EFFECT OF ADDED DIFFERENT DOSES OF ARSENIC ON GROWTH, YIELD AND ARSENIC ACCUMULATION IN BRRI dhan45 (*Oryza sativa* L.)

A Thesis

BY

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CERTIFICATE

This is to certify that the thesis entitled **"EFFECT OF ADDED DIFFERENT DOSES OF ARSENIC ON GROWTH, YIELD AND ARSENIC ACCUMULATION IN BRRI dhan45 (*Oryza sativa* L.)"** submitted to the Faculty of Agriculture**,** Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (MS) IN SOIL SCIENCE,** embodies the results of a piece of bonafide research work carried out by **Mst. Khadiza Khatun, Registration. No. 09-03650**, under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information as has been availed of during the course of this investigation has duly been acknowledged.

Dated:
Dhaka, Bangladesh

Professor Dr. Mohammad Mosharraf Hossain
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Dedicated
To
My Parents

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Place: Dhaka, Bangladesh **The Author**
Dated:

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ABSTRACT

A pot experiment was conducted in front of the Soil Science Department of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the period from December 2016 to March 2017 to evaluate the effect of arsenic on the growth and yield of BBRI dhan45. A pot experiment was conducted which comprised with six levels of arsenic doses 0, 0.1, 0.5, 1.0, 2.0 and 4.0 ppm. The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. Increased As showed adverse effect on plant height, effective tillers per plant, non-effective tillers per plant, filled grain per plant, unfilled grain per plant, grains per panicle, 1000-grain weight and grain & straw yield of rice. A popular Boro rice variety named BRRI dhan45 was cultivated with arsenic amended soil (0 mg, 0.1 mg, 0.5 mg, 1.0 mg, 2.0 mg and 4.0 mg/kg As containing soil). Sodium arsenate (Na_2HAsO_4) was added to soil for arsenic source. The tillers number, panicle length and grain yield of BRRI dhan45 rice were found to decrease significantly ($p \leq 0.05$) with increase of arsenic (As) concentration in soil. The highest values of plant height and straw yield was observed in 0.5 $mgkg^{-1}$ treatment, whereas highest tillers number, panicles number, panicle length and grain yield were found in control treatment. The lowest values of these parameters were observed in the treatment of 4.0 $mgkg^{-1}$ As containing soil. Rice growth and yield were significantly affected by arsenic in soil ($p \leq 0.05$). Lower concentration of arsenic in soil (up to 0.5 $mgkg^{-1}$) stimulated the rice growth and yield but higher concentration of arsenic in soil (above 0.5 $mgkg^{-1}$) reduced the rice growth and yield markedly. Farmers of Bangladesh should avoid above 0.5 $mgkg^{-1}$ arsenic contaminated ground water for irrigation in rice cultivation.

ABBREVIATIONS, ACRONYMS AND SYMBOLS

AAS	Atomic Absorption Spectrophotometer
AEZ	Agro-ecological Zone
As	Arsenic
As (III)	Arsenite
As (V)	Arsenate
AsB	Arsenobetaine
AsC	Arsenocoline
AsS	Arsenic disulphate
BARC	Bangladesh Agricultural Research Council
BAU	Bangladesh Agricultural University
BGS	British geological survey
BRRI	Bangladesh Rice Research Institute
BSMRAU	Bangabandhu Sheikh Mujibur Rahman Agricultural University
CEC	Cation exchange capacity
cfu	Colony forming unit
CMC	Carboxy methyl cellulose
CRD	Complete Randomized Design
CV	Co-efficient of variation
DMMA	Diemethylarsinic acid
DNA	Dioxy ribonucleic acid
DPHE	Directorate of public health and engineering
FI-HG-AAS	Flow Injection Hydride Generation Atomic Absorption Spectrophotometer
FW	Fresh weight

g	Gram
g/cc	Gram per cubic centimeter
GP	Germination percentage
IAA	Indole-3-acetic acid
Kb	Kilo base pair
Kg	Kilogram
LSD	Least significant differences
meq	Milli equivalent
mg kg^{-1}	Milligram per kilogram
MMA	Monomethylarsonic acid
MoP	Muriate of potash
NA	Nutrient agar
PCR	Polymer chain reaction
PGPR	Plant growth promoting rhizobacteria
pH	Hydrogen ion concentrations
ppb	Parts per billion
ppm	Parts per million
PSI	Phosphate solubilization index
RCBD	Randomized Complete Block Design
ROL	Radial oxygen loss
ROS	Reactive oxygen species
SRDI	Soil Resources Development Institute
TSP	Triple Superphosphate
USDA	United States of America

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CHAPTER I

INTRODUCTION

Bangladesh is currently facing the challenge of high arsenic (As) contamination in groundwater and the contaminated ground water is widely used for irrigation especially to grow boro rice. Most of the shallow groundwater in south-west part of Bangladesh is naturally contaminated with As (BGS and DPHE, 2001) through geogenic process. The source of As in groundwater of Bangladesh is arseno-pyrite which is originally derived from granite and metamorphic rocks of the Himalayas (Polizzotto et al., 2006). It has been transported to the Gangetic delta and deposited in the different layers of the aquifers. Arsenic can also occur geologically in the sediments of Bangladesh as an adsorbed coating with ferric oxy-hydroxide (Uddin et al., 2011). It has also been suggested that both As and ferric oxy-hydroxide derived from the oxidation of arseno-pyrite which is transported and deposited in the Gangetic delta along with abundant organic matters (Harvey et al., 2006; McArthur et al., 2004). The adsorbed As can be freed and mobilized to groundwater under reducing condition, which is likely to dominate when the organic matters consume oxygen (Escobar et al., 2006; Singh, 2006; Uddin et al., 2011). Soil pH decrease may boost the dissolution of As and iron. However, higher soil pH (Campbell et al., 1985) and soil organic matter can enhance or reduce As mobility (Wang and Mulligan, 2006). In this way shallow aquifer is contaminated with arsenic which is the main source of soil during the dry Boro season in Bangladesh. Approximately 95% of all groundwater extracted is used for irrigation, mainly for Boro rice production (Alam et al., 2002; Chakraborti et al, 2002; Chowdhury et al., 2000; Mukherjee and Bhattacharya, 2001).

The use of As contaminated ground water as irrigation has been shown to build up As in soil lead to elevated levels of As in paddy soil and soil solution (Meharg and Rahman, 2003; Van Geen et al. 2006; Dittmar et al. 2007).We are increasingly depending on shallow aquifer for soil for crop production. Large quantity of arsenic is being added in the agricultural fields each year with contaminated soil. The background As level in the subsoil of major part Bangladesh seem to be <10 ppm (Abedin et al., 2002a) and similar to or lower in the topsoil (Saha and Ali, 2006). Duxbury and Zavala (2005) reported topsoil As levels >10 ppm at 48% of 456 STWs at the south-west study sites of Bangladesh and Huq et al. (2006) also reported that 21% of samples from a 24-upazila showed As levels >20 ppm, with a highest level of 81 ppm. Alam and Sattar, (2000) also found that arsenic levels in top soils (0-15 cm) ranged from not detectable to 31.8 ppm and those arsenic levels in soil were higher in the 15-30 cm soil, with levels reaching 56 ppm.

Mainly two mechanisms are involved in As uptake into the roots of rice plants from contaminated soils, the phosphate transport pathway since arsenate is an analogue of phosphate (Wu et al., 2011) and aquaporin channels by which arsenite (silicic acid analog) and undissociated methylated As species (dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA)) are taken up into the root (Li et al., 2009; Ma et al., 2008). Arsenite (iAsIII; as H_3AsO_3) mobility is higher than arsenate (iAsV; H_3AsO_4) in submerged soils because of the reductive dissolution of iron (Fe) oxides/hydroxides and consequent reduction of iAsV to iAsIII (Takahashi et al., 2004).The uptake efficiency of methylated As species (DMA and MMA) into the root was much lower than inorganic arsenic species (iAsIII and iAsV), but the translocation efficiency in the rice plant of methylated As species was much higher than inorganic arsenic species (Raab et al., 2007a). The accumulations of As in rice shoots among different rice genotypes in As-contaminated soils depends on the translocation of As in rice plants (Syu et al., 2014).Rice cultivars containing high inorganic

As represent a greater risk to human health than rice with higher levels of organic As (Zavala et al., 2008).

Elevated As contents in rice were observed from several field studies conducted with As-contaminated soils (Hossain et al., 2008; Panaullah et al., 2009; Adomako et al., 2009) and in pot experiments with As-spiked soil or soil (Rahman et al., 2008; Khan et al., 2009) which can reduce growth and productivity of rice (Abedin et al. 2002b; Delowar et al. 2005; Islam et al. 2004).The reduction of rice plant growth, in terms of tillering, plant height and shoot biomass production, was the ultimate result of arsenic phytotoxicity at high soil arsenic concentrations (Jahan et al., 2003; Rahman et al., 2004; Xie and Huang, 1998). Significant yield reductions were also observed in pot experiments (Li et al., 2009) and in rice fields in Bangladesh with soil As concentrations up to ~80 ppm (Panaullah et al., 2009). Khan et al. (2010a) found that arsenic addition in either soil or as soil-applied arsenic resulted in yield reductions from 21 to 74 % in Boro rice (dry season) and 8 to 80 % in T. Aman rice (wet season). Hossain et al. (2008) also found yield reductions of more than 40 and 60% for two popular rice varieties (BRRI Dhan28 and Iratom-24), when 20 ppm of arsenic was added to soils, compared to the control.

All previous studies showed high As concentration in roots, straw and grains but no information is available on genotypic variation in As uptake and its distribution in rice plant parts. Quantitative recovery of As from rice grains without species transformation is very challenging, especially for inorganic species. Accurate determination of inorganic As concentration is essential to access the impact of As toxicity in rice on human health because large differences of toxicity among inorganic and organic As. A successful extraction is necessary to break As (III)-thiolate complexes (Guzman et al., 2009) and avoid any As redox transformation caused by an extraction matrix, e.g. extractants and thiolate compounds released from rice grains. To date, nearly all existing methods are not able to satisfactorily recover As from rice grains independent of types (Pizarro et al., 2003; Baba et al., 2008; Sanz et al., 2005; Guzman et al., 2009; Narukawa et al., 2008). So, a reliable method is still lacking.

In the present study, mild extraction procedure (0.1M HCl) was followed for maintaining species integrity. Moreover the research presented in this dissertation therefore seeks to link soils and plants together through conducting pot experiments over two consecutive rice growing seasons. To address the As concentrations in a rice plant throughout the growing season and its effect on agronomic characters and As species uptake by rice have been studied in detail. The extent of As uptake by rice genotypes into straw and grains is expected to change with the content of As in the soil. It is expected that the results of the research herein will provide the scientific community with a useful piece of information, being accessible for governments and policy makers concerned with the consequences of rice plant with As-rich soils, and that the conclusions would serve as a basis for the process of mitigating further As accumulation in soils and crops.

Limited literatures are available on As accumulation in different rice genotypes at various growth stages and As species taken by rice grain. Detail information is needed for the conclusive assessment on As availability, accumulation, translocation and speciation in rice grain of different rice genotypes and to find out rice varieties which are tolerant and or resistant to As phytotoxicity. Therefore, the dissertation research was conducted with different exotic and native rice genotypes under different soil As levels with following objective:

To investigate the effect of arsenic (As) on the growth, yield and As content in grain and straw in arsenic contamination soil of BRRI dhan45.

CHAPTER II

REVIEW OF LITERATURE

2.1 Arsenic behavior in soil and groundwater

Arsenic can be present in soils as a metalloid or as organic and inorganic chemical compounds (Escobar et al., 2006; Fendorf et al., 2010). It can liberate from their compounds through geologic processes and human activities. Since the As in soil is highly mobile, once liberated, it can enter the water-bearing aquifers leading to groundwater contamination (Acharyya and Shah, 2010). The exact causes for As mobilization are not known yet. The scientists and researchers have proposed two hypotheses- pyrite oxidation and hydroxide reduction on the mechanism of As mobilization into groundwater in Bangladesh. The pyrite oxidation hypothesis states that the As is released into groundwater due to the oxidation of arsenopyrite (Acharyya and Shah, 2010; Mallick and Rajagopal, 1996; Mandal et al., 1998; Singh, 2006). The hydroxide reduction hypothesis proposed by Nickson et al. (1998) presumes that the As is present in alluvial sediments as an adsorbed coating onto amorphous ferric oxy-hydroxide (FeOOH). The adsorbed As is released into groundwater by the reductive dissolution of arsenic-rich ferric oxy-hydroxide (Acharyya and Shah, 2010; Fendorf et al., 2010; Reza et al., 2010; Singh, 2006).

Ferric oxy-hydroxide (FeOOH) is mainly present in the clay size soil fraction (< 2 μm) of the soil and clayey soils, therefore, generally have a higher As content than the coarser textured soils, like sandy soils (Fitz and Wenzel, 2002; Mahimairaja et al., 2005). At the same total soil concentration, clayey soils are less toxic compared to sandy soils because As is more strongly bound in the clayey soils. In parts of high-As ground water affected south-eastern districts of Bangladesh, it was found that As in rice grains and straw was high although the total As in the soils was relatively low; the soils were sandy loams (Panaullah,et al., 2005).

Ferric oxy-hydroxide is readily dissolved through reduction under anaerobic condition, releasing mainly AsV into the soil solution which is then reduced to AsIII (Fitz and Wenzel, 2002). Reduction of AsV to AsIII prior to desorption from FeOOH has also been reported (Takahashi et al., 2004). They proposed that desorption of As from FeOOH was responsible for the high As concentrations in the soil water (Takahashi et al., 2004). Under aerobic conditions FeOOH is relatively insoluble and serves as a sequestering sink for As. Considering the cycles of aerobic and anaerobic soil conditions in paddy fields (wet season monsoon rice, a 2-month dry period, and then again winter rice under flooded conditions), the Fe and As chemistry is of a dynamic nature making it less likely that aging plays a role in paddy fields. Although AsIII predominates in the bulk soil under flooded conditions, it is unclear whether the oxidized rice rhizosphere (the microenvironment around the roots), influences As speciation prior to As uptake by the rice plants.

Xu et al. (2008) investigated the dynamics of As speciation in the soil solution under both flooded and aerobic conditions and compared As accumulation in rice shoot and grain in a greenhouse experiment. Flooding of soil led to a rapid mobilization of arsenic, mainly as arsenite, in the soil solution. Arsenic concentrations in the soil solution were 7-16 and 4-13 times higher under the flooded than aerobic conditions in the control without As addition and in the As treatments (10 ppm as arsenite or arsenate), respectively. Arsenate was the main As species in the aerobic soil.

2.2 Arsenic accumulation in rice soil

In Bangladesh, very few soil analytical works have been done on As issue. Uddin (1998) of Dhaka University accounted that mean arsenic concentration in uncontaminated agricultural soils in some districts of Bangladesh varied between 2.6 and 7.6 ppm (mean 4.64 ppm), which is comparable to the standard level of arsenic in other uncontaminated soils from various countries ranging from 0.1 to 40 ppm (mean 6 ppm) (Mandal and Suzuki, 2002). A detailed arsenic survey in soils of Bangladesh has been done by BGS (1999), and is presented in Fig. 2.1

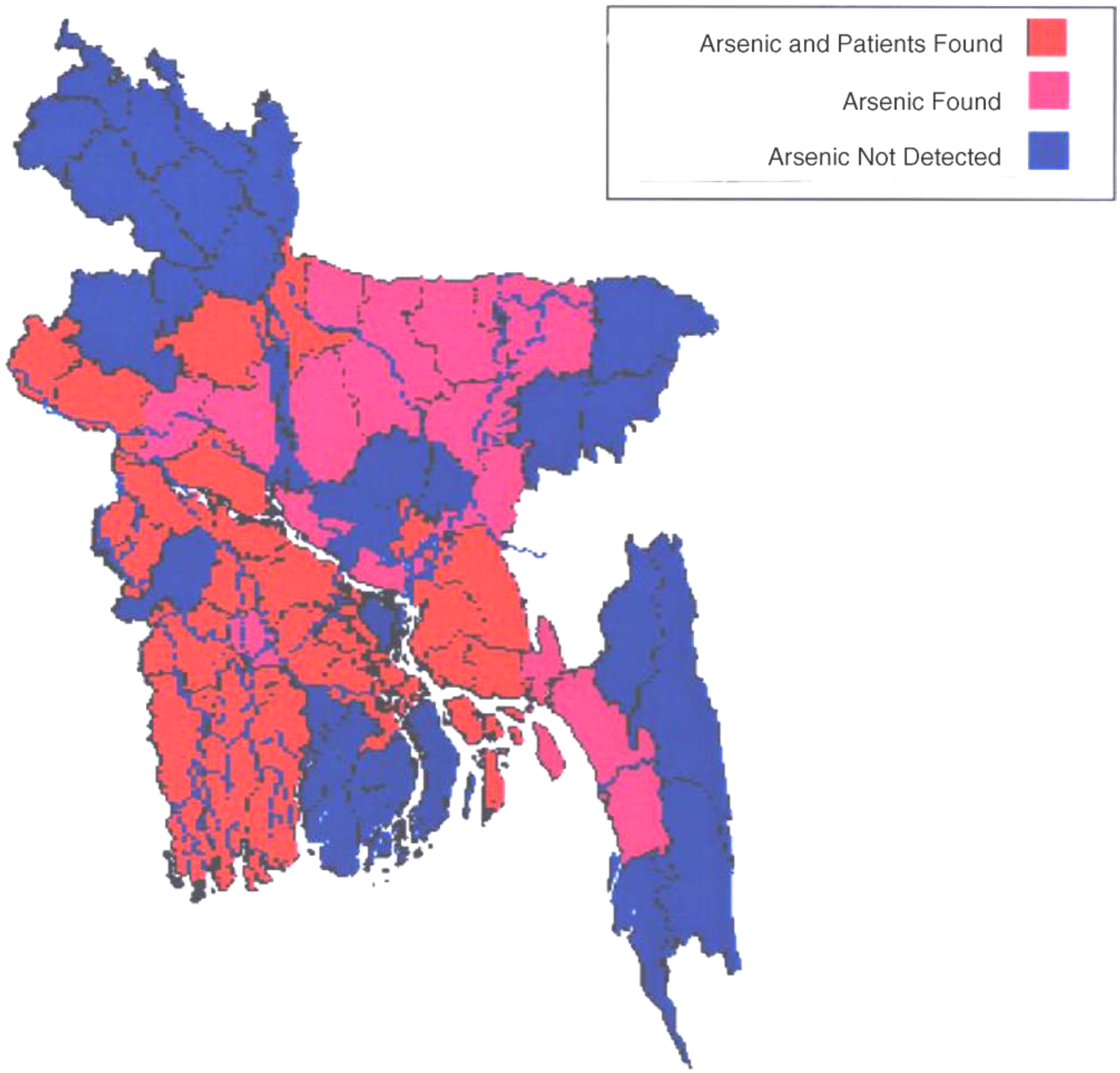

Fig. 2.1 Arsenic contaminated areas in Bangladesh (Source: Jakaria, 2000).

Another survey of arsenic levels in paddy soils was undertaken throughout Bangladesh by Meharg and Rahman, 2003 showed that arsenic levels were elevated in zones where arsenic in groundwater used for irrigation was high, and where these tube-wells have been in operation for the longest period of time. Arsenic levels in the 0-15 cm surface paddy soils varied between 3.1 and 42.5 ppm. These are in agreement with more geographically limited surveys of Bangladesh soils. Alam and Sattar, 2000 found that arsenic levels in surface soils (0-15 cm) ranged from not detectable to 31.8 ppm and those arsenic levels in soil were higher in the 15-30 cm soil, with levels reaching 56 ppm. They found that soil arsenic levels were correlated with local well water concentrations, suggesting that the soils had become contaminated through irrigation with arsenic contaminated water. Ullah et al., 1998 also reports, in 0-15 cm surface soil, levels up to 83 ppm arsenic in another survey of Bangladesh soils and suggests that these soils were contaminated through irrigation with arsenic contaminated groundwater.

Dittmar et al. (2010a) able to quantify gains and losses of soil arsenic in a rice paddy field during irrigation and monsoon flooding over a three-year period. Annual arsenic input with soil was estimated as 4.4 kg ha^{-1} a^{-1}. Within the top 40 cm of soil, the mean arsenic accumulation over three years amounted to 2.4 kg ha^{-1} a^{-1}), implying that on average 2.0 kg ha^{-1} a^{-1} were lost from the soil. Seasonal changes of soil arsenic showed that 1.05 to 2.1 kg ha^{-1} a^{-1} were lost during monsoon flooding. The remaining arsenics-loss (up to 0.95 kg ha^{-1} a^{-1}) was attributed to downward flow with percolating soil. Despite these losses, they estimate that total arsenic within the top 40 cm of soil at field site would further increase by a factor of 1.5 to 2 by the year 2050 under current cultivation practices.

2.3 Arsenic accumulation in rice root and translocation

The pH in the rhizosphere (microenvironment conditions around the rice root) may influence arsenic bioavailability and uptake. Such as NO_3^- (Important N source) is a strong oxidizing agent (increase pH) and its application to the soil may reduce As solubility by the oxidation of AsIII to AsV and the subsequent adsorption on FeOOH (Harvey et al., 2002; Nicolas et al., 2003).

Under anaerobic conditions, rice plants can transport O_2 from the leaves to the roots, resulting in a leakage of O_2 to the rhizosphere. This can create aerobic conditions in the rhizosphere and can cause precipitation of FeOOH on the roots, also known as Fe-plaque. Microbial oxidation processes may also contribute to the formation of Fe-plaque. This may influence As bioavailability and uptake (Fitz and Wenzel, 2002; Meharg, 2004).

Fe-plaque has been commonly observed in roots of rice and it may play an important role in As tolerance (Meharg, 2004). But contradictory data are available in the literature. Liu et al. (2004) showed in hydroponic experiments the potential importance of Fe-plaque formation induced by P-deficiency on AsV uptake by rice plants. In their experiments, formation of Fe-plaque was observed containing high levels of AsV after 24h transplantation from a P-sufficient solution to a P-deficient solution. Under P-deficient conditions, the translocation of AsV from the roots to shoots was reduced while the AsV concentrations in the roots were increased. The rice cultivar dependent level of Fe-plaque formation was negatively correlated to the AsV levels in shoots. The authors concluded that the influence of Fe-plaque formation on As uptake and translocation should be further investigated considering its suggested capability to limit As translocation to above ground parts of plants. A limitation was that only AsV was taken into account whereas AsIII is dominant in the paddy soil (Fitz and Wenzel, 2002).

A pot experiment was conducted by Pan et al., 2014 to investigate the effects of root oxidation on arsenic (As) dynamics in the rhizosphere and As sequestration on rice roots. There were significant differences ($P < 0.05$) in pH values between rhizosphere and non-rhizosphere soils, with pH 5.68–6.16 in the rhizosphere and 6.30–6.37 in non-rhizosphere soils as well as differences in redox potentials ($P < 0.05$). Percentage arsenite was lower (4%–16%) in rhizosphere soil solutions from rice genotypes with higher radial oxygen loss (ROL) compared with genotypes with lower ROL ($P < 0.05$). Arsenic concentrations in iron plaque and rice straw were significantly negatively correlated ($R = -0.60$, $P < 0.05$). Genotypes with higher ROL (TD71 and Yinjingruanzhan) had significantly ($P < 0.001$) lower total As in rice grains (1.35 and 0.96 ppm, respectively) compared with genotypes with lower ROL (IAPAR9, 1.68 ppm; Nanyangzhan 2.24 ppm) in the As treatment, as well as lower inorganic As ($P < 0.05$). This study showed that genotypes with higher ROL could oxidize more arsenite in rhizosphere soils, and induce more Fe plaque formation, which subsequently sequestered more As. This reduced As uptake in aboveground plant tissues and also reduced inorganic As accumulation in rice grains.

2. 4 Toxicity of soil arsenic on agronomic characters of rice

Arsenic is toxic to most plants at elevated concentration. It interferes with metabolic processes and reduces plant growth and development (Marin et al., 1993). When plants are exposed to excess arsenic in soil, they exhibits toxicity symptoms such as: decrease in plant height (Carbonell-Barrachina et al., 1995; Abedin et al., 2002b; Jahan et al., 2003; Karimi et al., 2010); lower in tillering (Kang et al., 1996; Rahman et al., 2004); lessen in shoot growth (Cox et al., 1996; Carbonell-Barrachina et al., 1998); lower fruit and grain yield (Carbonell-Barrachina et al., 1995; Abedin et al., 2002c; Kang et al., 1996); and sometimes, leads to death (Marin et al., 1992; Baker et al., 1976). Most toxicity experiments have been carried out with plants grown in hydroponics. Such type experiments can be useful to study, for example, uptake mechanisms, internal transport, metabolism, and toxic effects. Some reviews below:

Marin et al. (1992) in their hydroponic experiment found shorter rice plants when grown with $0.8 mgL^{-1}$ arsenite and MMAA. However, they found no significant reduction in plant height when plants were exposed to arsenate. Despite stunted plant height, rice straw biomass did not decrease significantly with increasing arsenate doses. They also reported AsIII was more toxic to root growth (dry weight production) than AsV, with the first inhibition observed at 0.8 mg/kg. Although there are number of reports of reduced shoot biomass/growth in rice (Milam et al., 1988; Marin et al.,1993) due to application of arsenate (AsV).

Abedin and Meharg (2002) exposed eight Bangladeshi rice varieties to AsIII and AsV and tested for germination and seedling growth. Germination was slightly inhibited at 0.5 and 1 ppm As. At 2 ppm As, inhibition was more than 10 percent. No significant difference between Boro and T.Aman cultivars in terms of germination was observed. Root growth was inhibited by ~20 percent at 0.5 ppm As and AsV was more toxic than AsIII. Their results are contrasting from the previous findings of Marin et al. (1992) of which AsIII was more toxic to root growth than AsV. Moreover Dasgupta et al. (2004) reported AsV a root growth inhibition of 90 percent for rice cultivar Azucena and 50 percent inhibition for Bala at 1 ppm. Abedin and Meharg (2002) also reported Boro cultivars were slightly more tolerant than T.Aman cultivars. Shoot height was also affected, at 0.5 ppm As, the shoot height was reduced by ~30 percent with no significant difference between cultivars and As species.

Toxicity experiments are also carried out with plants grown in soil to which a certain amount of As is added (spiked soil) shortly before the experiment. Adding As to reach a certain soil concentration suggests that the results are representative of the field. However, in the field As is added over a number of years. The prolonged contact time between As and the soil in the field can result in a lower solubility of As and therefore lower uptake by plants in the field. Therefore, experiments with spiked soils often result in an overestimation of the adverse effects compared to the actual field situation (Duxbury and Zavala, 2005).

Onken and Hossner (1995) spiked soil with 25 ppm AsIII and AsV. In the silt loam soil, a reduced dry matter was first observed after 40 days exposure. At the termination of the experiment (60 days exposure), the dry matter was reduced by approximately 50 percent with no significant difference between AsV and AsIII. In the clayey soil, no toxicity was observed, suggesting that a greater part of the added As was strongly bound to the soil. Water from the clayey soil contained 10 to15 times less As although the large uncertainties and fluctuations in soil water concentrations. However, Onken and Hossner (1995) also reported a contrasting result of increased dry matter production of rice plants grown in the Beaumont clay treated with 5 ppm As as sodium arsenite or sodium arsenate over the plants that received no arsenic (control).

The reduction of rice plant growth, in terms of tillering, plant height and shoot biomass production, was the ultimate result of arsenic phytotoxicity at high soil arsenic concentrations (Jahan et al., 2003; Rahman et al., 2004; Xie and Huang, 1998).

Azad et al. (2012) found filled spikelet number of BR 11 rice decreased with increase of arsenic concentration in soil. The highest filled spikelet number (678.33 ± 78.81) was found in control treatment and the lowest (197.33 ± 97.58) in 4 ppm As treatment. A significant ($p < 0.05$) negative correlation ($r = -0.552$) between filled spikelet number and soil arsenic was detected.

Rahman et al. (2007b) recorded tiller numbers, plant height and shoot biomass production of rice plant to determine the effect of soil arsenic concentrations on rice growth. The highest number of tiller (15.51±1.00 pot^{-1}) was observed in BRRI hybrid dhan1 at control arsenic treatment followed by 12.34±1.50, 10.24±0.69, 9.14±1.02 and 8.43±1.02 in BRRI dhan 29, BRRI dhan 35, BRRI dhan 28 and BRRI dhan 36, respectively. On the other hand, the lowest number of tiller (4.01±2.00) was observed in BRRI dhan36 at 30 ppm soil As treatment. Plant height and shoot biomass production were also decreased drastically with the increase of soil arsenic concentrations.

Abedin et al. (2002c) conducted a greenhouse experiment to evaluate the effects of different concentrations of arsenic in soil on the growth of rice. Treatments of the greenhouse experiment consisted of seven different arsenate concentrations ranging from 0 to 8 ppm of As applied regularly throughout the 170 day post-transplantation growing period until plants were ready for harvesting. The concentration of arsenate in soil has a marked effect on the height of rice plant. It was observed that plant height decreased significantly ($p < 0.001$) with increasing arsenate concentration in soil. With the lower range of arsenate doses (0-1.0 ppm of As), plant height ranged between 91.1 and 84.1 cm, while with higher arsenate doses (2.0-8.0 ppm of As), plant height decreased to 79.2-63.8 cm. Tsutsumi, 1980 in his pot experiment, with different concentrations of arsenate in soil, observed no reduction of rice plant height up to 125 ppm of As but did observe 63% reduction of height at 312.5 ppm of As.

2.5 Toxicity of soil arsenic on grain and straw yield of rice

The overwhelming majority of research evidence indicates that prolonged exposure to arsenic rich water via irrigation leads to reduced crop yields. Khan et al. (2010a) found that arsenic addition in either soil or as soil-applied arsenic resulted in yield reductions from 21 to 74 % in Boro rice (dry season) and 8 to 80 % in T. Aman rice (wet season), the latter indicating the strong residual effect of arsenic on subsequent crops. Hossain et al. (2008) also found yield reductions of more than 40 and 60% for two popular rice varieties (BRRI Dhan28 and Iratom-24), when 20 ppm of arsenic was added to soils, compared to the control.

Jahiruddin et al. (2004) spiked silt loam soil with arsenic. First, a Boro rice cultivar developed by the Bangladesh Rice Research Institute BRRI dhan29 and then an Aman cultivar BRRI dhan3 was grown. For Boro rice, the first significant effects occurred at 10 ppm soil As, causing a grain yield reduction of more than 45 percent. The As concentration in grains of Boro rice first increased with the exposure level but then decreased. A possible explanation is that the toxic effects became so severe that arsenic was hardly translocated anymore to the few grains that were produced at 25 ppm soil As and higher. For Aman rice, the first significant adverse effects were on the number of grains per panicle and straw yield at 10 ppm As. At 20 ppm soil As, grain yield became affected whereas the other parameters were not significantly affected below 40 ppm soil As. A shortcoming of Jahiruddin et al. (2004) was that chemical analysis did not include a certified reference material (CRM). The reported concentrations can therefore only be regarded as indicative.

Begum et al. (2008) found significantly reduction of grain and straw yield due to arsenic toxicity. The yield reductions due to two arsenic treatments (15 and 30 ppm arsenic) compared to control were 20.6 and 63.8% in grain and 21.0 and 65.2 % in straw respectively. They also found highly significant toxic effect on the effective tillers pot^{-1}, filled grains per panicle and 1000 grain weight in case of 30 ppm soil arsenic. The toxic effect on yield parameters due to 15 ppm arsenic treatment was not significant.

Panaullah et al. (2009) acknowledged progressively decreased of rough rice yield from about 7 to 2 t ha^{-1} in 2006 and 9 to 3 t ha^{-1} in 2007 across the soil arsenic gradient. The reduction in rice yield was allied with a decrease in the number of productive tillers. Plant growth was in the same way affected by soil-As concentration, with total above ground plant biomass production at the high soil-As level approximately one-third of that at the low soil-As level.

2.6 Total arsenic concentration of rice plant

Arsenic accumulation in rice is determined by many factors, such as soil conditions, the uptake capacity of roots, the efficiency of translocation, and the distribution and redistribution of arsenic among plant tissues.

The water used for the agricultural sectors in Bangladesh is mostly extracted from the groundwater sources. In the arsenic-affected areas of Bangladesh, groundwater contains up to

2 ppm of As (Tondel et al., 1999) as compared to the WHO recommended provisional limit of 0.01 ppm of As (WHO, 2001). Since the groundwater contains arsenic, the agricultural soils as well as the agricultural crops, particularly different rice varieties are vulnerable to arsenic contamination (Bhattacharya et al., 2010; Khan et al., 2010b; Martin et al., 2010).

The arsenic from both soils and soil is accumulating in rice grains during cultivation (Ahmed et al., 2003; Huq et al., 2006; Khan et al., 2010b; Rahman and Hasegawa, 2011; Saha and Zaman, 2011; Williams et al., 2004), as illustrated in Figure 2.2. As a result, the crop yields can be reduced significantly due to the phytotoxic effects of arsenic (ITNC, 2008).

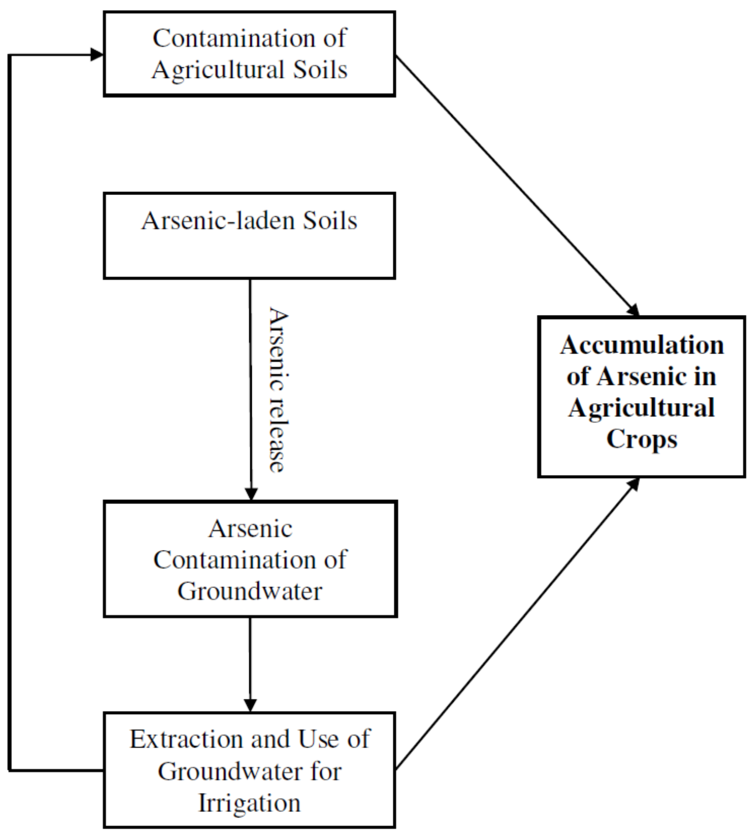

Fig. 2.2 Arsenic accumulation in agricultural crops (Source: Safiuddin et al., 2011)

Because of groundwater contamination with high level of arsenic, scientists and researchers become interested to investigate the effects of arsenic contaminated soil and soil on its accumulation and metabolism in rice (Oryza sativa L.). Recently, some reports focused on the effects of arsenic contaminated soils and soil on its uptake in root, shoot, husk and grain of rice and its metabolism in rice at greenhouse condition (Rahman et al., 2004; Rahman et al., 2007a; Abedin et al., 2002a; Abedin et al., 2002b). However, field level investigation on this aspect is inadequate. Limited literatures are found on arsenic accumulation in different fractions of rice gain as well as its retention in cooked rice following the traditional cooking methods used by the populations of arsenic epidemic areas.

Different plant parts showed different abilities to accumulate arsenic content. There have been reports on arsenic content in tissues of rice, which are distributed in fractions, although values vary greatly due to differences in varieties, fertilizers, or cultivating behavior in field sites or glasshouses (Marin et al., 1992; Abedin et al., 2002c; Alam and Rahman, 2003; Das et al., 2004; Rahman et al., 2004, 2007a,b).

The pathway of As translocation from roots to shoots and from shoots to grain is less well understood, although the sharp declining gradient in the concentration of As from roots to stems, leaves and grain (Liu et al., 2006; Zheng et al., 2011) suggests a limited mobility of inorganic As in rice. Abedin et al., 2002c; Rahman et al., 2004; Rahman et al., 2007a reported elevated content of arsenic in tissues of rice when the plant was grown in soils contaminated with higher concentrations of arsenic. Onken and Hossner (1995) also reported that plants grown in soil treated with arsenic had higher rate of arsenic uptake compared to those grown in untreated soil. In principle, arsenic concentration in rice straw increases significantly with increasing arsenic concentration in rice root. However, arsenic translocation from straw to rice grain does not differ significantly for variations in rice strain (Abedin et al., 2002c; Rahman et al., 2007b).

Abedin et al. 2002c conducted a greenhouse experiment to evaluate the effects of different concentrations of arsenic in soil on the growth of rice, including the partitioning of arsenic between different plant parts (grain, husk, root, and straw). The data for root arsenic concentrations clearly show that, irrespective of arsenate dose (including the control), roots contain higher concentrations of arsenic than any other part of the rice plant. This effect is quite marked for all arsenate treatments except for the highest arsenate treatment where concentrations of arsenic in both root and straw are similar. This suggests that the rice cultivar used in this study has an ability to store a certain amount of arsenic in its root system. However, at the highest arsenate treatment, this storage ability may have been exceeded, and arsenic is readily translocated to the shoot, resulting in similar arsenic concentrations for both straw and root. Marin et al., 1992 & 1993 and Xie and Huang, 1998 was also observed higher arsenic accumulation in roots than any other parts.

Dittmar et al. (2010b) investigated the arsenic contents of rice straw and grain over three consecutive harvest seasons (2005-2007) in a paddy field in Munshiganj, Bangladesh, which exhibits a documented gradient in soil As caused by annual irrigation with As-rich groundwater since the early 1990s. This may result in increasing levels of As in rice straw and grain. This straw and grain As contents from the field study indicated increased soil As to lead to elevated As uptake into rice plant. Some earlier studies also revealed that straw As contents as a function of soil As contents (Hossain et al., 2008; Panaullah et al., 2009; Lu et al., 2009). Additionally, a pot experiment with soils and rice seeds from the field site was carried out by dittmar et al., 2010b in which soil and soil As were influence As uptake during rice growth. At similar soil As contents, plants grown in pots exhibited similar grain and straw As contents as plants grown in the field.

Khan et al. (2010a) studied some paddy soils in the Bengal delta are contaminated due to irrigation of As-laden groundwater, resulting rice straw As concentrations increased with increasing soil As concentrations. Rice straw is used as cattle feed in many countries including Bangladesh. The high arsenic concentrations may have the potential for adverse health effects on the cattle and an increase of arsenic exposure in humans via the plant-animal-human pathway.

Talukder et al. (2012) conducted pot experiments to investigate the effects of water management (aerobic and anaerobic), arsenic rates (0, 20 and 40 ppm) and phosphorus rates (0, 12.5 and 25 ppm) on arsenic uptake in rice plants. They found that arsenic concentration in rice grain and straw increased significantly with increasing arsenic rates in the soil. Arsenic availability in soil water solution was less (58%) under aerobic water management as compared to anaerobic water management. The highest total grain arsenic content 2.23±0.12 ppm and 0.623±0.006 ppm was found in $P_{12.5}$ As_{40}-anaerobic and P_{25} As_{40}-anaerobic treatment combination in BRRI dhan29 and BRRI dhan32 respectively, which was significantly higher (41-45%) than in the same As and P treatments for pots under aerobic water management. The As content in rice straw (upto 24.7±0.49 ppm in BRRI dhan29, 17.3±0.49 ppm in BRRI dhan32 with the highest As level) suggested that As can more easily be translocated to the shoots udder anaerobic conditions than aerobic condition. BRRI dhan29 was more sensitive to arsenic than BRRI dhan32. Under aerobic water management, P soil amendments reduced arsenic uptake by rice plants. The study demonstrated that aerobic water management along with optimum P amendment and selection of arsenic inefficient rice varieties are appropriate option that can be applied to minimize arsenic accumulation in rice which can reduce effects on human and cattle health risk as well as soil contamination.

Bhattacharya et al. (2010) were investigated arsenic contaminated soil and paddy field soil to assess the accumulation of arsenic and its distribution in the various parts (root, straw, husk, and grain) of rice plant. Results showed that the paddy soil get contaminated from the soil and thus enhancing the bioaccumulation of arsenic in rice plants. The total soil arsenic concentrations ranged from 1.34 to 14.09 ppm. Soil organic carbon showed positive correlation with arsenic accumulation in rice plant, while soil pH showed strong negative correlation. Higher accumulation of arsenic was noticed in the root (6.92 ± 0.241–28.63 ± 0.225 ppm) as compared to the straw (1.18 ± 0.002–2.13 ± 0.009 ppm), husk (0.40 ± 0.004–1.05 ± 0.006 ppm), and grain (0.16 ± 0.001–0.58 ± 0.003 ppm) parts of the rice plant. However, the accumulation of arsenic in the rice grain of all the studied samples was found to be between 0.16 ± 0.001 and 0.58 ± 0.003 ppm dry weights of arsenic, which did not exceed the permissible limit in rice (1.0 ppm according to WHO recommendation). Two rice plant varieties, one high yielding and another local had been chosen for the study of arsenic translocation. Higher translocation of arsenic was seen in the high yielding variety (0.194–0.393) compared to that by the local rice variety (0.099–0.161). An appreciable high efficiency in translocation of arsenic from shoot to grain (0.099–0.393) was observed in both the rice varieties compared to the translocation from root to shoot (0.040–0.108).

A study conducted by Lin et. al. (2013) with the objective to measure arsenic content in different parts of rice and paddy soils, and eventually explains the arsenic distribution in ratoon rice, including its relationship to the soil. Arsenic levels of rice in grains, straws, roots, and soils were obtained from 15 rice paddies, selected based on different arsenic soil concentrations ranging from 67 to 438 ppm (n = 15). The mean arsenic content in grains was measured at 0.20 ppm (n = 60) and the highest grain arsenic of the survey was at 1.183 ppm. Meanwhile, the mean total arsenic levels were 244 ppm (n = 28) in root and 4.4 ppm (n = 28) in straw. In comparison, regression of topsoil arsenic levels with rice grains (r^2 = 0.00) and straws (r^2 = 0.56) were less significant compared to that with rice roots (r^2 = 0.93), and the mean arsenic level in rice from root to grain was also shown to have a decreasing trend.

Over a wide range of environments total As in the grain has been correlated with total As in the shoots at harvest, for a single rice cultivar, and modeled by a hyperbolic relationship (Ying, et al., 2009). Norton et al. (2010) found positive quadratic correlation between shoot As and grain As at harvest. This suggests that there is genetic regulation for both grain and shoot As but that the two are only closely related when there is a low concentration of shoot As. This is further emphasized by same authors by the analysis of the same cultivars across the four field sites in Bangladesh and found significant genetic as well as environmental effect, with the environmental effect dominating. This trend is in agreement with previous studies where the grain to shoot ratio across multiple environments is observed as a hyperbolic relationship, with a decrease in translocation efficiency alongside increasing shoot As accumulation (Williams, et al., 2007*;* Adomako et al.,2009; Ying, et al., 2009). In other words, the higher the shoot As concentration, the lower the proportion (but not the amount) of As that reaches the grain.

Norton et al. (2009) was measured grain As in 76 cultivars consisting of Bangladeshi landraces, improved Bangladesh Rice Research Institute (BRRI) cultivars, and parents of permanent mapping populations grown in two field sites in Bangladesh, Faridpur and Sonargaon, irrigated with As-contaminated tubewell water. Grain As ranged from 0.16 to

0.74 ppm at Faridpur and from 0.07 to 0.28 ppm at Sonargaon. Highly significant cultivar differences were detected and a significant correlation (*r*=0.802) in the grain As between the two field sites was observed, indicating stable genetic differences in As accumulation.
Norton et al. (2010) also observed the significant correlation between percentage Asi and total shoot As suggests that the percentage of Asi in the shoot is mechanistically linked to the total concentration of As within the shoot. There As speciation in shoots at harvest was in agreement with Abedin, et al., (2002c) who found that in rice >95% of shoot As was in the As*i* form, either arsenate or arsenite, at harvest.

A study was conducted by Rahman et al. (2007b) to investigate the accumulation and distribution of arsenic in different fractions of rice grain (*Oryza sativa* L.) collected from arsenic affected area of Bangladesh. The agricultural soil of study area has become highly contaminated with arsenic due to the excessive use of arsenic-rich underground water (0.070±0.006 mg L^{-1}, *n*=6) for irrigation. Arsenic content in tissues of rice plant and in fractions of rice grain of two widely cultivated rice varieties, namely BRRI dhan28 and BRRI hybrid dhan1, were determined. Regardless of rice varieties, arsenic content was about 28 and 75 folds higher in root than that of shoot and raw rice grain, respectively. In fractions of parboiled and non-parboiled rice grain of both varieties, the order of arsenic concentrations was; rice hull > bran-polish > brown rice > raw rice > polish rice. Arsenic content was higher in non parboiled rice grain than that of parboiled rice. Arsenic concentrations in parboiled and non-parboiled brown rice of BRRI dhan28 were 0.8±0.1 and 0.5±0.0 ppm dry weight, respectively while those of BRRI hybrid dhan1 were 0.8±0.2 and 0.6±0.2 ppm dry weight, respectively. However, parboiled and non-parboiled polish rice grain of BRRI dhan28 contained 0.4±0.0 and 0.3±0.1 ppm dry weight of arsenic, respectively while those of BRRI hybrid dhan1 contained 0.43±0.01 and 0.5±0.0 ppm dry weight of As, respectively. Both polish and brown rice are readily cooked for human consumption. The concentration of arsenic found in the present study is much lower than the permissible limit in rice (1.0 ppm) according to WHO recommendation. Thus, rice grown in soils of Bangladesh contaminated with arsenic of 14.5±0.1 ppm could be considered safe for human consumption.

A root-feeding experiment was conducted by Zhao et al., (2012) to evaluate As translocation from roots to shoots and from shoot tissues to rice grain. This study demonstrated that As (mainly arsenite) has a relatively low mobility within rice plants. Arsenite was transported to rice grain mainly through the phloem. A small proportion of arsenite fed to flag leaves can be transported to grain.

CHAPTER III

MATERIALS AND METHODS

The pot experiment was conducted at the Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the period from December 2016 to April 2017 to study the effect of different doses of arsenic on yield of rice. The details of the materials and methods have been presented below:

3.1 Description of the experimental site

3.1.1 Location

The present piece of research work in pot was conducted in the experimental area of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka. The location of the site is 230 74′ N latitude and 900 35′ E longitude with an elevation of 8.2 meter from sea level.

3.1.2 Soil

The soil of the experimental area that used in the pot for rice grown belongs to "The Modhupur Tract", AEZ 28 .Pot soil was silty clay in texture. Soil pH was 5.6 and has organic carbon 0.45%. The experimental area was flat having available irrigation and drainage system. The details of the pot soil have been presented in Appendix I.

3.1.3 Climate

The geographical location of the experimental site was under the subtropical climate, characterized by three distinct seasons, winter season from November to 20 February and the pre-monsoon period or hot season from March to April and monsoon period from May to October (Edris*et al.,* 1979). Details of the meteorological data of air temperature, relative humidity, rainfall and sunshine hour during the period of the experiment was collected from the Weather Station of Bangladesh, Sher-e Bangla Nagar, Dhaka and has been presented in Appendix II.

3.2 Experimental details

3.2.1 Treatments

There were 06 (six) treatments of arsenic level (6 levels): 0, 0.1, 0.5, 1.0, 2.0 and 4.0.

i. As0: Control (No arsenic)

ii. As0.1: 0.1 ppm As

iii. As0.5: 0.5 ppm As

iv. As1.0: 1.0 ppm As

v. As2.0: 2.0 ppm As

vi. As4.0: 4.0 ppm As

3.2.2 Experimental design and layout

The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. There were 45 pots for 15 treatment combination in each of 3 replications. The 15 treatment combinations of the experiment were assigned at random in 45 pots of each replication.

3.3 Growing of crops

3.3.1 Raising seedlings

3.3.1.1 Seed collection

The seeds of the test crop i.e. BRRI dhan45 is collected from Bangladesh Rice Research Institute (BRRI), Joydevpur, Gazipur.

3.3.1.2 Seed sprouting

Healthy seeds were selected by specific gravity method and then immersed in water bucket for 24 hours and then they were kept tightly in gunny bags. The seeds started sprouting after 48 hours and were sown after 72 hours.

3.3.1.3. Preparation of seedling nursery bed and seed sowing

According to BRRI recommendation seed bed was prepared with 1 m wide seed bed adding nutrients as per the requirements of soil. Seeds were sown in the seed bed on December 8, 2016, in order to transplant the seedlings in the pot as per experimental treatment.

3.3.2 Preparation of the pot

The pot for the experiment was filled up with soil at 02 January, 2017. Weeds and stubble were removed from the soil and finally obtained a desirable tilth of soil for transplanting of seedlings.

3.3.3 Fertilizers and manure application

The fertilizers N, P, K, S, Zn and B in the form of urea, TSP, MoP, gypsum, zinc sulphate and borax, respectively were applied. The entire amount of TSP, MoP, gypsum, zinc sulphate and borax were applied during the final preparation of pot land. Urea was applied in two equal installments at tillering and before panicle initiation. Different concentration of As was mixed the soil as per treatment. The dose and method of application of fertilizers are shown in Table 1.

Table 1. Dose and method of application of fertilizers

Fertilizers	Dose (kg/ha)	Application (%)		
		Basal	1st installment	2nd installment
Urea	150	33.33	33.33	33.33
TSP	100	100	-	-
MP	100	100	-	-
Gypsum	60	100	-	-
Borax	10	100	-	-

Source: Anon., 2010, BRRI, Joydevpur, Gazipur

3.3.4 Uprooting of seedlings

The nursery bed was made wet by application of water one day before uprooting of the seedlings. The seedlings were uprooted on January 8, 2017 without causing much mechanical injury to the roots.

3.3.5 Transplanting of seedlings in the pots

The rice seedlings were transplanted in the pot at 9 January, 2017 and 2 healthy seedlings were transplanted in the pot in a hill.

3.3.6 After care

After establishment of seedlings, various intercultural operations were accomplished for better growth and development of the rice seedlings.

3.3.6.1 Irrigation and drainage

Sprinkler irrigation was provided to maintain a constant level of standing water up to 6 cm in the early stages to enhance tillering and 10-12 cm in the later stage to discourage late tillering. The pot was finally dried out at 15 days before harvesting.

3.3.6.2 Gap filling

First gap filling was done for all of the pots at 10 days after transplanting (DAT) by planting same aged seedlings.

3.3.6.3 Weeding

Weeding were done to keep the pots free from weeds, which ultimately ensured better growth and development. The newly emerged weeds were uprooted carefully at tillering stage and at panicle initiation stage by manual means.

3.3.6.4 Top dressing

After basal dose, the remaining doses of urea were top-dressed in 2 equal installments in the soil.

3.3.6.5 Plant protection

Furadan 57 EC was applied at the time of final land preparation and later on other insecticides were applied as and when necessary.

3.4 Harvesting, threshing and cleaning

The rice was harvested depending upon the maturity of plant and harvesting was done manually from each pot. The harvested crop of each pot was bundled separately, properly tagged and brought to threshing floor. Enough care was taken during harvesting, threshing and also cleaning of rice seed. Fresh weight of grain and straw were recorded pot wise. The grains were cleaned and finally the weight was adjusted to a moisture content of 13%. The straw was sun dried and the yields of grain and straw pot^{-1} were recorded and converted to t/ ha.

3.5 Data recording

3.5.1 Plant height

The height of plant was recorded in centimeter (cm) at the time of harvest. The height was measured from the ground level to the tip of the tiller.

3.5.2 Number of tillers hill^{-1}

The number of tillers hill^{-1} was recorded at the time of harvest by counting total tillers in a hill.

3.5.3 Total tillers hill-1 (at harvest)

The total number of total tillers hill^{-1} was counted as the number of panicle bearing and nonbearing tillers hill^{-1}. Data on total tillers hill^{-1} were counted at harvest and value was recorded.

3.5.4 Effective tillers hill^{-1}

The total number of effective tillers hill^{-1} was counted as the number of panicle bearing tillers plant^{-1}. Data on effective tiller hill^{-1} were counted and value was recorded.

3.5.5 Length of panicle

The length of panicle was measured with a meter scale from 5 selected panicles and the average value was recorded.

3.5.6 Number of panicle

The number of total panicle per pot were counted.

3.5.7 Filled grain hill^{-1}

The total number of filled grain per hill were counted manually.

3.5.8 Un-filled grain hill^{-1}

The total number of unfilled grain per hill were counted manually.

3.5.9 Filled grains panicle^{-1}

The total number of filled grains was collected randomly from selected 3 panicles of a pot on the basis of grain in the spikelet and then average number of filled grains panicle^{-1} was recorded.

3.5.10 Unfilled grains panicle^{-1}

The total number of unfilled grains was collected randomly from the same 3 panicles where filled grains were counted of a pot on the basis of no grain in the spikelet and then average number of unfilled grains panicle^{-1} was recorded.

3.5.11 Weight of 1000 seeds

One thousand seeds were counted randomly from the total cleaned harvested seeds of each individual pot and then weighed in grams and recorded.

3.5.12 Grain yield

Grains obtained from each unit pot were sun-dried and weighed carefully. The dry weight of grains of each pot was measured and grain yield pot^{-1}.

3.5.13 Straw yield

Straw obtained from each unit pot were sun-dried and weighed carefully. The dry weight of the straw of each pot was measured.

3.6 Statistical analysis

The data obtained for different characters were statistically analyzed using Statistix 10 software to observe the significant difference among the treatments. The mean values of all the characters were calculated and factorial analysis of variance was performed. The significance of the difference among the treatment means was estimated by the Least Significant Difference Test (LSD) test at 5% level of probability (Gomez and Gomez, 1984).

Chapter IV
RESULTS AND DISCUSSION

The present investigation included the response of rice variety (BRRI dhan45, susceptible to arsenic) to different levels of arsenic (As). The results of different growth, yield parameters and arsenic concentration in straw and grain of BRRI dhan45 presented in tables and figures are discussed systematically under the following heads:

3.1 Effect of arsenic on growth of BRRI dhan45

3.1.1Plant height

The effects of arsenic (As) showed remarkable effect on plant height in rice variety BRRI dhan45 (Table 3.1). It was observed that plant height of rice plant decreased significantly ($p<0.05$) with increasing soil As concentration. Plant Height was affected markedly due to the effects of different arsenic (As) levels in BRRI dhan45 (Table 1, Fig.3.1). The highest plant height (96.64) was found in As3 treated pot and lowest plant height (82.66) was found in As4 treated pot. Furthermore, the reduction of plant height due to application of arsenic was also recorded and highest reduction was observed from As4 ppm treated pot (13.30%) and lowest plant height reduction found from As0.1 ppm treated pot (0.18%) (35.1%) (Fig. 3.1). Similar results were in agreement with Islam and Jahiruddin (2010), who reported that arsenic treatment resulted in a marked decrease in effective tillers per pot, filled grains per panicle and 1000-grain weight; these together contributed reduced grain yield. Rice grain yield was reduced by 20.6% for 15 $mgkg^{-1}$ As treatment and 63.8% due to 30 $mgkg^{-1}$ As. Plant height might be reduced due to increase in arsenic concentration in soil. Azad et al. (2009) found that arsenic (As) had a significant ($p\leq0.5$) effect on the reduction of plant height of T-aman rice. We found that plant height was increased up to the application of 0.5 mgL^{-1} arsenic and thereafter at higher concentration of arsenic had caused a gradual decrease of plant height.

Xie and Huang (1994) also reported that lower concentration of arsenic through soil had stimulatory effect for rice. The tallest (96.64±0.73cm) and smallest (82.66±7.6 cm) plant height were found in 0.5 and 4.0 mgL^{-1} arsenic amended plots . This study found a negative correlation (r^2=0.951) between arsenic in soil and plant height (Fig. 3.2). Abedin *et al*. (2002) also found that arsenic contaminated soil significantly reduced the plant height.

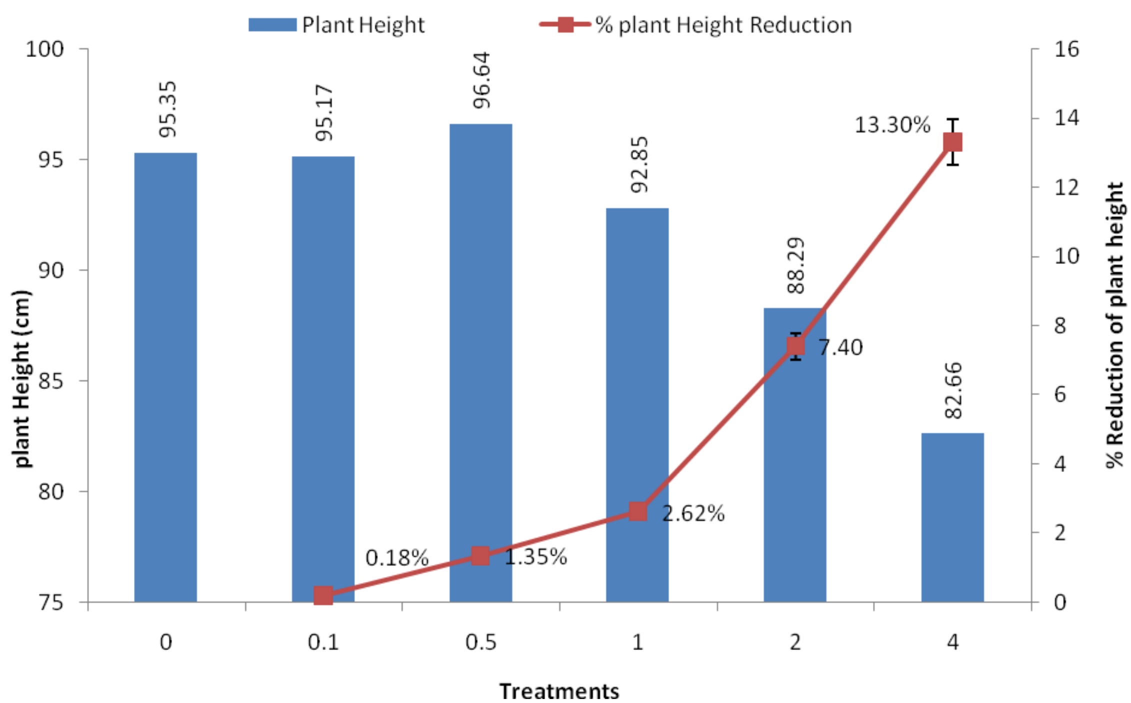

Fig.: 3.1 Effect of arsenic (As) on plant height (cm) and % reduction in plant height of BRRI dhan45

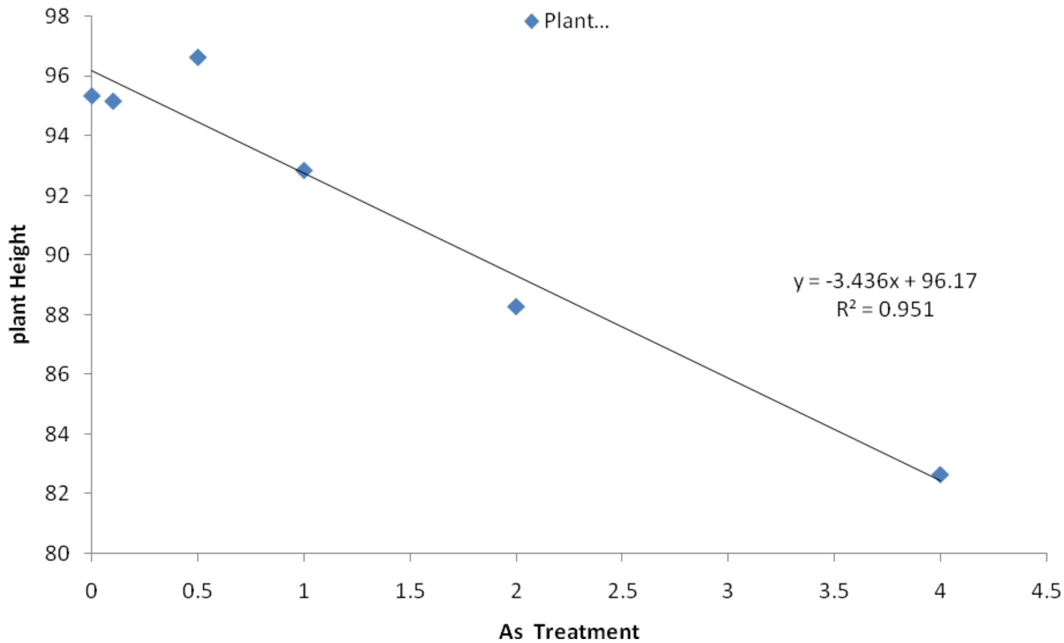

Fig.: 3.2 Correlation of arsenic treatments and plant height of BRRI dhan45

3.1.2 Tillers per plant

The effects of arsenic (As) showed remarkable effect on tillers per plantin rice variety BRRI dhan45 (Table 3.1). It was observed that tillers per plantof rice plant decreased significantly ($p<0.05$) with increasing soil As concentration. Tillers per plant was affected markedly due to the effects of different arsenic (As) levels in BRRI dhan45 (Table 1, Fig.3.3). The highest tillers per plant (24.0) was found in As0 treated pot and lowest tillers per plant (15.0) was found in As4 treated pot. Furthermore, the reduction of tillers per plant due to application of arsenic was also recorded and highest reduction was observed from As4 ppm treated pot (37.5%) and lowest tillers per plant reduction found from As0.1 ppm treated pot (13.87%) (35.1%) (Fig. 3.3). Similar results were in agreement with Islam and Jahiruddin (2010), who reported that arsenic treatment resulted in a marked decrease in effective tillers per pot, filled grains per panicle and 1000-grain weight; these together contributed reduced grain yield.

Rice grain yield was reduced by 20.6% for 15 $mgkg^{-1}$ As treatment and 63.8% due to 30 $mgkg^{-1}$ As. Chino (1981) reported that tillers of rice to be severely depressed with high concentration of As .We found that arsenic in soil up to 1.0 mg/kg did not affect the tillers number significantly. But a higher concentration of arsenic significantly decreased the total number of tillers per plant. Abedin *et al*. (2002) also observed that tillers number was reduced significantly with increase of arsenic concentration in soil up to 8 mgL^{-1}. The highest number (24.00a) of tillers was observed in control treatment and lowest number (15.00b) was observed in 4.0 mg/kg arsenic treated plot. We found a negative correlation (r^2=0.611) between arsenic in soil and tillers number. Khan *et al*. (2010) also found that the addition of arsenic significantly reduced tillering.

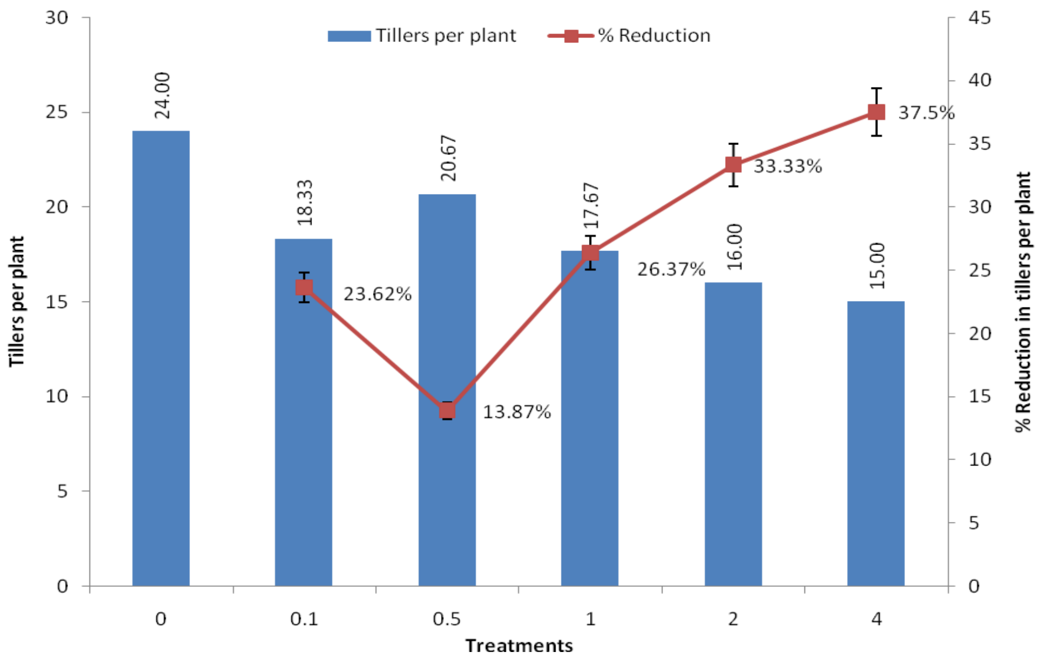

Fig.: 3.3 Effect of arsenic (As) on tillers per plant and % reduction in tillers per plant of BRRI dhan45

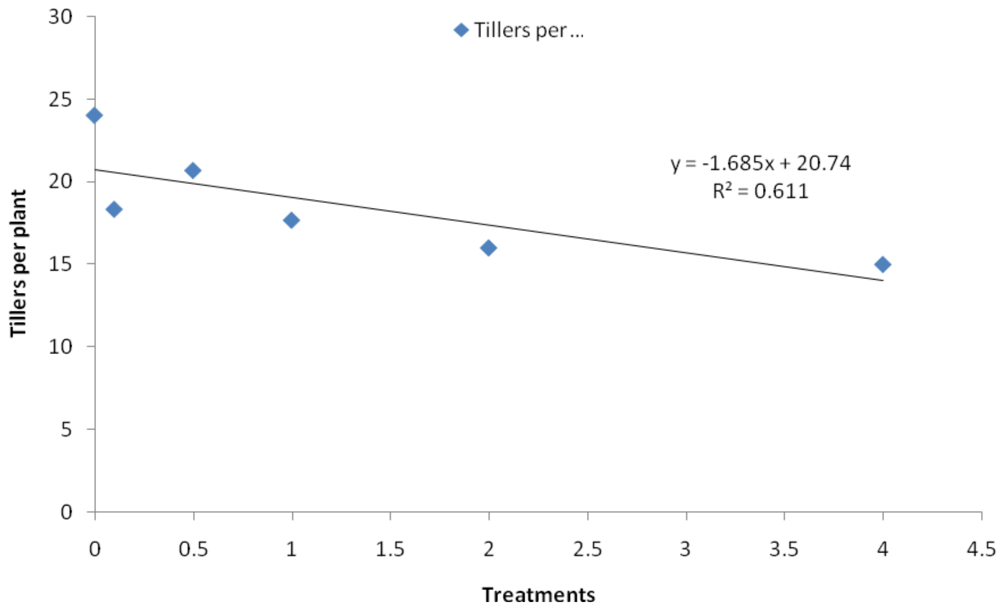

Fig.: 3.4 Correlation of arsenic treatments and plant height of BRRI dhan45

3.1.3 Panicle number per plant

The effects of arsenic (As) showed remarkable effect on panicle number per plant in rice variety BRRI dhan45 (Table 3.1). It was observed that panicle number per plantof rice plant decreased significantly ($p<0.05$) with increasing soil As concentration. Panicle number per plant was affected markedly due to the effects of different arsenic (As) levels in BRRI dhan45 (Table 1, Fig.3.1). The highest panicle number per plant (16.0) was found in As0 treated pot and lowest panicle number per plant (9.67) was found in As4 treated pot. Furthermore, the reduction of panicle number per plant due to application of arsenic was also recorded and highest reduction was observed from As4 ppm treated pot (39.56%) and lowest panicle number per plant reduction found from As0.5 ppm treated pot (2.06%) (35.1%) (Fig. 3.1). Azad *et al.* (2009) reported that the panicles number of T-aman rice were not affected at low doses of As in soil but significantly affected the panicles number at higher doses. This study found that panicles number was decreased with increase of arsenic concentration in soil but the differences were not statistically significant. The highest panicle number (16.00±02a) was observed in control treatment and the lowest panicle number (9.67±3.18a) was found in 4.0 mgL^{-1} treatment. Arsenic in soil and panicle number had negative relation (R^2=0.442).

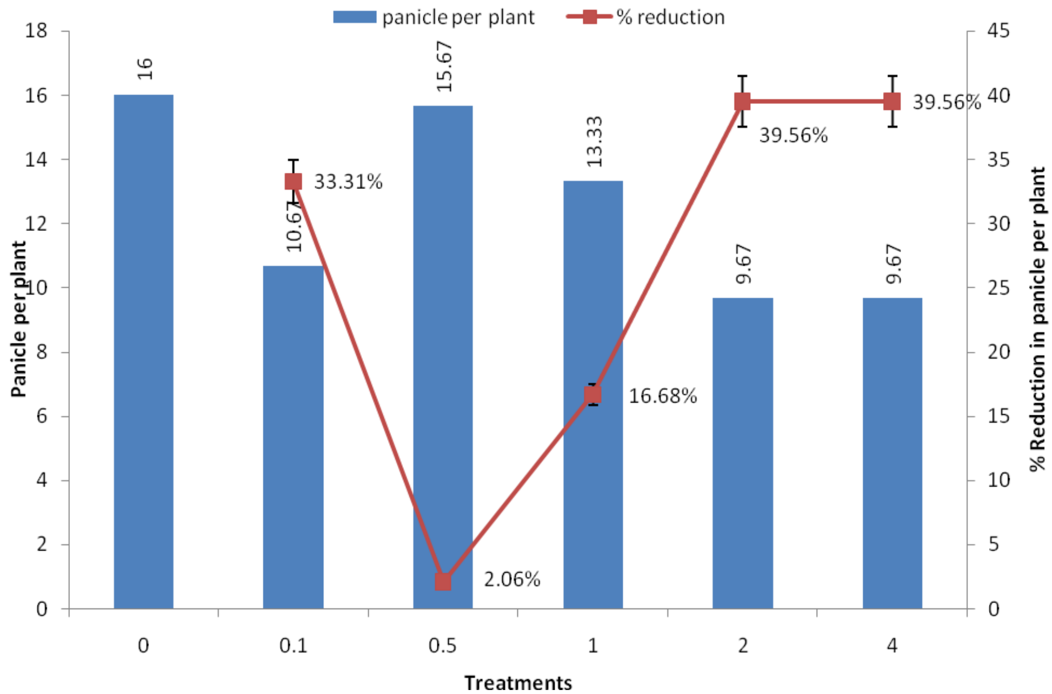

Fig.: 3.5 Effect of arsenic (As) on panical per plant and % reduction in panical per plant of BRRI dhan45

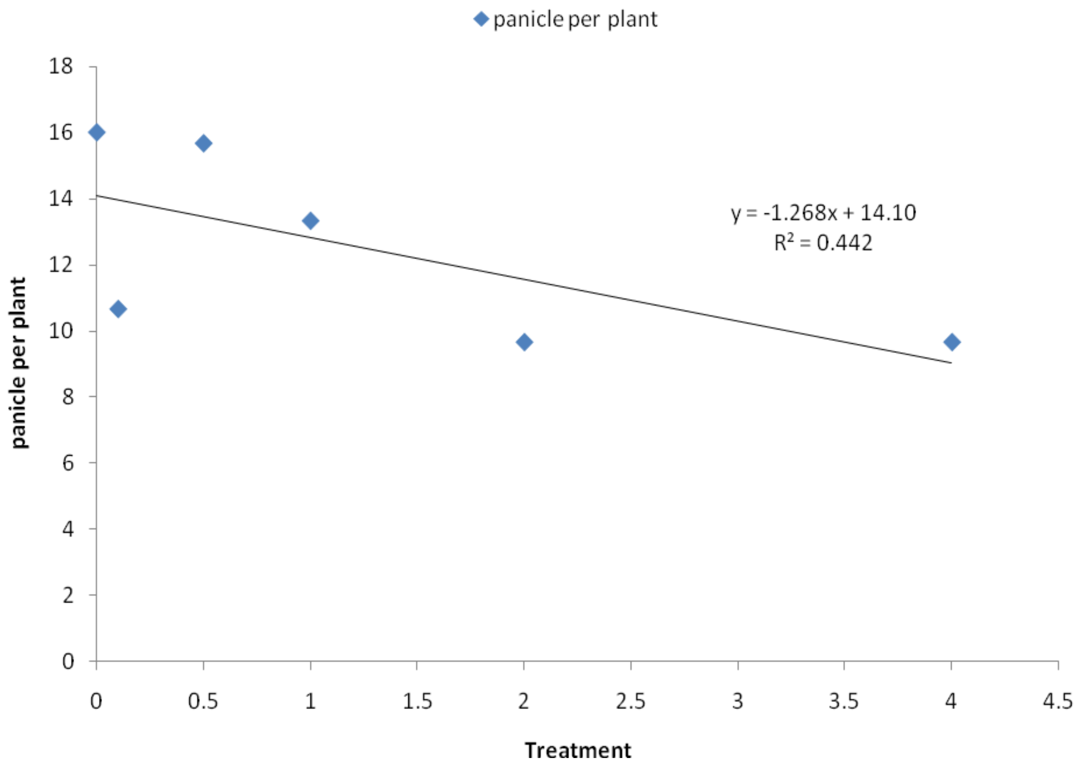

Fig.: 3.6 Correlation of arsenic treatments and plant height of BRRI dhan45

3.1.4 Panicle length

The effects of arsenic (As) showed remarkable effect on panicle length in rice variety BRRI dhan45 (Table 3.1). It was observed that panicle lengthof rice plant decreased significantly ($p<0.05$) with increasing soil As concentration. Panicle length was affected markedly due to the effects of different arsenic (As) levels in BRRI dhan45 (Table 1, Fig.3.1). The highest panicle length (24.2) was found in As0 treated pot and lowest panicle length (21.83) was found in As4 treated pot. Furthermore, the reduction of panicle length due to application of arsenic was also recorded and highest reduction was observed from As4 ppm treated pot (9.79%) and lowest panicle length reduction found from As0.1 ppm treated pot (5.78%) (Fig. 3.1). The panicles length were not affected significantly up to 1.0 mgL^{-1} arsenic treatment, but thereafter the panicles length were decreased significantly ($p \leq o.5$) with increase of arsenic in soil. Azad *et al.* (2009) also found that the panicles length of T-aman rice were not affected at low doses of As in soil but affected significantly at higher doses of As. The highest panicle length (24.20±0.69cm a) and the lowest panicle length (21.83±0.84cm b) were observed in control and 4.0 mgL-1 arsenic treated plot, respectively (Table 1). Arsenic in soil and panicles length had a negative correlation (r^2=0.465).

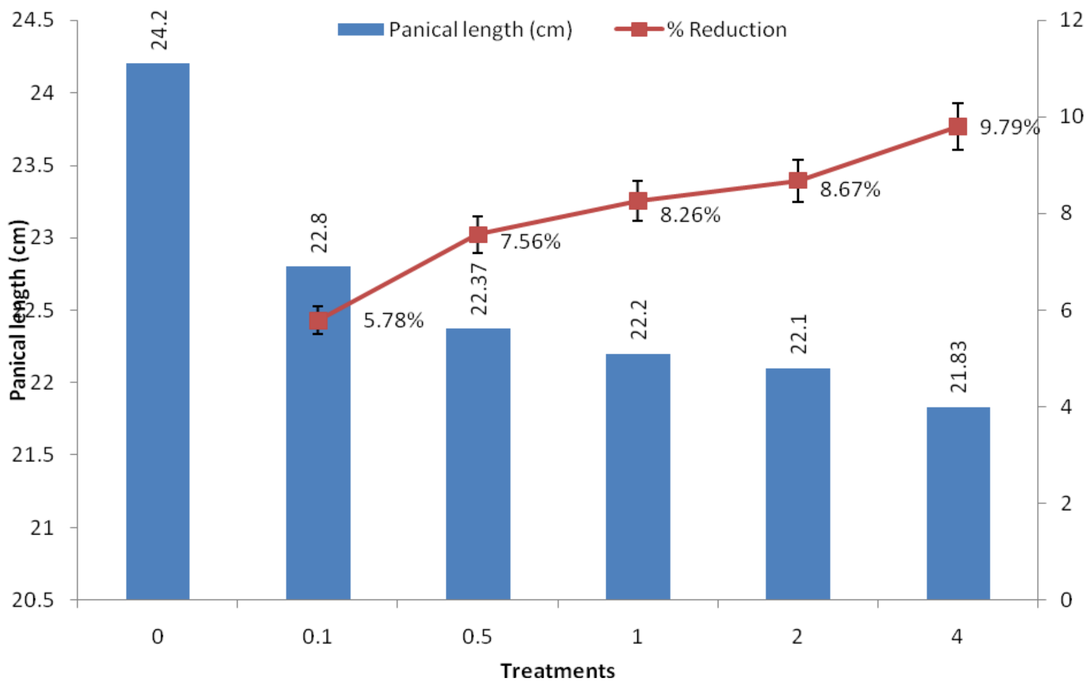

Fig.: 3.7 Effect of arsenic (As) on panical length (cm) and % reduction in panical length of BRRI dhan45

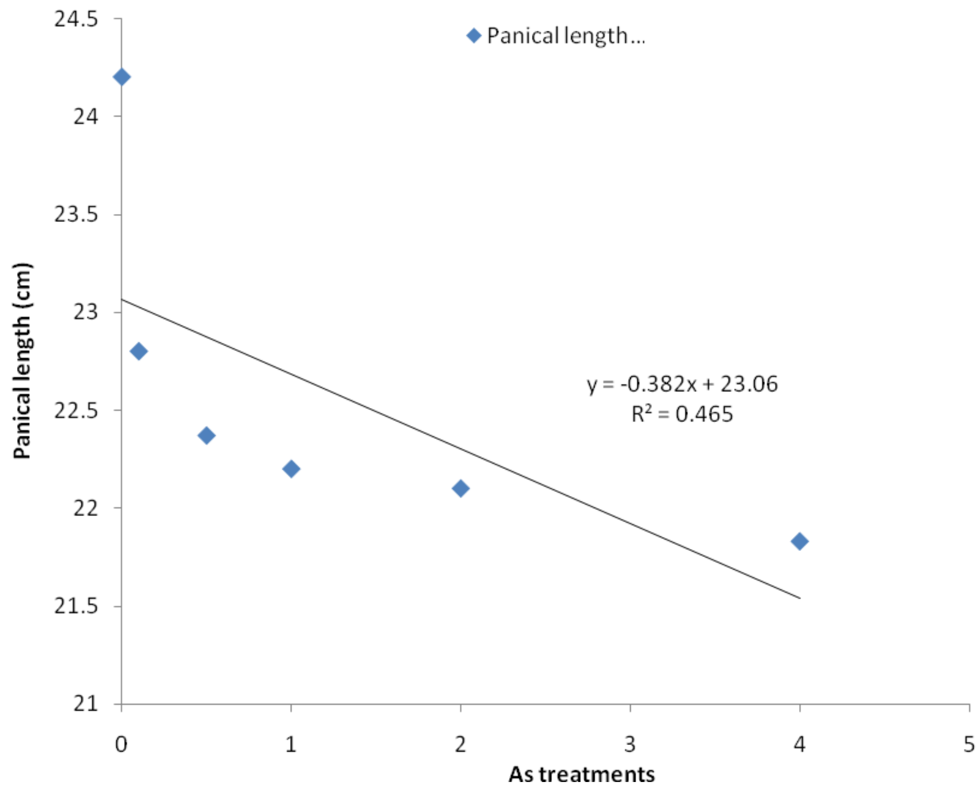

Fig.: 3.8 Correlation of arsenic treatments and plant height of BRRI dhan45

Table 3.1. Effect of arsenic amended soil on growth of BRRI dhan45

Arsenic added in water (mg/kg)	Plant height (cm)	Tillers/Plant (no.)
0	95.35±1.78a	24.00a
0.1	91.17±2.00a	18.33ab
0.5	96.64±0.73a	20.67ab
1.0	92.85±2.13a	17.67ab
2.0	88.29±6.17a	16.00b
4.0	82.66±7.61a	15.00b

Table 3.2. Effect of arsenic amended soil on growth of BRRI dhan45

Arsenic added in water (mg/kg)	Panicles/plant (no.)	Panicle length (cm)	1000-grain weight (g)
0	16.00±0.02a	24.20±0.69a	18.84±0.94a
0.1	0.67±3.18a	22.80±0.50ab	17.81±0.06a
0.5	15.67±0.67a	22.37±0.67ab	14.21±1.14a
1.0	13.33±1.45a	22.20±0.25ab	13.90±1.81a
2.0	9.67±2.33a	22.10±0.58b	13.68±2.14a
4.0	9.67±3.18a	21.83±0.84b	12.50±5.11a

3.1.5 1000-grain weight (g)

The effects of arsenic (As) showed remarkable effect on 1000-grain weight (g) in rice variety BRRI dhan45 (Table 3.1). It was observed that 1000-grain weight (g)of rice plant decreased significantly ($p<0.05$) with increasing soil As concentration. 1000-grain weight (g) was affected markedly due to the effects of different arsenic (As) levels in BRRI dhan45 (Table 1, Fig.3.1). The highest 1000-grain weight (g) (18.84) was found in As0 treated pot and lowest 1000-grain weight (g) (12.5) was found in As4 treated pot. Furthermore, the reduction of 1000-grain weight (g) due to application of arsenic was also recorded and highest reduction was observed from As4 ppm treated pot (33.65%) and lowest 1000-grain weight (g) reduction found from As0.1 ppm treated pot (5.46%) (Fig. 3.1). Abedin (2002) found that presence of arsenic as arsenate at a higher concentration in soil significantly reduced ($p<0.001$) the 1000 grain weight. We found that thousand grain weights were decreased with increasing of arsenic in soil but the differences were not statistically significant. Tsutsumi (1980) also reported that arsenic could reduced 1000 grain weight. The highest grain weight (18.84±0.94g) and lowest grain weight (12.5±5.11g) were recorded in control and 4.0 mgL-1 arsenic treatment plot, respectively (Table 1). Arsenic in soil and thousand grain weight were related antagonistically (r^2=0.602). Wang et al. (2006) also reported that1000 grain weight was significantly reduced with increased As level in soil treated with two organoarsenic compound ($p<0.01$).

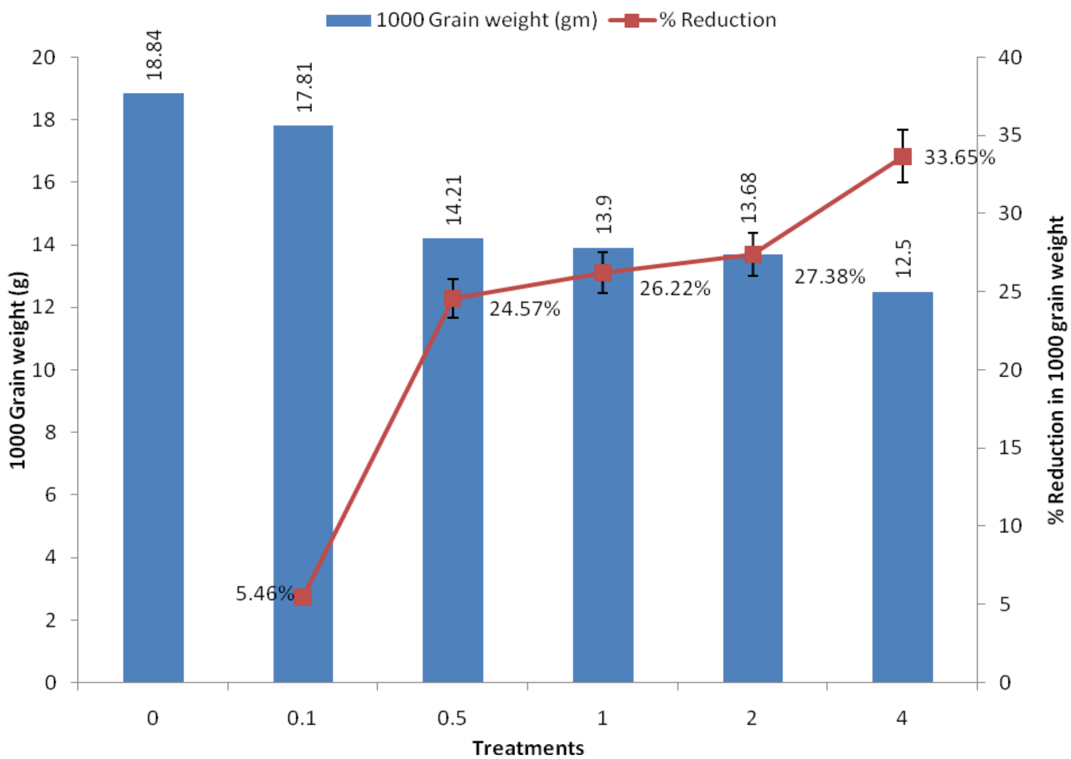

Fig.: 3.9 Effect of arsenic (As) on 1000-grain yield (g) and % reduction in 1000-grain yield of BRRI dhan45

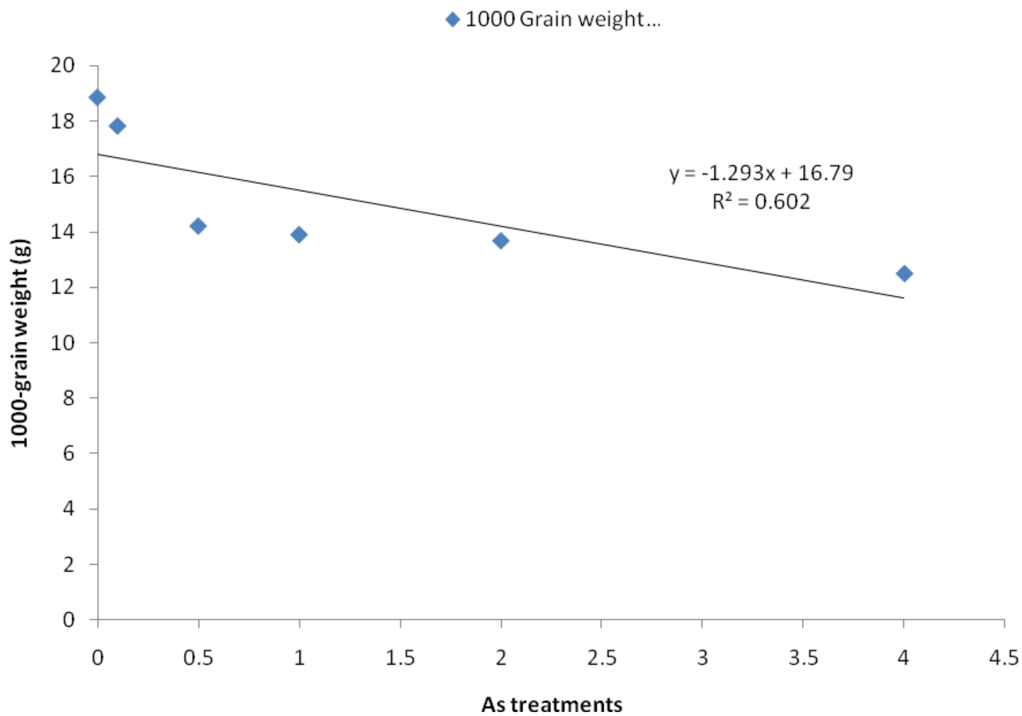

Fig.: 3.10 Correlation of arsenic treatments and plant height of BRRI dhan45

Table 3.3. Effect of arsenic amended soil on growth of BRRI dhan45

Arsenic added in water (mg/kg)	Grain yield (g)	Straw yield (g)
0	23.38±5.55a	31.50±4.53a
0.1	15.28±1.28ab	30.60±4.29a
0.5	9.84±1.31ab	39.07±4.08a
1.0	11.06±6.22ab	34.14±3.17a
2.0	9.81±4.64ab	29.05±11.52a
4.0	7.24±2.32b	27.01±6.74a

3.1.6 Grain yield (g)

The effects of arsenic (As) showed remarkable effect on grain yield (g) in rice variety BRRI dhan45 (Table 3.1). It was observed that grain yield (g)of rice plant decreased significantly ($p<0.05$) with increasing soil As concentration. Grain yield (g) was affected markedly due to the effects of different arsenic (As) levels in BRRI dhan45 (Table 1, Fig.3.1). The highest grain yield (g) (23.48) was found in As0 treated pot and lowest grain yield (g) (7.24) was found in As4 treated pot. Furthermore, the reduction of grain yield (g) due to application of arsenic was also recorded and highest reduction was observed from As4 ppm treated pot (69.03%) and lowest grain yield (g) reduction found from As0.1 ppm treated pot (34.64%) (35.1%) (Fig. 3.1). Abedin *et al.* (2002) reported that grain yield was decreased significantly ($p<0.001$) with increase of arsenic concentration in soil. We found that grain yield of BRRI DHAN45 rice was decreased significantly with increase of arsenic concentration in soil. There were also some reports of rice grain yield reduction due to As application for rice (Farn *et al.* 1988; Milan *et al.* 1988; Gilmour and Wells, 1988; Liu and Gao, 1987; Tsutsumi, 1980). The highest grain yield (23.38±5.55g a) and lowest grain yield (7.24±2.3g b) were found in control and 4.0 mgL^{-1} arsenic treated plot, respectively (Table 2). The grain yield was found to decrease drastically by 58.04% and 69.03% compared to control in 2.0 and 4.0 mg/kg arsenic treatments, respectively (Table 2). Hossain *et al*. (2009) also reported that grain yield of rice was decreased as the level of arsenic addition was increased, and the yield was reduced drastically with the 30 mg $Askg^{-1}$ addition. We found that grain yield and arsenic concentration in soil had a negative correlation ($r^2=0.485$). Panaullah *et al.* (2009) also found rice grain was negatively correlated with soil-As concentration ($r^2=0.91$).

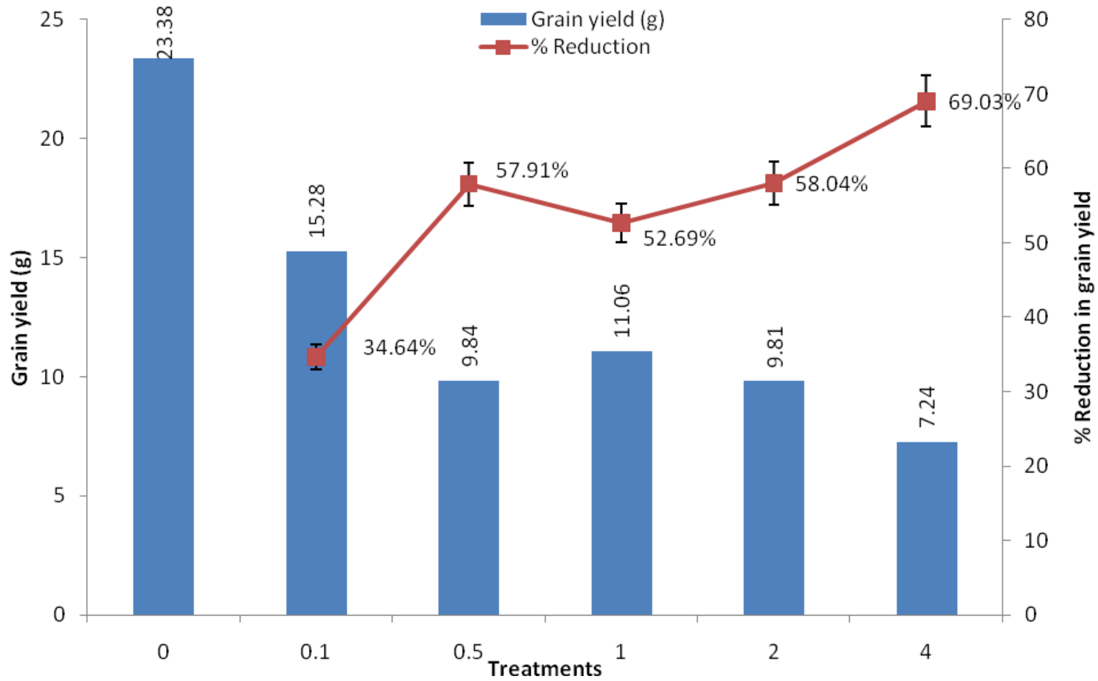

Fig.: 3.11 Effect of arsenic (As) on grain yield (g) and % reduction in grain yield of BRRI dhan45

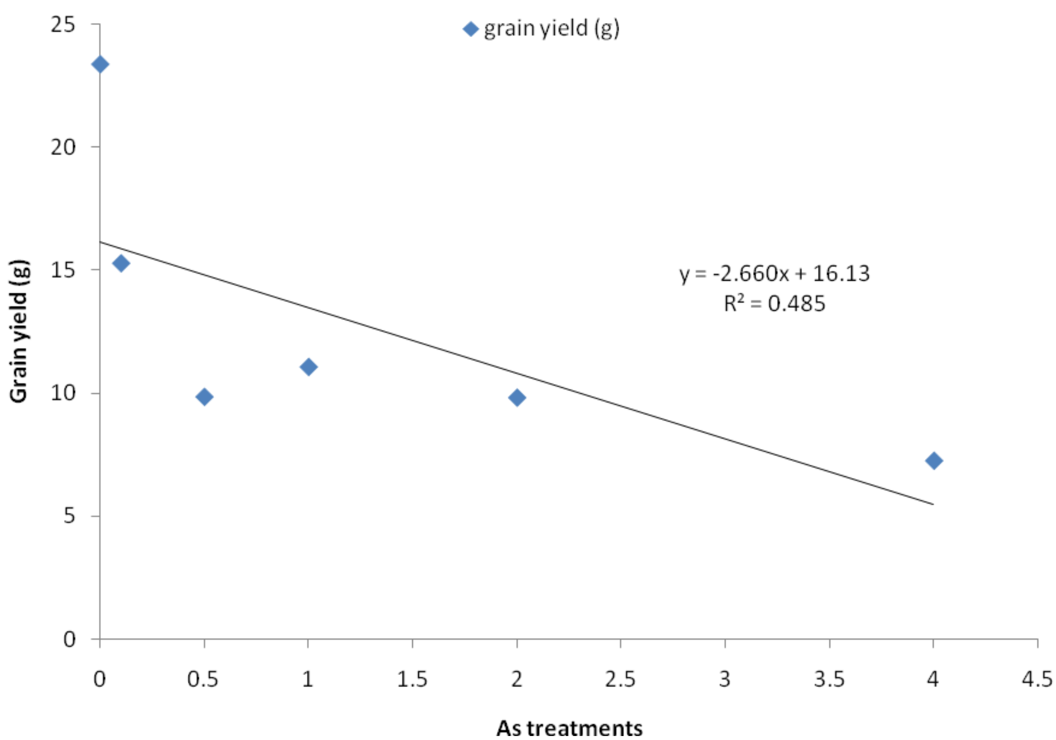

Fig.: 12 Correlation of arsenic treatments and plant height of BRRI dhan45

3.1.7 Straw yield (g)

The effects of arsenic (As) showed remarkable effect on straw yield (g) in rice variety BRRI dhan45 (Table 3.1). It was observed that straw yield (g)of rice plant decreased significantly ($p<0.05$) with increasing soil As concentration. Straw yield (g) was affected markedly due to the effects of different arsenic (As) levels in BRRI dhan45 (Table 1, Fig.3.1). The highest straw yield (g) (39.07) was found in As0.5 treated pot and lowest straw yield (g) (27.01) was found in As4 treated pot. Furthermore, the reduction of straw yield (g) due to application of arsenic was also recorded and highest reduction was observed from As4 ppm treated pot (14.25%) and lowest straw yield (g) reduction found from As0.1 ppm treated pot (2.85%) (35.1%) (Fig. 3.1). Khan *et al*. (2010) reported that straw yield was decreased significantly with As addition in irrespective of season, year, method and level of As application. We found that the straw yield of BRRI dhan45 rice was increased at 0.5 mgL^{-1} arsenic treatment and thereafter straw yield were decreased with increase of arsenic concentration in soil but the differences were not statistically significant. The highest straw yield (39.07±4.08g a) and lowest straw yield (27.01±6.74g a) were found in 0.5 mgL^{-1} and 4.0 mgL^{-1} arsenic treatment (Table 2). Arsenic concentration in soil and straw yield had a negative correlation ($r^2=0.362$). Hossain *et al.* (2009) also found a negative relationship between straw yield and As dose. The straw yield decreased by 7.77% and 14.25% compared to control in 2.0 mg/kg and 4.0 mg/kg arsenic treatments, respectively (Table 2). Abedin *et al*. (2002) also found that straw yield were significantly ($p<0.001$) reduced with increase of arsenate concentration in soil.

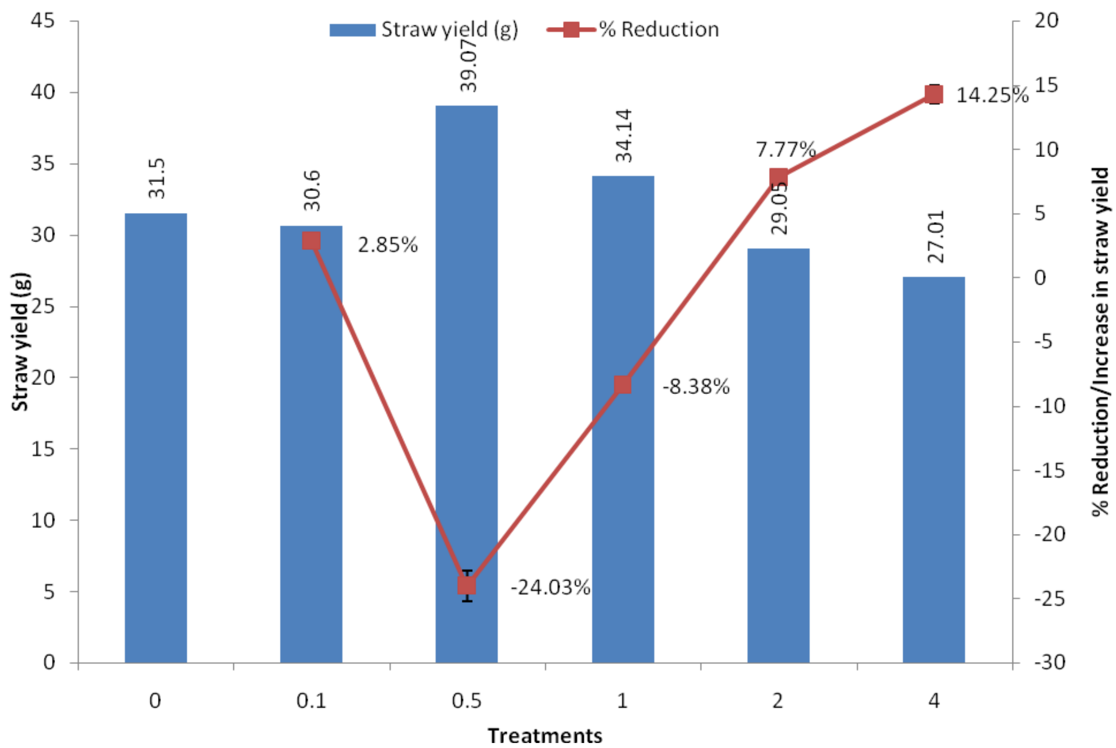

Fig.: 3.13 Effect of arsenic (As) on straw yield (g) and % reduction/increase in straw yield of BRRI dhan45

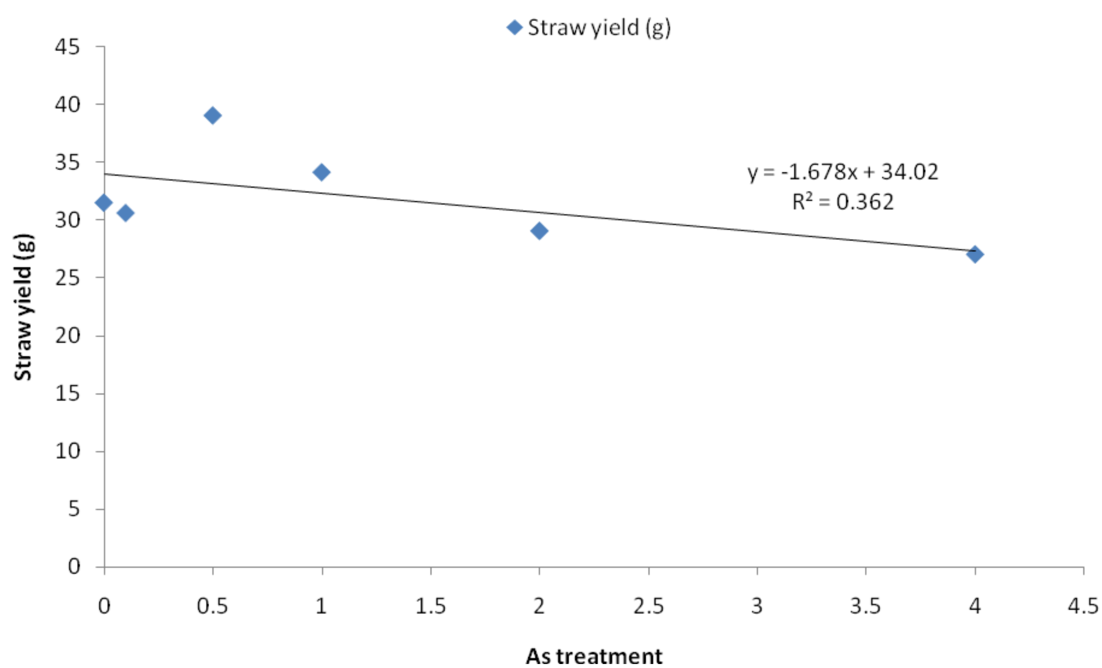

Fig.: 3.14 Correlation of arsenic treatments and plant height of BRRI dhan45

3.1.8 Arsenic (As) concentration in straw (ppm)

The effects of arsenic (As) showed remarkable effect on arsenic (As) concentration in straw (ppm) in rice variety BRRI dhan45 (Table 3.1). It was observed that arsenic (As) concentration in straw (ppm)of rice plant decreased significantly ($p<0.05$) with increasing soil As concentration. Arsenic (As) concentration in straw (ppm) was affected markedly due to the effects of different arsenic (As) levels in BRRI dhan45 (Table 1, Fig.3.1). The highest arsenic (As) concentration in straw (ppm) (2.40) was found in As4 treated pot and lowest arsenic (As) concentration in straw (ppm) (0.43) was found in As0 treated pot.

3.1.9 Arsenic (As) concentration in grain (ppm)

The effects of arsenic (As) showed remarkable effect on arsenic (As) concentration in grain (ppm) in rice variety BRRI dhan45 (Table 3.1). It was observed that arsenic (As) concentration in grain (ppm)of rice plant decreased significantly ($p<0.05$) with increasing soil As concentration. Arsenic (As) concentration in grain (ppm) was affected markedly due to the effects of different arsenic (As) levels in BRRI dhan45 (Table 1, Fig.3.1). The highest arsenic (As) concentration in grain (ppm) (0.425) was found in As4 treated pot and lowest arsenic (As) concentration in grain (ppm) (0.212) was found in As0 treated pot.

Table 3.4. Effect of arsenic amended soil on growth of BRRI dhan45

Arsenic added in water (mg/kg)	Arsenic (As) concentration (ppm)	
	Grain	Straw
0	0.212	0.43
0.1	0.215	0.79
0.5	0.373	0.98
1.0	0.359	0.98
2.0	0.416	1.83
4.0	0.425	2.40
SE±	0.0256	0.077

Table 3.6. Effect of arsenic amended soil on growth of BRRI dhan45

Arsenic added in water (mg/kg)	Percent of yield reduction over control	
	Grain yield	Straw yield
0	0	0
0.1	-34.64	-3.85
0.5	-57.91	24.03
1.0	-52.69	8.38
2.0	-58.04	-7.77
4.0	-69.03	-14.25

CHAPTER V

SUMMARY AND CONCLUSION

A pot experiment was conducted at the Soil Science Farm of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the period from December 2016 to March 2017 to evaluate the effect of arsenic on the growth and yield of BBRI dhan45. A pot experiment was conducted which comprised with six levels of arsenic doses 0, 0.1, 0.5, 1.0, 2.0 and 4.0 ppm. The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. Increased As showed adverse effect on plant height, effective tillers per plant, non-effective tillers per plant, filled grain per plant, unfilled grain per plant, grains per panicle, 1000-grain weight and grain & straw yield of rice. A popular Boro rice variety named BRRI dhan45 was cultivated with arsenic amended soil (0 mg, 0.1 mg, 0.5 mg, 1.0 mg, 2.0 mg and 4.0 mg/kg As containing water). The effects of arsenic (As) showed remarkable effect on plant height in rice variety BRRI dhan45 (Table 3.1). It was observed that plant height of rice plant decreased significantly ($p<0.05$) with increasing soil As concentration. Plant Height was affected markedly due to the effects of different arsenic (As) levels in BRRI dhan45 (Table 1, Fig.3.1). The highest plant height (96.64) was found in As3 treated pot and lowest plant height (82.66) was found in As4 treated pot. Furthermore, the reduction of plant height due to application of arsenic was also recorded and highest reduction was observed from As4 ppm treated pot (13.30%) and lowest plant height reduction found from As0.1 ppm treated pot (0.18%) (35.1%) (Fig. 3.1). Tillers per plant was affected markedly due to the effects of different arsenic (As) levels in BRRI dhan45 (Table 1, Fig.3.3). The highest tillers per plant (24.0) was found in As0 treated pot and lowest tillers per plant (15.0) was found in As4 treated pot. Furthermore, the reduction of tillers per plant due to application of arsenic was also recorded and highest reduction was observed from As4 ppm treated pot (37.5%) and lowest tillers per plant reduction found from As0.1 ppm treated pot (13.87%) (35.1%) (Fig. 3.3). It was observed that panicle number per plantof rice plant decreased significantly ($p<0.05$) with increasing soil As concentration. Panicle number per plant was affected markedly due to the effects of different arsenic (As) levels in BRRI dhan45 (Table 1, Fig.3.1). The highest panicle number per plant (16.0) was found in As0 treated pot and lowest panicle number per plant (9.67) was found in As4 treated pot. Furthermore, the reduction of panicle number per plant due to application of arsenic was also recorded and highest reduction was observed from As4 ppm treated pot (39.56%) and lowest panicle number per plant reduction found from As0.5 ppm treated pot (2.06%) (35.1%) (Fig. 3.1).

Panicle length was affected markedly due to the effects of different arsenic (As) levels in BRRI dhan45 (Table 1, Fig.3.1). The highest panicle length (24.2) was found in As0 treated pot and lowest panicle length (21.83) was found in As4 treated pot. Furthermore, the reduction of panicle length due to application of arsenic was also recorded and highest reduction was observed from As4 ppm treated pot (9.79%) and lowest panicle length reduction found from As0.1 ppm treated pot (5.78%) (Fig. 3.1). It was observed that 1000-grain weight (g) of rice plant decreased significantly ($p<0.05$) with increasing soil As concentration. 1000-grain weight (g) was affected markedly due to the effects of different arsenic (As) levels in BRRI dhan45 (Table 1, Fig.3.1). The highest 1000-grain weight (g) (18.84) was found in As0 treated pot and lowest 1000-grain weight (g) (12.5) was found in As4 treated pot. Furthermore, the reduction of 1000-grain weight (g) due to application of arsenic was also recorded and highest reduction was observed from As4 ppm treated pot (33.65%) and lowest 1000-grain weight (g) reduction found from As0.1 ppm treated pot (5.46%) (Fig. 3.1).

The highest grain yield (g) (23.48) was found in As0 treated pot and lowest grain yield (g) (7.24) was found in As4 treated pot. Furthermore, the reduction of grain yield (g) due to application of arsenic was also recorded and highest reduction was observed from As4 ppm treated pot (69.03%) and lowest grain yield (g) reduction found from As0.1 ppm treated pot (34.64%) (35.1%) (Fig. 3.1). It was observed that straw yield (g)of rice plant decreased significantly ($p<0.05$) with increasing soil As concentration. Straw yield (g) was affected markedly due to the effects of different arsenic (As) levels in BRRI dhan45 (Table 1, Fig.3.1). The highest straw yield (g) (39.07) was found in As0.5 treated pot and lowest straw yield (g) (27.01) was found in As4 treated pot. Furthermore, the reduction of straw yield (g) due to application of arsenic was also recorded and highest reduction was observed from As4 ppm treated pot (14.25%) and lowest straw yield (g) reduction found from As0.1 ppm treated pot (2.85%) (35.1%) (Fig. 3.1). The effects of arsenic (As) showed remarkable effect on arsenic (As) concentration in straw (ppm) in rice variety BRRI dhan45 (Table 3.1). It was observed that arsenic (As) concentration in straw (ppm)of rice plant decreased significantly ($p<0.05$) with increasing soil As concentration. Arsenic (As) concentration in straw (ppm) was affected markedly due to the effects of different arsenic (As) levels in BRRI dhan45 (Table 1, Fig.3.1). The highest arsenic (As) concentration in straw (ppm) (2.40) was found in As4 treated pot and lowest arsenic (As) concentration in straw (ppm) (0.43) was found in As0 treated pot. The effects of arsenic (As) showed remarkable effect on arsenic (As) concentration in grain (ppm) in rice variety BRRI dhan45 (Table 3.1). It was observed that arsenic (As) concentration in grain (ppm)of rice plant decreased significantly ($p<0.05$) with increasing soil As concentration. Arsenic (As) concentration in grain (ppm) was affected markedly due to the effects of different arsenic (As) levels in BRRI dhan45 (Table 1, Fig.3.1). The highest arsenic (As) concentration in grain (ppm) (0.425) was found in As4 treated pot and lowest arsenic (As) concentration in grain (ppm) (0.212) was found in As0 treated pot.

Rice growth and yield were significantly affected by arsenic in soil ($p \leq 0.05$). Lower concentration of arsenic in soil (up to 0.5 mgL^{-1}) stimulated the rice growth and yield but higher concentration of arsenic in soil (above 0.5 mgL^{-1}) reduced the rice growth and yield markedly. Farmers of Bangladesh should avoid above 0.5 mgL^{-1} arsenic contaminated ground water for irrigation in rice cultivation.

CHAPTER VI

RECOMMENDATIONS

Rice growth and yield were significantly affected by arsenic in soil ($p \leq 0.05$). Lower concentration of arsenic in soil (up to 0.5 $mgkg^{-1}$) stimulated the rice growth and yield but higher concentration of arsenic in soil (above 0.5 $mgkg^{-1}$) reduced the rice growth and yield markedly. Farmers of Bangladesh should avoid above 0.5 mgL^{-1} arsenic contaminated ground water for irrigation in rice cultivation.

CHAPTER VII

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CHAPTER VIII

APPENDIX

Appendix I. Characteristics of the soil of experimental field

Physical and chemical properties of the initial soil

Characteristics	Value
% Sand	27
% Silt	43
% Clay	30
Textural Class	Silty-clay
pH	6.1
Organic matter (%0	1.13
Total N (%)	0.03
Available P (ppm)	20.00
Exchangeable K (me/100g soil)	0.10
Available S (ppm)	23
As (ppm)	4.83