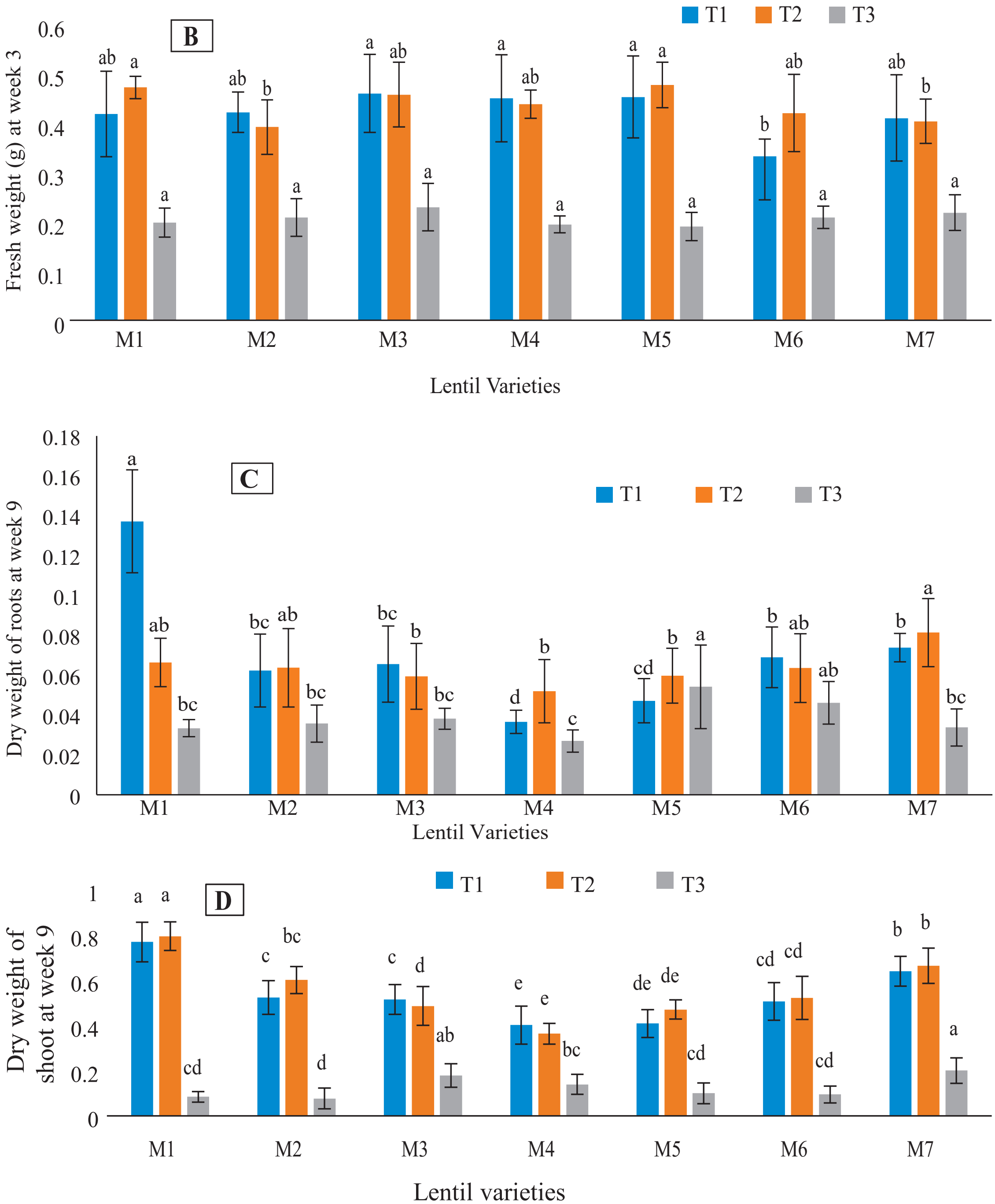


Figure 1. Effect of As uptake on a) shoot length (Mean ± SEM), b) fresh weight (Mean± SEM) of lentil varieties at week 3; c) dry weight of roots (Mean ± SEM), d) dry weight of shoots (Mean ± SEM) of Lentil varieties at week 9. Means denoted by different letters under the same As level indicate significant differences at 0.1% level of significance. M1, M2, M3, M4, M5, M6 & M7 means BARI Mashur 1, 2, 3, 4, 5, 6 & 7, respectively.

BARI Mashur 1 and 4 ($0.05 \leq p < 0.0.1$), BARI Mashur1 & 5(*$0.001 \leq p < 0.01$*), BARI Mashur1 and 6 ($0.01 \leq p < 0.05$), BARI Mashur 2 and 3 (*$p < 0.001$*), BARI Mashur 2 and 4 (*$0.001 \leq p < 0.01$*), BARI Mashur 2 and 5 (*$p < 0.001$*), BARI Mashur 2 and 6 (*$0.001 \leq p < 0.01$*), and BARI Mashur 2 and 7 (*$0.01 \leq p < 0.05$*) were found statistically significant on As uptake in their roots (Table 1).

Experiment: 2

Arsenic uptake in the grain of lentil varieties

The seven lentil varieties were grown in field plots with soil containing 5 mgkg-1 As. Among the varieties, BARI Mashur 1 was the highest As (0.45 mgkg-1) accumulator in grain and the lowest As (0.029 mgkg-1) accumulator was BARI Mashur 7 (Figure 2). On the other hand, As concentrations were 0.237, 0.133, 0.298, 0.17, and 0.262 mgkg-1 in grains of BARI Mashur 2, BARI Mashur 3, BARI Mashur 4, BARI Mashur 5, and BARI Mashur 6, respectively.

Experiment: 3

Effect of AMF on lentil

In non- AMF soil, shoot lengths of BARI Mashur 1 and BARI Mashur 5 were 6.8 and 6.2 cm in T_1 (As concentration 8 mg in each kg soils) treated lentil seedlings. Shoot length of lentil was 5.8, and 3.8 cm were in BARI Mashur 1, and BARI Mashur 5 at T_2 (arsenic concentration 45 mg in each kg soil) treated seedlings. AMF-treated shoot length in BARI Mashur 1 and 5 at 8 $mgkg^{-1}$ and 45 $mgkg^{-1}$ As concentrated both soils were found significantly higher than non AMF soils during week 4 (Figure 3a). Fresh and dry weight of shoot both were found significantly lower in non-AMF treated 45 $mgkg^{-1}$ As concentrated soils at week 5 (Figure 3b and 3c). As well as AMF has a significant effect on the increasing of dry and fresh weight of roots in lentil genotypes (Figure 3d and 3e).

Table 1. Mean comparisons of Arsenic uptake in the roots and shoots of lentil genotypes according to the treatment and varieties

Treatment interaction	As in root	As in shoot	Interaction of varieties	As in root
-	-	-	BARI Mashur 1 & BARI Mashur 3	-3.73317*
-	-	-	BARI Mashur 1 & BARI Mashur 4	-2.81283$^{.}$
-	-	-	BARI Mashur 1 & BARI Mashur 5	-4.86325**
-	-	-	BARI Mashur 1 & BARI Mashur 6	-3.39101*
-	-	-	BARI Mashur 1 & BARI Mashur 7	-2.2915
-	-	-	BARI Mashur 2 & BARI Mashur 3	-5.45291***
Treatment 1 & 2	-3.31224**	-0.9072943^{NS}	BARI Mashur 2 & BARI Mashur 4	-4.53258**
Treatment 1 & 3	-29.949***	-23.7215229***	BARI Mashur 2 & BARI Mashur 5	-6.58299***
Treatment 2 & 3	-26.6368***	-22.8142286***	BARI Mashur 2 & BARI Mashur 6	-5.11076**

***significant difference at *$p < 0.001$* level of significance, **significant difference at *$0.001 \leq p < 0.01$* level of significance, *significant difference at *$0.01 \leq p < 0.05$* level of significance, $^{(.)}$significant difference at *$0.05 \leq p < 0.1$* level of significance, ^{NS} insignificant difference

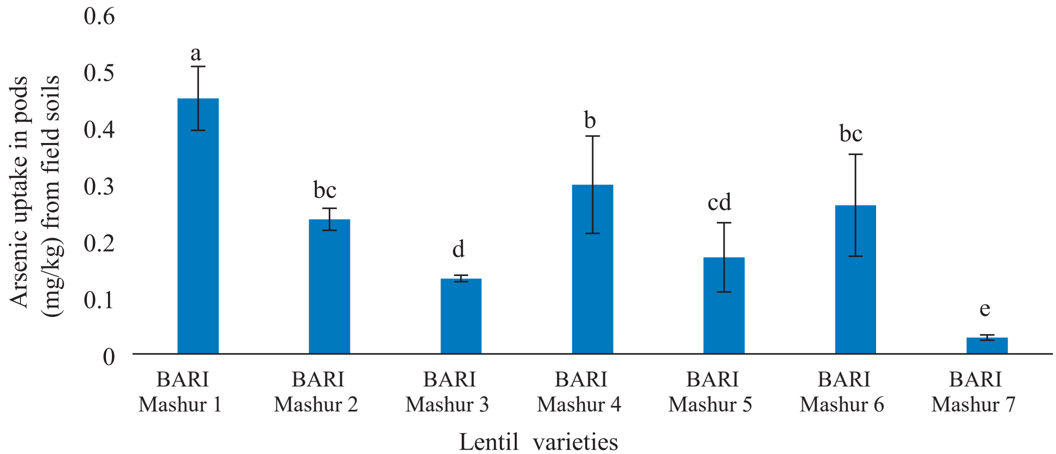
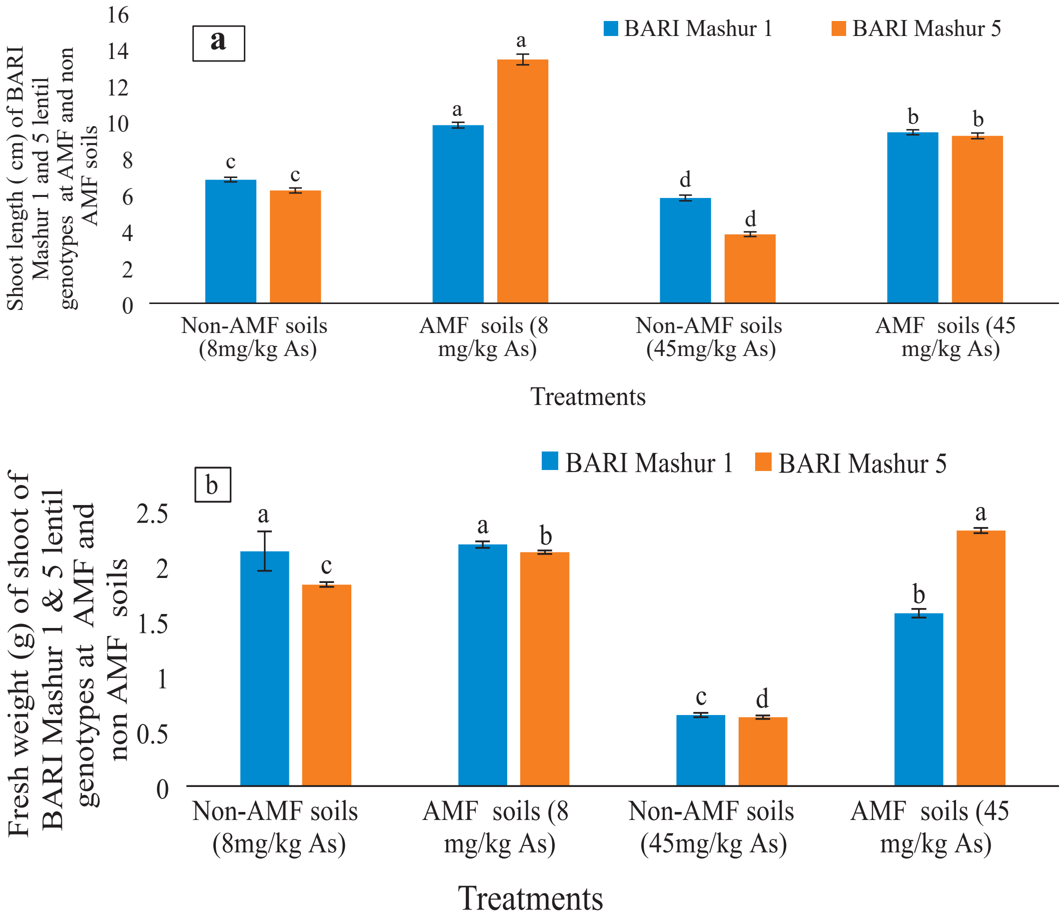


Figure 2. Arsenic accumulation in lentil grains (Mean ± SEM) from As contaminated field soils. Means denoted by different letters under the same As level indicate significant difference at 0.1% level of significance.



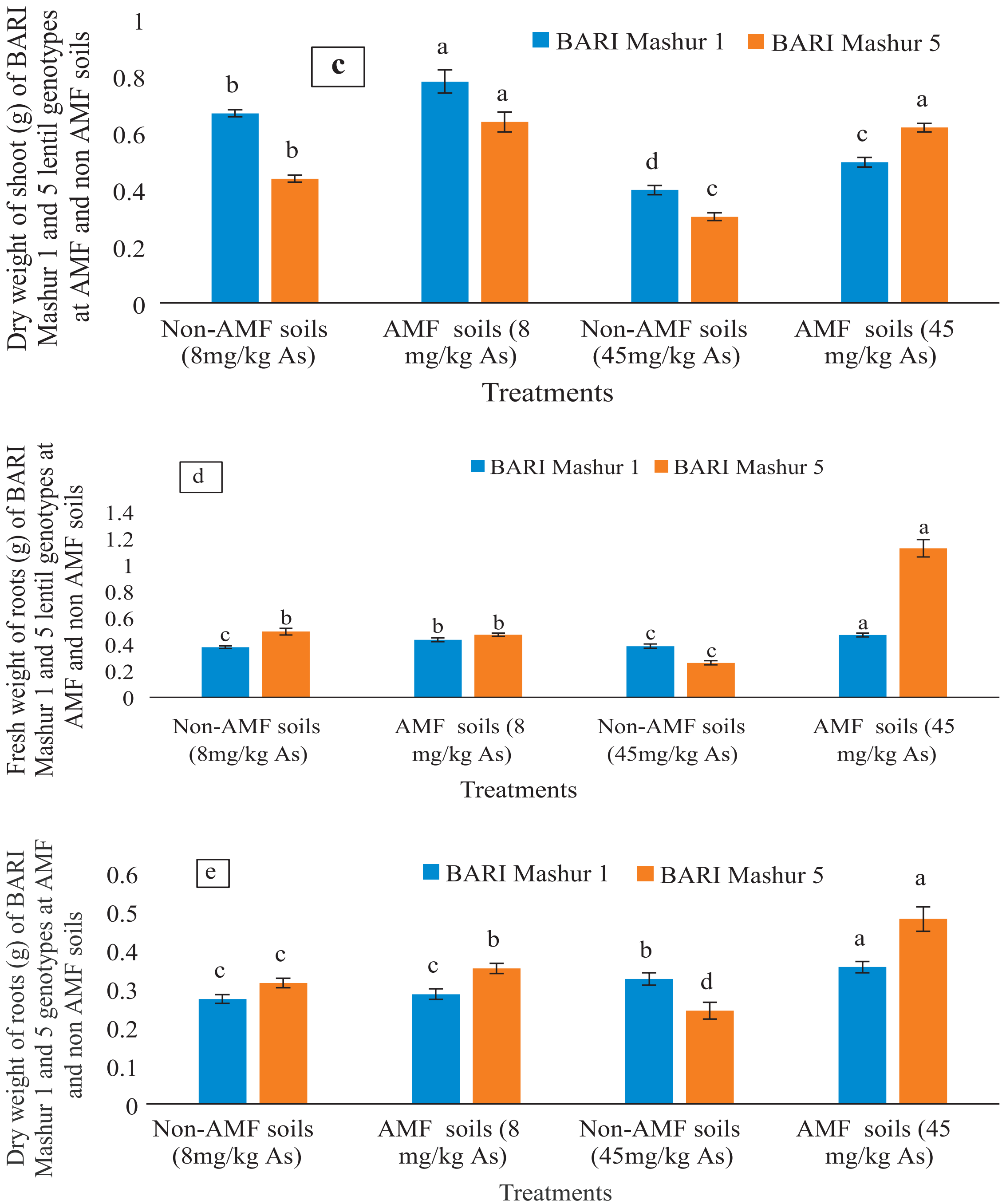


Figure 3. Effect of arbuscular mycorrhizal fungus (AMF) on a) shoot length (Mean± SEM), b) fresh weight of shoot (Mean ± SEM), c) dry weight of shoot (Mean± SEM), d) fresh weight of roots (Mean ± SEM), e) dry weight of roots (Mean± SEM) at arsenic treated BARI Mashur 1 & 5 lentil genotypes. Means denoted by different letters under the same As level indicate significant difference at 0.1% level of significance.

Effect of AMF on reduction of As uptake

Treatment and varietal interaction effects on the reduction of As uptake in the roots and shoot of lentil genotypes in AMF soil were found highly significant (*p<0.001)*. Likewise, AMF had a significant effect on the reduction in As uptake in both treated lentils. The mean comparison effects of the interaction between treatments and varieties *(p<0.001)* on the reduction of As uptake in the root and shoot mass of lentil varieties in the AMF-applied soils were significantly different (Table 2).

Discussion

Faridpur is one of the most severely As-contaminated districts in Bangladesh. Most of these areas became As polluted due to extensive groundwater use for irrigation in the crop fields. We found about 15 $mgkg^{-1}$ As in the background soils of these regions, this concentration is definitely toxic for the development of root, shoot, and grains for many cereal crops as well as lentil plants (Tables 1-2). Similarly, As contamination in food crops is also highly visible in the southern region of Bangladesh including West Bengal, India (Alam *et al.,* 2017; Alam *et al*., 2011; Ullah, 1998).

Arsenic in soil significantly affected As accumulation in lentil genotypes (Figs. 1, 2 & 3). In addition, different vegetative indexes such as root, length, shoot height, root and shoot mass of lentil plants were studied in the present experiment (Fig. 1). The sensitivity of the vegetative response in the following order: root length>root mass>shoot length>total mass (root + shoot)>shoot mass>germination. Consequently, we also found that As sensitivity was higher in lentil roots, followed by shoots and pods. Shoot height, plant biomass (root + shoot + pod), and root length were significantly affected by increasing As concentrations in soils (Alam *et al.,* 2011). For example, the total biomass of lentil crops was found to be in soils concentrated with greater danger in 100 $mgkg^{-1}$ As than other treated pots (5 $mgkg^{-1}$ As; 15 mg kg^{-1} As) of lentil seedlings (Fig. 1).

Table 2. AMF reduce As uptake in the root and shoot of lentil genotypes

Treatment: Variety-Treatment : Variety	As in root at non-AMF soil	As in shoot at non- AMF soil	AMF reduce As uptake in root	AMF reduce As uptake in shoot
T_2 : BARI Mashur 1- T_1 : BARI Mashur 1	26.7598***	6.5734***	20.6952***	4.4832***
T_1 : BARI Mashur 5- T_1 : BARI Mashur 1	-0.1566	0.9032.	-0.2166	0.5796
T_2 : BARI Mashur 5- T_1 : BARI Mashur 1	17.1094***	12.1932***	12.3146***	7.1598***
T1 : BARI Mashur 5- T2 : BARI Mashur 1	-26.9164***	-5.6702***	-20.9118***	3.9036***
T_2 : BARI Mashur 5- T_2 : BARI Mashur 1	-9.6504***	5.6198***	-8.3806***	2.6766***
T_2 : BARI Mashur 5- T_1 : BARI Mashur 5	17.266***	11.29***	12.5312***	6.5802***

***indicates a significant difference at *p<0.001* level of significance; (.) Indicates significant difference at *0.05≤p< 0.1* level of significance

All the lentil varieties released by BARI are promising in Bangladesh. However, before this study, no systematic experiment was conducted to explore the uptake of As from soil to root, shoot and grain in these lentil genotypes. Despite the lower genotypic variations, all lentil varieties showed significant differences in As accumulation in concentrated soils of their roots in 5, 15 and 100 $mgkg^{-1}$. It was found that all lentil varieties were in good conditions during the seedling stage in concentrated 5 and 15 $mgkg^{-1}$ As soils compared to the 100 $mgkg^{-1}$ concentrated soils (Fig. 1). A significant amount of As was transported from soil to lentil pods in the current study. In particular, the BARI Mashur 1 genotype accumulated a higher concentration of As (0.45 $mgkg^{-1}$) in the grains than in other genotypes (Fig 2). Similarly, regardless of As dose, roots accumulated higher concentrations of As than shoots and grains. Consistent with our results, higher As in roots of food crops were reported by Chen *et al.* (2013). However, there have been no previous reports of elevated As concentrations in lentil grains. Thus, this research is of significant importance in terms of human food chain contamination through the consumption of As-contaminated lentils.

The colonization of arbuscular mycorrhizal fungus (AMF) can alleviate multiple abiotic stresses in a variety of plant species (Chen *et al.*, 2013). While low As accumulation lentil genotypes are important for human consumption, AMF could reduce As uptake in roots, shoots and grains of lentil crops (Orlowska *et al*., 2012). The AMF colonized with the lentil roots inhibits As uptake and reduces As toxicity through the symbiotic relationship between each other (Li *et al.,* 2016). Due to the reduction in As toxicity, plants generally show increases in growth compared to non-AMF controls grown at the same As and P supplies in soil (Chen *et al.*, 2013). We found that BARI Mashur 1 and BARI Mashur 5 both lentil genotypes performed better for their growth of roots and shoots in 8 $mgkg^{-1}$ and 45 $mgkg^{-1}$ As concentrated applied AMF soils than non-AMF. The shoot length, root, and shoot mass of the lentil were found to be higher in As-contaminated soils. In other words, the growth of roots and shoots was satisfactory in both varieties of lentils when mutually treated with AMF in As-contaminated soils (Fig. 3).

In the current study, As uptake increased significantly in roots and shoots of the BARI Mashur lentil genotype (Table 1). We also provide convincing evidence that AMF can reduce As uptake in the roots and shoots in lentil genotypes (Table 2). Research also showed that AMF have a substantial effect on plant growth (Shi *et al.,* 2017). The growth parameters of the BARI Mashur 1 and 5 lentil genotypes improved significantly with the application of AMF in soils concentrated with As (Fig. 3). It emphasized that inoculation with AMF reduced As translocation from soil to plant and increased the growth and the nutrient uptake and chlorophyll content of food crops (Elahi *et al.,* 2010). Similarly, there is growing evidence that mycorrhizal fungi might alleviate As toxicity in the host plant by acting as a barrier in soils (Shi *et al.,* 2017). Mycorrhizal fungi have been widely reported to increase the tolerance of their host plants to heavy metals when present at toxic levels (Elahi *et al.,* 2010). Consistently, (Elahi *et al.,* 2010) demonstrated that, at high levels of As concentrations in soils, AMF infection reduced the concentration of As in plant biomass.

There is also evidence that AMF can be effective in soils concentrated in 8 and 45 $mgkg^{-1}$ As to reduce As uptake in root and shoot from soils (Table 2). AMF not only colonizes the root cortex but also extends the network of hyphae into the surrounding environment. These external hyphae can contribute to improving plant nutrients to increase biomass growth as well as can alleviate heavy metal toxicity by modulating metal acquisition in plants from contaminated soils (Ferrol *et al.,* 2017). Since most of the cations are essential, complete exclusion is not possible, and selective efflux would be more likely. That's why arsenate uptake by the hyphae of AMF (*R. irregularis)* occurs via the high-affinity phosphate transporter GiPT (Ferrol *et al.,* 2017). However, the direct involvement of arbuscular mycorrhizal fungi (AMF) in detoxification mechanisms remains largely unclear. Soils treated with AMF show that fungal colonization could dramatically increase plant biomass accumulation (Ferrol *et al.,* 2017). Furthermore, a positive effect of mycorrhizal inoculation on lentil growth (*L. culinaris*), P nutrition, and attenuation of As toxicity has also been reported in plant soil interaction (Chen *et al.,* 2007). Reduced uptake of As by lentil roots and, subsequently, attenuated translocation to shoots and grains will reduce the risk associated with the consumption of As-contaminated food. Therefore, lentil crops grown in As-contaminated soils with AMF colonization can potentially reduce As entry into the human body through food chains.

Conclusion

Results of present investigation reaveled that the BARI Mashur 1 genotype is a high As accumulating lentil genotype compared to the other lentil varieties grown in Bangladesh. Application of AMF decreased As concentrations in the roots and shoots of lentil genotypes. We also found that AMF could effectively reduce the transport of As from soils to roots, shoots and grains of lentil plants. These results explained the important role of AMF in the mitigation of As uptake in root and shoots, and reallocation to grains of lentil genotypes, which might have important implications in supplying toxin-free lentil grains in As-affected areas of the world.

Data Availability

The data used to support the findings of this study are included in the article.

Funding statement

The authors are also immensely grateful to the Research Management Wing of BSMRAU for their funding.

Author contributions

Mohammad Zahangeer Alam: Writing - original draft preparation, funding acquisition & editing; **Emrul Kayes**: review, and investigation; **Mimi Talukder:** writing & statistical analysis**,** Moumita Choudhury**:** Revised and editing

Competing interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgments

The authors are grateful to the Laboratory of Environmental Science at BSMRAU. The authors are especially grateful to the BCSIR for their laboratory support in conducting this research.

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