

MITIGATION OF ARSENIC STRESS IN BRRI dhan29 WITH COWDUNG AND INORGANIC FERTILIZERS

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**MITIGATION OF ARSENIC STRESS IN BRRI dhan29 WITH
COWDUNG AND INORGANIC FERTILIZERS**

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CERTIFICATE

This is to certify that the research work entitled, “**MITIGATION OF ARSENIC STRESS IN BRRI dhan29 WITH COWDUNG AND INORGANIC FERTILIZERS**” submitted to the department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Dhaka in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (M.S.) in AGRICULTURAL CHEMISTRY**, embodies the results of a piece of *bona fide* research work carried out by **MD. TANVIR HASAN**, Registration No. **12-04813**, under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma in any other institution.

I further certify that any help or sources of information received during the course of this investigation has duly been acknowledged.

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ABSTRACT

An experiment was conducted in the net house and the agro-environmental chemistry laboratory of the Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Dhaka-1207 during the Boro season of the year 2017-18 to evaluate the mitigation of arsenic stress in BRRI dhan29 with cowdung and inorganic fertilizers. The two factorial experiment was laid out in a Completely Randomized Design (CRD) with three replications. Factor A: different doses of arsenic on required water basis [As_0 =No arsenic applied, As_1 = 20 ppm arsenic, As_2 = 40 ppm arsenic, As_3 = 60 ppm arsenic] and Factor B: different doses of cowdung and inorganic fertilizers [T_0 = Recommended dose of cowdung + inorganic fertilizer, T_1 = Recommended dose of cowdung without inorganic fertilizer, T_2 = Recommended dose of inorganic fertilizer without cowdung, T_3 = Reduction of 20% recommended dose of inorganic fertilizer + addition of 20% recommended dose of cowdung, T_4 = Reduction of 40% recommended dose of inorganic fertilizer + addition of 40% recommended dose of cowdung]. Arsenic was added from Sodium Arsenate ($Na_2HAsO_4 \cdot 7H_2O$). In case of fertilizers, T_4 gave higher results in most growth, yield and yield contributing parameters (tiller number, effective tiller, filled grain, panicle length, grain and straw yield) and T_1 gave lower results; whereas for arsenic, maximum results were recorded from As_0 and minimum from As_3 . In interaction, As_0T_4 produced higher results and As_3T_1 produced lower results in most cases. The treatment T_4 gave higher N, P and K content in straw whereas, T_1 gave lower N, P and K content in straw; highest As content by T_1 and lowest by T_4 . In case of arsenic content in shoot, maximum results were recorded from As_3 and minimum from As_0 . In interaction, the maximum N, P and K content in straw were found in As_0T_4 and the minimum were observed from As_3T_1 treatment; whereas, highest As by As_3T_1 and lowest by As_0T_4 . Therefore, the treatment T_4 showed lower arsenic in shoot and T_1 showed the highest content.

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LIST OF ABBREVIATIONS

%	Percent
@	At the rate of
^o C	Degree Celsius
AEZ	Agro-Ecological Zone
As	Arsenic
BRRI	Bangladesh Rice Research Institute
cm	Centimeter
CRD	Completely Randomized Design
CV%	Percentage of Coefficient of Variance
DAT	Days After Transplanting
e.g.	As for example
<i>et al.</i>	and others
g	Gram
ha	Hectare
i.e.	that is
K	Potassium
kg	Kilogram
kg ha ⁻¹	kg per hectare
L	Liter

m	Meter
ml/L	Milliliter per Liter
mg/L	Milligram per Liter
MoP	Muriate of Potash
N	Nitrogen
nm	Nano Meter
ng	Nano Gram
P	Phosphorus
pH	Hydrogen ion concentration
ppm	Parts Per Million
S	Sulphur
SAU	Sher-e-Bangla Agricultural University
S.E.	Standard Error of Variance
tha ⁻¹	Ton per hectare
TSP	Triple Super Phosphate
µg/kg	Microgram per kg
Zn	Zinc

CHAPTER I

INTRODUCTION

Rice (*Oryza sativa* L.) botanically referred to the family Poaceae is one of the most important and popular cereal staple food for 160 million people of Bangladesh. Rice sector contributes one-half of the agricultural GDP and one-sixth of the national income in Bangladesh. Rice provides nearly 48% of rural employment, about two-third of total calorie supply of an average person in Bangladesh and this covers about 81% of the total cropped area in Bangladesh (BBS, 2012). It is the main source of food for more than 60% of the world's population and the second most important staple crop in the world after wheat. More than 90% of rice produced in Asia. In Bangladesh, over 80% of the irrigated area and about 75% of the total cropped area is planted to rice (BRKB, 2017). In financial year 2014-15 rice cultivated in 114.15 lac hectare area, which produced 347.1 Lac M.ton of rice (BBS, 2017).

The total rice production in our country is about 34 million tons to feed her 149.69 million people (Mandal and Choudhury, 2014). The increase in rice production becomes increasing due to adoption of rice modern rice varieties on around 66% of the rice land which contributes to about 73% of the country's total rice production in Bangladesh (BRKB, 2017). BBS (2010) reported that the population will have possibly increased to 230 million by the year 2030 which need more food for meet their demand. The population of Bangladesh is increasing at an alarming rate but the cultivable land is reducing day by day due to urbanization and industrialization resulting in more shortage of food. So management practices can help to produce better yield unit⁻¹ area.

Bangladesh is one of the major rice growing countries in the world. Rice plays a significant role in the livelihood of the people of Bangladesh. But cultivation is always vulnerable to natural disasters and abiotic stresses. Growth and development of the rice depend on environmental factors such as atmosphere, temperature, light, humidity, nutrients etc. Many abiotic factors such as heat, cold, drought, salinity and heavy metal contamination reduce the growth and development of the crops. Arsenic (As) stress is one kind of heavy metal stress with generally alters the morpho-physiology, yield contributing characters and yield of agricultural crops. It inhibits the growth with fresh and dry biomass accumulation (Stoeva and Bineva, 2003) and causes physiological disorders (Wells and Gillmor, 1997) as well as reduction of the crop productivity (Stepanok, 1998).

Intensive irrigation of rice with As contaminated ground water is increasing the soil As level in many areas of Bangladesh (Panauallah *et al.*, 2007). A large number of tube wells in 61 districts including 321 upazila of the country have been identified to have arsenic concentration above the national recommended limit of 0.05 mg L⁻¹. Smith *et al.* (2000) assume that in countries where the population had a long-term exposure to arsenic in groundwater, one in every 10 people who drink water containing 0.5 mg As L⁻¹ may die from arsenic induced cancer. The World Health Organization (WHO, 2001) estimates that arsenic in drinking water may cause deaths of 2,00,000 to 2,70,000 people from cancer in Bangladesh. It has been estimated that 9,00,000 to 13,60,000 kg As per year is brought onto the arable land due to irrigation with As contaminated groundwater (Ali, 2003).

Now-a-days, twenty countries including Bangladesh have been suffering from groundwater contamination by arsenic, which is the most severe problem occurring in Asia (Biswas *et al.*, 1998). Arsenic contamination of groundwater is a serious problem in Bangladesh. Use of groundwater for irrigation is a route of arsenic which enters the food chain and indirectly affects human health. Duxbury *et al.* (2003) mentioned the presence of arsenic in food chain food could be a way of arsenic entry into human body through water-soil-plant transfer. Arsenic contaminated water used in irrigation contaminates soils and then uptake by plants cause arsenic contamination of the edible portions of rice grains that consumed by humans. Most groundwater used for irrigation in Bangladesh is contaminated with arsenic. Rai *et al.* (2015) reported that accumulation of arsenic (As) in grain is a serious concern worldwide which affects nutritional status in rice grain and is associated with higher rates of skin, bladder, lung cancer and heart disease.

The deposition of As on the arable land is high, especially in southwest and south of Bangladesh. Arsenic is the causal factor of several physiological disorders in human namely edema, skin cancer, bladder cancer, lung cancer, hyperkeratosis, premature birth and black foot disease (Das *et al.*, 1996). It is estimated that about 40 million people are now exposed to the risk of being affected by arsenic contaminated water (Alam and Rahman, 2003). As concentration in Bangladesh soils is in the range of 4- 8 mg As kg⁻¹ soil. However, in areas where irrigation performed with arsenic contaminated groundwater, soil arsenic level can reach upto 83 mg As kg⁻¹ soil (Ullah, 1998). Arsenic uptake and accumulation is greatly affected by arsenic contamination in soil and increased greatly with increasing arsenic levels (Meharg and Rahman, 2003).

Arsenic uptake, transport to the plant system and toxicity are strongly influenced by soil characteristics and widely variable among plant species and cultivars within a species (Dush *et al.*, 1990 and Islam *et al.*, 2004b). Kiss *et al.* (1992) reported red yellow discoloration of rice leaf due to arsenic poisoning. Also there are a few arsenic induced diseases of rice, most remarkable is “straight head” disease (Gilmour and Wells, 1980). Paddy rice is more efficient in As uptake than other cereal crops, and the underlying reasons have been identified recently. Singh *et al.* (2006) stated that arsenic reduces the rate of photosynthesis by drainage mechanism of different cell organelles such as plasma membrane, chloroplast which causing electrolyte leakage and an increase in malondialdehyde (C₃H₄O₂), a product of lipid peroxidation, pointing to the role of oxidative stress in arsenic toxicity.

Paddy rice is more efficient in As uptake than other cereal crops, and the underlying reasons have been identified recently. First, anaerobic conditions in paddy soil leads to arsenite mobilization and thus enhanced bioavailability to rice plants. Rice grown under flooded conditions was found to accumulate much more As than that grown under aerobic condition. Growing rice aerobically during the entire rice growth duration resulted in the least As accumulation in rice straw and grain significantly compared with rice grown under flooded conditions (Li *et al.*, 2009).

A number of mitigation approaches have been tried to control arsenic accumulation in plants like chemical precipitation, ion-exchange, reverse osmosis, solvent extraction, and bioremediation by microbes which include the removal of heavy metals by microorganisms (bacteria, fungi, yeast and algae) as absorbents. Phyto-remediation that the removal of contaminants with the help of green plants and organic soil amendments such as cowdung, vermicompost etc. also includes the mitigation of arsenic accumulation in plants which is eco-friendly and available to farmers. Methylating bacteria and methanogens, which are most likely to be involved in arsenic methylation, are higher in cowdung than in soil. Mohapatra *et al.* (2008) reported volatilization of As by methanogenic bacteria from a system where cowdung was used as the major substrate. Though little is known about the transformation of As into volatile forms in the presence of only cowdung, many researchers have suggested disposing the wastes from arsenic removal system on cowdung bed (e.g., Hwang, 2002; Sarkar *et al.*, 2011).

Considering the above fact, the present study was under taken with the following objectives:

- To assess the effect of arsenic stress on the growth and yield of BRRI dhan29.
- To observe the role of cowdung and inorganic fertilizers on mitigation of arsenic stress in BRRI dhan29 with reference to growth, yield and nutrient content of rice.
- To find out the suitable combination of cowdung and inorganic fertilizers to mitigate the detrimental effects of arsenic stress for rice production in Bangladesh.

CHAPTER II

REVIEW OF LITERATURE

Rice is the staple food crop of Bangladesh and farmed in watery conditions. Farmers are applying imbalanced doses of fertilizer, insecticide and pesticide to their rice field to harvest maximum yield from their small piece of land which is creating environmental pollution as well as one of the causes of climate change. Besides these consequences rice cultivation with arsenic contaminated irrigation water brought another risk for human health. The higher arsenic contents have been observed in the top layer of soil (Haq *et al.*, 2013). Growth and yield of rice plants are also greatly affected by the environmental factors i.e. air, day length or photoperiod, temperature, variety and agronomic practices like transplanting time, spacing, number of seedlings, depth of planting, fertilizer management etc. and abiotic stresses like salinity, drought, flood, contamination by heavy metals like Arsenic, Cadmium, Lead etc. Arsenic is one of the most toxic heavy metals in many parts of the world. It is one of the major pollutants in paddy fields near industrial areas and creates adverse effect to plant growth and development. But the available relevant review related to arsenic reduction in rice is very limited in the context of Bangladesh as well as the World. Some of the recent past information on arsenic reduction in rice have been reviewed under the following headings:

2.1 Environmental Source of Arsenic Exposure

Arsenic, a metalloid and naturally occurring element, is one of the most ample elements in the earth's crust and is found throughout our environment. Arsenic can attach to very small particles in the air, stay in the air for many days, and travel long distances. Arsenic is primarily used as an insecticide and herbicide or preservatives for wood due to its germicidal power and resistance to rotting and decay respectively. Arsenic is also pISSN 1975-8375 eISSN 2233-4521 used in medicine, electronics, and industrial manufacturing (Nriagu and Azcue, 1990).

Arsenic is one of the major toxic environmental pollutants which has recently attract attention because of its chronic and epidemic effects on human health through widespread water and crop contamination due to the natural release of this toxic element from aquifer rocks in Bangladesh (Fazal *et al.*, 2001; Smith *et al.*, 2000; Ahmed, 2000 and Hopenhayn, 2006). During last decade, arsenic pollution has been considered an important environmental issue in many countries worldwide. However, the environmental route of exposure causing arsenic

contamination in the general population has not been fully determined. Recently, the most significant concern related to human health risks from arsenic toxicity is thought to be transported through drinking water, worldwide food distribution, smoking, and global cosmetics (Buchet et al., 1996).

According to the World Health Organization (WHO, 2003), environmental exposure to arsenic imposes a big health issue worldwide. Since the middle of the 19th century, production of heavy metals increased steeply for more than 100 years, with concomitant emissions to the environment. Arsenic is an environmental toxicant with wide distribution in rock, soil, water and air. Arsenic compound is classified into two viz. inorganic arsenic and organic arsenic. Inorganic arsenic is generally abundant in groundwater used for drinking in several countries all over the world (e.g. Bangladesh, Chile and China), whereas organic arsenic compounds are primarily found in fish, which thus may give rise to human exposure. Mazumder (2008) reported that arsenic is a great environmental contaminant in the Bengal delta basin and is responsible for causing carcinogenicity to millions of people and animals. Emissions of heavy metals to the environment occur through wide range of processes and pathways, including to the air to surface waters and to the soil.

Ferguson (1990) reported that in the environment, arsenic and its compounds are mobile and cannot be destroyed. However, interaction with oxygen or other molecules present in air, water, or soil, as well as with bacteria that live in soil or sediment can cause arsenic to change form, attach to different particles, or separate from these particles. Elevated concentrations of arsenic have primarily resulted from natural sources, such as erosion and leaching from geological formations or anthropogenic sources. In addition, arsenic use for industrial purposes, mining activities, metal processing, and pesticides and fertilizers are other major sources of contamination.

Soil is being contaminated with arsenic through irrigated water and rice, vegetables, plants are thereby contaminated with arsenic through its uptake to the toxic level (Das et al., 2000). Gibb *et al.* (2011) and Argos *et al.* (2010) stated that many common arsenic compounds can dissolve in water, thus arsenic can contaminate lakes, rivers, or underground water by dissolving in rain, snow, or through discarded industrial wastes. Therefore, arsenic contamination in groundwater is a serious public health threat worldwide. In addition, the effect of chronic arsenic exposure from ingested arsenic-contaminated food and water or

inhaled contaminated air has been investigated in various countries and found to be associated with detrimental health effects such as hyperpigmentation, keratosis, various types of cancer and vascular diseases.

Most arsenic used in industrial processes is used to produce antifungal wood preservatives that can lead soil contamination. Incineration of preserved wood products, pressure treated with chromate copper arsenate was found to be a source of environmental arsenic contamination (Lu, 1990). According to the publication by the US Agency for Toxic Substance and Disease Registry, since 2003, the use of arsenic-containing wood preservatives have been phased out for certain residential uses such as play structures, picnic tables, decks, fencing, and boardwalks, but are still used in industrial applications. Arsenic is also used in the pharmaceutical and glass industries, in the manufacturing of alloy, sheep dips, leather preservatives, arsenic-containing pigments, antifouling paints, and poison baits. Arsenic compounds are also employed in the microelectronics and optical industries (WHO, 2002).

Hughes (2002) stated that arsenic is found in natural and anthropogenic sources. It occurs naturally in rocks and soil, water, air, plants and animals. Volcanic activity, erosion of rocks and minerals, and forest fires are natural sources. Arsenic occurs naturally in soil, water, air, plants and animals. There are two forms of arsenic: organic and inorganic. Both are easily absorbed, but the inorganic form is more harmful. It accumulates in body organ, is classified as a carcinogen and may affect different chemical and metabolic processes in the body.

USDA (1970) reported that arsenic compounds are used in insecticides and pesticides due to its germicidal power. The inorganic arsenic compounds, primarily, sodium arsenite, have been widely used as a weed killer, and non-selective soil sterilant.

EPA (1983) reported that methylated arsenic is a minor component in the air of urban and industrial areas, and that the main inorganic portion of air is composed of the trivalent and pentavalent compounds.

Arsenic occurs mainly as inorganic species, but it also can bind to organic material in soils (BGS, 1999; Mandal and Suzuki, 2002). Arsenic may accumulate in soils through the use of arsenical pesticides, herbicide, fertilizer etc. Inorganic arsenic may be converted to arsenic

compounds by soil microorganisms (Wei *et al.*, 1991). The total amount of arsenic in soils and its chemical forms has adverse effects on plant, animal and human health (Nriagu and Lin, 1994). Accumulation of arsenic can cause toxic effects to plants and enter into the human by food chain. Arsenic retention and release by sediments depends on the chemical properties of the sediments, especially on the amount of iron and aluminium oxides and hydroxides they contain (BGS, 1999). The amount of sedimentary iron is an important factor that influences arsenic retention in sediments (Mandal and Suzuki, 2002).

2.2 Potential Pathways of arsenic exposure in humans

Groundwater contamination is one of the major pathways of human exposure to inorganic arsenic and the risk of arsenic contamination is generally much higher in groundwater than that in surface water (Argos *et al.*, 2012). Bhattacharya *et al.* (2002) stated that groundwater is a main source of drinking water, and elevated concentration of arsenic in groundwater has been associated with various adverse health effects in humans. Arsenic in drinking water is one of the most significant environmental causes of cancer. In 1963, WHO has recommended limits to the maximum concentrations of arsenic in drinking water and their recommendation was of 50 µg/L, but after new evidence relating low arsenic concentrations with cancer risk, WHO further reduced their recommendation to 10 µg/L in 1992 (WHO, 2001).

Akter *et al.* (2005) reported that once arsenic compounds are absorbed, they are generally processed via the liver's metabolic pathway, and then converted into several different types of inorganic and organic species including arsenite (As^{3+}), arsenate (As^{5+}), dimethylarsinate (DMA), and monomethylarsonate (MMA). Inorganic arsenic and organic arsenic are absorbed promptly into the blood and circulated to the human gastrointestinal tract. Organic arsenic species are generally considered innocuous since they are poorly absorbed into body cells. In contrast, inorganic arsenic species are highly reactive and affect a series of intercellular reactions (Drobna *et al.*, 2010).

Chowdhury *et al.* (2000) reported the high concentrations of arsenic in groundwater of Bangladesh, Vietnam (Berg *et al.*, 2001), China (Lianfang and Jianzhong, 1994), Taiwan (Chen *et al.*, 1994), Argentina (Smedley *et al.*, 2005), and Canada (Grantham and Jones, 1977). Chakraborti *et al.* (2001) reported that contaminated used to cultivate rice and vegetables for human consumption is a significant pathway of arsenic ingestion. Le *et al.* (1994) stated that some crustaceans contain arsenobetaine and some seaweed contains

arsenosugar, but seafood usually contains organic arsenic compounds that are less toxic than inorganic compounds.

Cigarette smokers have a significantly greater total urinary arsenic concentration than non-smokers do because some chemicals in cigarettes compete for many enzymes or co-factors involved in the arsenic methylation process (Tseng, 2005). Ferreccio *et al.* (2000) found that cigarette smoking and ingesting arsenic in drinking water had a synergistic effect. Cigarette smokers exposed to extreme concentrations of arsenic in drinking water (200 µg/L) had a greater risk of bladder cancer than smokers exposed to low concentrations of arsenic did (Morales *et al.*, 2000 and Steinmaus *et al.*, 2003).

Cosmetics are also considered an unlikely source of arsenic exposure and as a mild impurity, but are a leading cause of direct exposure among a many individuals. Assessing the amount of dermal absorption from a single component in a cosmetic product is complex and depends on several factors such as the concentration of arsenic in the product, the amount of product applied, the length of time left on the skin and the presence of emollients and penetration enhancers in the cosmetic products (Hostynek, 2014).

EPA (2004) reported that human exposure to arsenic through the air generally occurs at very little concentrations ranging from 0.4 to 30 ng/m³. They also estimated that approximately 40 to 90 ng of arsenic per day are commonly inhaled by humans. In unpolluted areas, approximately 50 ng or less arsenic is inhaled per day.

Duxbury and Panaullah (2002) reported that the most serious effects have been found in Bengal Delta region in Bangladesh and West Bengal, India where the groundwater has been widely developed to supply drinking and irrigation water. An estimated more than 30 million people drink water from arsenic contaminated Shallow Tube Wells (STWs) and approximately 900000 STWs are used in irrigating 2.4 million out of 4 million ha under irrigation in Bangladesh, mainly paddy fields. Also, about 95% of the groundwater extracted is used for irrigation.

FAO (2007) reported that the problem started from the arsenic-rich bedrock of the Brahmaputra river basin that filters drinking water pumped to the surface through millions of tube wells. High concentrations of arsenic enter the food chain through absorption by crops

from roots to straw and grain contaminated from irrigated water. It was also estimated that water pumping from shallow aquifers for irrigation adds one million kilogram of arsenic in every year to the arable soil in Bangladesh, mostly in the paddy fields.

Stroud et al. (2011); Brammer and Ravenscroft (2009) reported that arsenic pollution in groundwater has been reported in over 70 countries and population of about 150 million people worldwide with excessive concentration discovered in 10 countries in south and south-east Asia namely, Bangladesh, Cambodia, China, India, Laos, Myanmar, Nepal, Pakistan, Taiwan and Vietnam with over 110 million people living in these areas have serious health threats due to their dependence on As-contaminated water for drinking and irrigation purposes.

Mirdar-Ul-Haq *et al.* (2005) reported that among many other factors responsible for heavy metal such as arsenic contamination in soils are long term usage of sewage or effluents for irrigation purposes which in turn will have negative effects on plants, animals and human health.

Islam and Islam (2007) reported that arsenic is a toxin. It is a great health risk to millions of people worldwide when it is there in food and drink. It is highly toxic at higher doses but chronic exposure to lower levels increases the risk of cancer of skin, bladder, lungs, kidney, liver, colon, prostate; cardiac disease, pulmonary disease, cardiovascular disease, diabetes; diseases of arteries and capillaries; increased sensitivity to Hepatitis B infection, infertility, and other ailments. Observable symptoms to the arsenic poisoning can be thickening and discoloration of skin, stomach pain, nausea, vomiting, diarrhea, paralysis and blindness.

2.3 Pathways of arsenic availability to plants

As is primarily taken up by plants via root absorption, although some submerged plants can absorb As from water via their leaves (Wolterbeek and Meer 2002). Three main forms of As in soil are available to plants, namely arsenate, arsenite and methylated As [monomethylarsinic acid (MMA) and dimethylarsinic acid (DMA)]. Different forms of As exist simultaneously in the soil. Microorganisms in the soil transform As species from arsenate to arsenite and further to MMA and DMA. Plant roots selectively take up specific As species via distinct pathways and transporters.

Arsenic accumulated by green algae at a significantly high concentration while grown in boro rice field in Bangladesh. This arsenic accumulation by green algae from irrigation water may cause lower arsenic accumulation by rice plant which would be useful to the people of Bangladesh (Huq *et al.*, 2001).

A test was conducted by Das *et al.* (2003) for arsenic in rice grown on the soils adjacent to water source contaminated with arsenic. They found that the highest concentration of arsenic accumulated by roots of paddy followed by shoot and rice grain (0.23 ppm).

Chakraborti *et al.* (2001) reported that 95% inorganic and 5% organic arsenic species present in rice. Irrespective of chemical forms root arsenic concentration was 10.5 mg/kg in the 0.05 mg/L treatment, which increased to 212.7 mg/kg in the 0.8 mg/L treatment (Marin *et al.*, 1992).

A test was conducted by Duxbury *et al.* (2002) to determine the concentration of arsenic in rice grain of 150 samples collected from different districts of Bangladesh including Barisal, Comilla, Dinajpur, Rajshahi and Rangpur. Arsenic concentration was found in the range from 0.01 to 00415 mg/kg dry weight. As expected boro rice grain contained higher arsenic concentrations (mean value 183 µg/kg dry weight.) compared to aman rice (mean value 117 µg/kg dry weight).

Heitkemper *et al.* (2001) concluded that rice grain has lower concentration of arsenic and the concentration remain much below than maximum permissible limit of 1 mg/kg.

Xie and Huang (1998) posed that arsenic accumulation is affected by concentration of arsenic in soil or nutrient media and increased significantly with increasing levels of arsenic. They also reported that the pattern of arsenic concentration in rice plant parts generally follow the pattern: root > straw > husk > whole grain > husked rice.

Yan Chu (1994) stated a relationship between concentrations of arsenic in soil solution and rice tested to quantify the effect of level of arsenic uptake into rice. The regression equation found between the amount of arsenic present in the rice plant, Y, and the amount of arsenic in aqueous solution, X, to be: $Y = 0.042X - 0.0413$.

2.4 Performance of arsenic on rice

Rice is very potent in taking up arsenic, because it is grown in water-flooded situation. That declines the binding of arsenic by soil. It makes arsenic more accessible to rice. The semi-aquatic nature of rice plant and grain gives the opportunity to pull arsenic up from readily available sources. It is a fact that arsenic is a naturally occurring pollutant and because it is in soil and water, so it is going to get into food. Rice takes more arsenic from the soil than any other crops. This is because of the way rice is grown. Rice is grown in flooded areas which charge the soil readily releasing arsenic from the soil. Rice is most damaged by arsenic uptake. The flooded soil is anaerobic and strongly declined. In this situation arsenic is readily available to rice plants roots. Moreover, higher amount of water is used for irrigated rice than is used for aerobic culture. Different varieties of rice give different performance in arsenic tolerance. Seriously affected varieties develop straight head disease, empty panicle at maturity (Brammer, 2008).

Abedin *et al.* (2002a) reported that irrigation water contaminated with arsenic reduce seed germination, plant height, root growth and yield of rice. In Bangladesh, groundwater of 61 out of 64 districts is contaminated with arsenic in various concentrations. Concentration of arsenic exceeding 1.00 mg per liter of water is observed in 17 districts of Bangladesh including Chandpur, Comilla, Noakhali, Feni, Munshiganj, Brahmanbaria, Faridpur, Madaripur, Laksmipur, Gopalganj, Shariatpur, Narayanganj, Narail, Satkhira and Chapainawabganj.

Duxbury and Panaullah (2007) stated that rice yield reduced from 8.9 t/ha at 26.3 ppm soil arsenic to 3 t/ha at 57.5 ppm arsenic. The results indicated that the practical limit for paddy cultivation might lie between 25-50 mg/kg soil arsenic.

Panaullah *et al.* (2009) reported that irrigation water contaminated with arsenic decrease yield from 7-9 to 2-3 t/ha with rising soil arsenic content and the average yield loss was 16 percent. They also reported that growth suppression of rice was associated with straw arsenic concentrations of >5-10 mg/kg.

Abedin *et al.* (2002b) reported that increase in the content of arsenic in irrigation water led to increasing arsenic content in rice plants and consequent reduce in plant yield. Islam *et al.*

(2004) reported that household survey on dietary habits revealed women drunk on an average 3.1 liter of water, 1.1 kg of cooked rice and 42 g dry weight of curry in every day. The total ingestion rates ranged from 31.1-129.3 $\mu\text{g}/\text{day}$ and the result indicated that the main route of arsenic in Bangladesh is rice followed by water and curry.

Bhattacharjee *et al.* (2014) stated that higher level of arsenic negatively affected the nutrient uptake in rice and nutrient content except N. Nitrogen content increased with the accumulation of more arsenic in plant roots zone. Greater amount of nutrient uptake and nutrient content was recorded in BRRI dhan48 and flooding enhanced higher nutrient content and uptake in rice. This study suggests the possible management of moisture regime and considering low arsenic susceptible variety, which might decrease the toxic effects of arsenic on nutrient uptake.

Khan *et al.* (2010) reported that low mobility of applied arsenic and the likely continued detrimental accumulation of arsenic within the rooting zone. Arsenic present in irrigation water or in soil resulted in decreasing of yield from 21-74% in Boro rice and 8-80% in T. Aman rice, the later indicating the strong residual effect of arsenic on subsequent crops. The concentrations of arsenic in rice grain (0.22-0.81 μg), straw (2.64-12.52 μg) and husk (1.20-2.48 μg) increased with accumulation addition of arsenic. In pot experiment, the growth of rice was retarded when soil contained >15 mg As/kg, and severe toxicity symptoms were apparent when soil contained 60 mg As/kg.

Bhattacharya *et al.* (2010) stated that the arsenic uptake in rice varies with different rice varieties; the maximum accumulation was recorded in White Minikate (0.31+or-0.005 mg/kg) and IR 50 (0.29+or-0.001 mg/kg) rice varieties and minimum was found in the Jaya rice variety (0.14+or-0.002 mg/kg). In rice plant, higher arsenic accumulation found in the straw part (0.89+or-0.019-1.65+or-0.021 mg/kg) compared to the accumulation in husk (0.31+or-0.011-0.85+or-0.016 mg/kg) and grain (0.14+or-0.002-0.31+or-0.005 mg/kg) parts. For any rice sample concentration of arsenic in the grain did not exceed the WHO recommended permissible limit in rice (1.0 mg/kg).

Wang *et al.* (2010) stated that arsenic is one of the most serious contaminants as noxious element-especially inorganic arsenic and it has a chronic poisoning effect in human body. Some studies have shown that rice is much more efficient accumulator of arsenic into its straw and grains than other cereal crops, and rice consumption constitute a great proportion

of dietary intake of arsenic. The total arsenic content in rice varies from 0.005-0.710 mg/kg. The content of inorganic arsenic in rice varies from 10-90% of total arsenic.

Jahan *et al.* (2003); Rahman *et al.* (2004); Xie and Huang (1998) reported that plant height and shoot biomass production reduced with the increase of soil arsenic concentrations. Reduction in growth of rice plant in terms of tillering, plant height and shoot biomass production due to the result of arsenic phytotoxicity at high soil arsenic concentrations.

Duel and Swoboda (1972) and Jacobs *et al.* (1970) stated that displacement of soil phosphate by arsenate at low soil arsenic concentration increased the accessibility of phosphate to the plant resulted in the increase of plant growth.

Schoof *et al.* (1999) stated that rice has higher inorganic arsenic concentrations than most other food, and consequently, diets that rely massively on rice may contain the most inorganic arsenic.

About 80% inorganic arsenic contamination in rice was reported in Bangladesh which is far more noxious than organic species. This was in sharp contrast to 58% Arsenic in U.S. rice, 64% in rice from Europe and 81% contamination in rice from India. However, basmati rice imported from India and Pakistan and jasmine rice from Thailand were found to contain low arsenic (Meharg, 2009).

Islam *et al.* (2005); Delowar *et al.* (2005) Bhattacharya *et al.* (2009) stated that rice accumulates up to 2 mg/kg which is much above the permissible limit of 1.0 mg/kg, according to the WHO recommendation. Mehrag and Rahman (2002) concluded that the average contribution to total arsenic intake from drinking water was 13%, whereas from cooked rice, it was 56%, thus making it clear that rice contributed most to the daily arsenic uptake.

Van Geen *et al.*(2006) reported that the health hazards due to ingestion of arsenic contained in rice therefore appear to be dwarfed in countries such as Bangladesh. Many studies observe that rice (*Oryza sativa* L.) in different growth stages accumulates arsenic in different levels but at maturing stage uptakes maximum amount significantly than at other stages (Wang *et al.*, 2006).

Abedin *et al.* (2002a) reported that percent of rice seed germination over control reduced significantly with the increasing concentrations of arsenite (As-III) and arsenate (As-V) and found that arsenite was more noxious than arsenate for rice seed germination.

Dahal *et al.* (2008) reported that the significant presence of arsenic contaminated irrigation water on alkaline soils and arsenic uptake in agricultural plants at field level in Nepal. He concluded his study by giving the mean arsenic content of edible plant material (dry weight) in the order of onion leaves (0.55 mg As kg⁻¹) > onion bulb (0.45 mg As kg⁻¹) > cauliflower (0.33 mg As kg⁻¹) > rice (0.18 mg As kg⁻¹) > brinjal (0.09 mg As kg⁻¹) > potato (<0.01 mg As kg⁻¹) indicating that in Nepal, onion leaves had maximum and rice (fourth in order of concentration) As uptake.

Bhattacharya *et al.* (2009) and Nahar (2009) reported that over 1000 tons of arsenic is transferred to arable land each year from irrigation by groundwater contaminated with arsenic in Bangladesh. Long term use of arsenic contaminated groundwater for irrigation purposes may result in further increase in arsenic concentration in agricultural soil and eventually led to hyper-accumulation in crops, including rice.

Abedin *et al.* (2002a); Delowar *et al.* (2005); Islam *et al.* (2004) and Rauf *et al.* (2011) reported that elevated levels of arsenic in irrigation water or soil either naturally or artificially can decline growth and productivity of rice due to its toxicity. Arsenic impairs metabolic processes and thus declines plant growth and development (Marin *et al.*, 1993). Soil arsenic, declines plant height (Carbonell-Barrachina *et al.*, 1995; Abedin *et al.*, 2002b; Jahan *et al.*, 2003 and Karimi *et al.*, 2010); decreases tillering ability (Kang *et al.*, 1996 and Rahman *et al.*, 2004); lessen shoot growth (Cox *et al.*, 1996 and Carbonell-Barrachina *et al.*, 1998); lowers fruit and grain yield (Carbonell-Barrachina *et al.*, 1995; Abedin *et al.*, 2002c and Kang *et al.*, 1996) and sometimes leads to death (Marin *et al.*, 1992 and Baker *et al.*, 1976).

Dittmar *et al.* (2010) investigated that concentrations of arsenic in grain and straw were elevated in the field and maximum near the irrigation water inlet, where arsenic concentrations in both irrigation water and soil were highest. Based on a recently published scenario of long term accumulation of arsenic at the study site, it was estimated that, under unchanged irrigation practice, average arsenic concentrations in grain increase from currently ~0.15 mg/kg to 0.25-0.58 mg/kg by the year 2050. This translates to a 1.5-3.8 times higher

intake of arsenic by the local population through rice, possibly exceeding the provisional tolerable intake value of arsenic defined by FAO/WHO.

Begum *et al.* (2008) stated that the grain yield of Boro rice was decreased by 20.6% for 15 ppm arsenic and 63.8% due to 30 ppm arsenic treatments. Such reductions for straw yield were 21.0% and 65.2% with these two treatments of arsenic, respectively. Residual effect of arsenic was also important and negative in T. Aman rice. The grain-As concentration in all cases was below 1 ppm, and the straw-As content was well above 1 ppm. The concentrations of arsenic in both grain and straw were higher in Boro rice than in T. Aman rice.

Hossain (2005) reported that reduction of yield more than 40% and 60% for two popular rice varieties (BRRI Dhan28 and Iratom-24) when 20 mg/kg of arsenic was added to soils, compared to the control. They investigated the effects of different concentrations of arsenic in irrigation water on Boro (dry-season) rice and their residual effects on the following Aman (wet-season) rice. All the growth and yield parameters of Boro rice responded positively at lower concentrations of up to 0.25 mg/L in irrigation water but reduced sharply at concentrations more than 0.5 mg/L. The concentrations of arsenic in both grain and straw of Boro rice increased significantly with increasing concentration of arsenic in irrigation water. The grain arsenic concentration was in the range of 0.25 to 0.97 µg/g and its concentration in rice straw varied from 2.4 to 9.6 mg/g over the treatments. Residual effect of arsenic from previous Boro rice showed a very similar pattern in the following Aman rice, although arsenic concentration in Aman rice grain and straw over the treatments was almost half of the levels of arsenic in Boro rice grain.

Delowar *et al.* (2005) reported the extent of arsenic accumulation in rice plants and its effects on growth and yield of rice. Arsenic concentrations in paddy soils (irrigated with 0, 2.5, 5, 10, 15 and 20 mg/L of arsenic water) were 0-0.2, 0-0.95 and 0-0.27 mg/kg at tillering, heading and ripening stages. Arsenic accumulated in rice grains from soil/water and arsenic accumulation varied greatly in the two rice varieties studied. The concentrations in rice grains were 0-0.07 and 0-0.14 mg/kg dry weight in rice varieties BRRI dhan28 and Iratom 24, respectively. The growth and yield of rice plants were decreased significantly with increased doses of arsenic but the grain weight was not affected. Among the different yield components, the number of tillers per pot, number of effective tillers per pot and grain yield per pot reduced greatly with the greater dose (20 mg/L) of arsenic applied. Yield reduction of

more than 60% and 40% for Iratom-24 and BRRI dhan28, was found with 20 mg/L of arsenic as compared to control. The reduction in straw yield was also significantly greater for both of rice varieties with the 20 mg/L arsenic application.

Williams (2003) observed that 64% of European, 80% of Bangladeshi and 81% of Indian rice arsenic were inorganic, with As (III) predominating. Arsenic present in ground water affects people in Bangladesh via seed grains and forages. Samples of rice and rice straw were collected from arsenic-contaminated areas and arsenic concentration was measured using Flow Injection Hydride Generator Atomic Absorption Spectrophotometer (FI-HG-AAS) method. The concentrations in rice and rice straw were 0.235 ± 0.014 ppm ($n = 48$) and 1.149 ± 0.119 ppm ($n = 51$), respectively. Both were greater than the maximum permissible concentration in drinking water (0.05 ppm; WHO).

Jahiruddin *et al.* (2004) studied the effects of arsenic contamination on crop yield and arsenic accumulation under control conditions. The levels of soil added arsenic were 0, 5, 10, 15, 20, 30, 40 and 50 ppm, and that of irrigation water arsenic were 0, 0.1, 0.25, 0.5, 0.75, 1.0, 1.5 and 2 ppm. The effect of added arsenic (plus 2.6 ppm soil arsenic) was tested directly on Boro rice (BRRI dhan29) and its residual effect on T. Aman rice (BRRI dhan33). The pots for both crops received an equal amount of fertilizers. They found that the grain protein was adversely affected due to arsenic contamination. 40% grain yield reduction for 10 mg/kg addition of arsenic to BAU farm soil.

Kang *et al.* (1996) reported that increasing the level of arsenic declined plant height, number of effective tillers, dry weight of aboveground parts and 1000-grain weight. Yields reduced from 48.7 g/pot with the minimum rate of arsenic to 17.9 g with the maximum rate. Arsenic content was higher in roots than in stems plus leaves or in grain, but in all parts the content increased as soil arsenic increased. The contents of arsenic in stems plus leaves were more closely related to soil total and available arsenic than those of roots or grain.

Kabata and Pendias (1992) recommended the safe level of arsenic in agricultural soil as 20 mg/kg. 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) though the phytotoxicity at lower soil arsenic concentrations was not significant.

Abedin and Mehrag (2002) reported that the elevated levels of soil arsenic resulting from long-term use of arsenic contaminated ground water for irrigation in Bangladesh may retard seed germination and seedling establishment of rice, the country's prime food crop. A germination study on rice seeds and a short-term toxicity experiment with different concentrations of arsenite and arsenate on rice seedlings were conducted. Percent (%) of germination over control decreased significantly with increasing concentrations of arsenite and arsenate. Arsenite was found to be more poisonous than arsenate for rice seed germination. There were varietal differences among the test varieties in response to arsenite and arsenate exposure. The performance of the dry season cultivar Purbachi was the best among of all cultivars. Germination of Purbachi was not inhibited at up to 4 mg/L arsenite and 8 mg/L arsenate treatment. Root tolerance index (RTI) and relative shoot height (RSH) for rice seedlings reduced with increasing concentrations of arsenite and arsenate. Reduction of RTI caused by arsenate was higher than that of arsenite. Normally, dry season varieties have more tolerance to arsenite or arsenate than the wet season varieties.

2.5 An overview of arsenic removal processes

Brown *et al.* (2003) and Hartley *et al.* (2009) reported that organic soil amendments such as compost, manures and sludges are now established amongst *in-situ* alternatives to expensive and/or disruptive hard engineered removal or capping of contaminated substrates to reduce contaminant-associated risk.

Agrafioti *et al.* (2014) and Samsuri *et al.* (2013) reported that biochars had high efficiency to reduce inorganic As (III) and As (V) from aqueous solution, especially for the activated biochars produced from agricultural wastes.

Warren *et al.* (2003) and Tighe *et al.* (2005) stated that the combination of biochar with iron-oxides can reduce arsenic mobility in soil by anion exchange.

Das *et al.* (2001) claim that the microbes residing in the cowdung helped convert arsenic into volatile arsenic species since analyses of the soil failed to produce concentrated As values. Arsenic waste can be disposed of by converting it into volatile organic forms through the activities of the microbes in soil or sediments. One such disposal method was used in Bangladesh where arsenic waste was disposed in soil in the backyard with cowdung added.

Epps and Sturgis (1939) stated that application of sulfur exerted a depressive effect on arsenic toxicity to paddy rice.

Leupin and Hug (2005) and Ghurye *et al.* (2004) stated that oxidation or precipitation is very effective technology for the removal of arsenic from water.

Lara *et al.* (2006) reported that instead of UV-light, solar-light can also remove arsenic from natural water upon addition of iron and citrate.

Katsoyiannis and Zouboulis (2006) reported that iron oxidizing bacteria reduce arsenic more efficiently than those of manganese oxidizing bacteria.

Saalfeld and Bostick (2009) demonstrated a process in the laboratory, where the mobility of arsenic was affected by biologically mediated redox processes by binding it to iron oxides through dissimilatory sulphate reduction and secondary iron reduction processes in reducing aquifers.

Appelo *et al.* (1999) stated that in-situ oxidation can remove the arsenic content in the pumped groundwater by pumping the oxygenated water into the groundwater aquifer. The dissolved oxygen content in water oxidizes As (III) to less mobile As (V) in the aquifer causing the reduction in the content of arsenic.

Ma *et al.* (2001) reported that the *Pteris vittata* (Chinese brake fern) was found to be resistant to arsenic, having the capability of hyper accumulating large amounts of arsenic in its fronds by area contaminants are picked up by the roots of plants and transported to their over ground parts, and then removed together with the crops (phytostabilization, phytoextraction and phytovolatilization).

Cao *et al.* (2003) reported that when bio solid was added to either acidic or neutral soil the adsorption of arsenic was increased and reduce water soluble arsenic.

Baig *et al.* (2010) reported that the use of native biomasses (powdered) reduced arsenic from surface water. For example, biomass from the stem of a thorny *Acacia nilotica* was used for the removal of arsenic from arsenic contaminated water bodies.

Takamatsu *et al.* (1982) reported that water management practices influenced the physiochemical properties of the paddy soils through reduction-oxidation process, which decrease the availability of arsenic to the soil solution through transformation of less toxic and available (arsenate) form, and subsequent entry to plant systems.

Das *et al.* (2008) stated that the addition of organic matter in paddy field decreased the arsenic availability through the formation of an insoluble and stable arseno-organic complexes and their adsorption on to organic colloids of soil solutions.

Walker *et al.* (2004) reported that the addition of organic amendments to the soils decrease the heavy metal bioavailability by changing them from bio-available forms to the fractions associated with organic matter or metal oxides or carbonates.

Shiralipour *et al.* (1992) reported that the application of organic matter to soil would increase soil anion and cation exchange capacity, which may increase arsenic adsorption by increasing the amount of positive charge on the oxide surface and/or forming a positively charged surface and enhanced sorption capacity of the soil matrix.

Mukhopadhyay and Sanyal (2004) reported that the combined application of manures and compost have great effect in reducing the plant and soil arsenic content, due to the release of higher amount of organic acid (humic/falvic acid), binding site of the arsenic in the soil rather than release of nutrients and change the physiochemical properties of soil-water.

Wang and Muligan (2006) stated that humic acid (HA) and falvic acids (FA) from organic matter compete strongly with arsenic for active adsorption sites on mineral surfaces result in lowering the levels of arsenic retention, mobility and bioavailability of arsenic.

Redman *et al.* (2002) stated that the formation of humic acid (HA)/falvic acid (FA)-metal complexes strongly bind arsenate and arsenite anions through metal-bridging mechanism contribute to immobilization of arsenic.

Chen *et al.* (2000) reported that organic amendments such as manures and compost which contain a high amount of humified organic matter can reduce the bioavailability of heavy metals through adsorption and by forming stable complexes with humic substances.

Jones *et al.* (2000) reported that the reduced accumulation of arsenic in plants are due to low availability of the toxicant from soil due to amended through compost, manures etc.

McLaren *et al.* (2001) stated that the microorganism present in cowdung may also enhance the mobility of arsenic in soil. Again, chemical fertilizers are also applied to agricultural lands to enhance crop yield especially for irrigated crops. Arsenic from irrigation water may be adsorbed on the soil particles. The adsorbed arsenic may be transformed or bio-transformed and released in the presence of cowdung and phosphate fertilizer. But the processes of transformation/ biotransformation and release of arsenic in the agricultural fields are not clearly understood and quantified. Increased bio-methylation and volatilization of gases from soil can reduce arsenic accumulation in agricultural fields.

CHAPTER III

MATERIALS AND METHODS

The experiment was conducted during the period from December 2017 to May 2018 to see the mitigation of arsenic stress on BRRI dhan29 with cowdung and inorganic fertilizers. This chapter presents a brief description about the materials and methods that were used for conducting this experiment. It includes a short description of different parameters to correlate with establishing rice plant. It further covers the data collection procedure, source of data and ways of data were analyzed.

3.1 Experimental site

The pot experiment was conducted in the net house of the Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Dhaka-1207 during the Boro season of 2017-18 to evaluate the mitigation of arsenic stress in BRRI dhan29 with cowdung and inorganic fertilizers. Geographically, the experiment site stands 24.09⁰N latitude and 90.26⁰E longitudes. The altitude of the area was 8 m from the sea level as per the Bangladesh Metrological Department, Agargaon, Dhaka-1207 (Anonymous., 1989).

3.2 Climate

The University area is located in the sub-tropical humid climate and is characterized by high temperature accompanied by moderately high rainfall during kharif season (April-September) and low temperature in rabi season (October-March).

3.3 Description of soil that used in pot

The soil collected for this experiment from the field of Sher-e-Bangla Agricultural University (SAU) Farm. The soil was Shallow Red Brown Terrace soil under Tejgaon series and that belongs to the Agro-Ecological Zone 28 (Modhupur Tract). The soil texture of the experiment was clay loam with common fine medium distinct dark yellowish brown mottles.

3.4 Soil Collection and preparation

Soil was collected at a depth of 0-15 cm from the experimental field of Sher-e-Bangla Agricultural University. After collection, the plant roots and unnecessary materials were removed from the soil and dried under sunlight for four days. Then the soil sieved and mixed up thoroughly to ready for potting.

3.5 Pot preparation

An amount of 8 kg soil was taken in a series of pots. The diameter of the pot was 24 cm top, 18 cm bottom diameter and 22 cm depth were collected from the local market and cleaned before use. There were altogether 60 pots comprising 4 different treatments of arsenic to 5 different treatments of cowdung and inorganic fertilizers to BRRI dhan29 with 3 replications. Arsenic was added from sodium arsenate. Water was added to the pot to bring the soil up to saturation.

3.6 Treatments of the experiment

Four rates of arsenic *viz.* 0, 20, 40, and 60 ppm arsenic (on water per pot basis) and five rates of cowdung and inorganic fertilizers were applied on BRRI dhan29. The source of arsenic was Sodium Arsenate ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$).

Design: CRD with two factorials

Factor A: Different doses of arsenic: 04

As₀= (No arsenic applied)

As₁= (20 ppm arsenic on required water basis)

As₂= (40 ppm arsenic on required water basis)

As₃= (60 ppm arsenic on required water basis)

Factor B: Different doses of cowdung and inorganic fertilizers: 05

T₀ = Recommended doses of cowdung + inorganic fertilizers (BRRI, 2016)

T₁ = Recommended doses of cowdung without inorganic fertilizers

T₂ = Recommended doses of inorganic fertilizers without cowdung

T₃ = Reduction of 20% recommended dose of inorganic fertilizer + addition of 20% recommended dose of cowdung

T₄ = Reduction of 40% recommended dose of inorganic fertilizer + addition of 40% recommended dose of cowdung

Treatment combinations = 4 x 5 = 20

Replications: 3

3.7 Description of BRRI dhan29

BRRI dhan29 is a mega variety because it widely cultivate in Bangladesh. BRRI dhan29 was developed by Bangladesh Rice Research Institute (BRRI). BRRI dhan29 was officially released in 1994. It is a high yielding variety which can yield up to 7.5 t/ha. Its crop duration is 152 days (BRKB, 2017).

3.8 Seed collection

Seeds of BRRI dhan29 was collected from BRRI (Bangladesh Rice Research Institute), Joydebpur, Gazipur-1701, Dhaka.

3.9 Sterilization of seed

Before germination test seeds were surface sterilized with 1% sodium hypochlorite solution. The glass vials containing distilled water for seed rinsing was sterilized for 20 minutes in an autoclave at $121\pm 10^0\text{C}$ and at 15 bar air pressure.

3.10 Raising of seedlings

The seedlings were raised at the wet seed bed in SAU farm. The seeds were sprouted by soaking for 72 hours. The sprouted seeds were sown uniformly in the well-prepared seed bed in 2nd December 2017.

3.11 Fertilizer and manure application

All the pot received fertilizers according to BRRI's recommended fertilizer dose (BRRI, 2016). The amounts of nitrogen, phosphorus, potassium and sulphur required for each pot were calculated as per their rates of application. The sources of N_2 , P_2O_5 , K_2O as urea, TSP and MP were applied, respectively. Except nitrogen, the entire amount of P, K and cowdung were added at the time of final pot preparation. Nitrogen was added in three equal splits at 7, 30 and 45 days after transplanting (DAT). Arsenic was added to soil before transplanting.

3.12 Transplanting of seedlings

The healthy and uniform seedlings were uprooted carefully from the seedbed in the morning and transplanted in the same day. Healthy and uniform seedlings of 40 days age were transplanted in the pots on 11th January, 2018. One hill in each pot with two seedlings were maintained. Before uprooting the seedlings, seedbed was watered so as to minimize damage to the roots.

3.13 Intercultural operations

The hand weeding and loosening of soils around the hills were done when felt necessary to keep the pots free from weeds. Top dressing of urea was done when felt necessary. Observation was regularly made. Every stages of plants and plants response as per treatments were observed carefully.

3.14 Irrigation

Three cm water was added above ground after transplanting and maintained for after transplanting up to maturity.

3.15 Harvesting

The crop was harvested at full maturity on 6th May 2018. Plants of each pot was bundled separately and carefully with tag mark indicating the respective treatment combinations and brought to the laboratory for recording data on yield and yield contributing parameters.

3.16 Sampling and data collection

Data were collected from the experiment on different growth stages of plants under the following heads as per experimental requirements.

3.16.1 Plant height

The heights (cm) of the plants were taken by measuring the distance from base of the plant to the tip of the flag leaf at 70 and 140 DAT to observe the growth rate of plants and finally averaged.

3.16.2 Number of tillers hill⁻¹

Number of tillers hill⁻¹ was counted from each pot at 70 and 140 DAT to see the arsenic effects and finally averaged.

3.16.3 Number of effective and non-effective tillers hill⁻¹

Number of effective and non-effective tillers hill⁻¹ were counted from the plants of the each pot after harvesting and finally averaged.

3.16.4 Length of panicle

The length (cm) of panicle was taken from each effective tiller after harvesting and finally averaged.

3.16.5 Number of filled grains and unfilled grains panicle⁻¹

Number of filled grains and unfilled grains panicle⁻¹ were counted from each pot. Lack of any food materials inside the spikelets were denoted as unfilled grains.

3.16.6 Grain yield (g/pot)

Grains obtained from each pot were sun-dried and weighed carefully. The dry weight of grain of the respective pot was recorded carefully.

3.16.7 Straw yield (g/pot)

Straw obtained from each pot were oven-dried and weighed carefully. The dry weight of straw of the respective pot was recorded carefully.

3.16.8 Root weight (g/pot)

Root obtained from each pot was oven-dried and weighed carefully. The dry weight of root of the respective pot was recorded carefully.

3.17 Sample threshing and processing

The plant samples were dried in an oven at 60⁰C for 48 hours and then cut into small pieces using clean scissors. The plant materials were stored in desiccators to analyze total As, N, P and K concentrations in the plants.

3.18 Chemical Analysis

3.18.1 Preparation of plant extract for P and K determination

The samples were dried in an oven at 70⁰C to obtain constant weight. Oven-dried straw samples were ground in a Wiley Hammer Mill, passed through 40 mesh screens, mixed properly and stored in plastic vials. Exactly 1g oven-dried samples of rice plants were measured with the help of electrical balance. Then 1g dried samples were taken in conical flask. About 20 mL of di-acid mixture (nitric acid : perchloric acid = 2:1) was taken in a conical flask and left to stand for 20 minutes and then transferred to a digestion block and continued heating at 100⁰C. The temperature was increased to 365⁰C gradually to prevent frothing (50⁰C steps) and left to digest until yellowish color of the solution turned to whitish color. Then the digestion tubes were removed from the heating source and allowed to cool at the room temperature. About 40 mL of distilled water was carefully added to the conical flask and the contents filtered through Whatman No. 40 filter paper into a 100 mL volumetric flask

and the volume was made up to the mark with distilled water. The samples were then stored at room temperature with clearly marked containers.

After digestion, approximately 100 mL of each digest samples was stored in a clearly marked plastic bottle for determination of the P and K. Content of P was determined by Spectrophotometer and content of K was determined by Flame Photometer. After that, the percent of P and K values were also calculated from the concentration of P and K in the plant tissues.

3.18.2 Determination of Arsenic

Sample information: Digest sample, pH<2 with HCl 5 mL/L

Sample storage: Refrigerator, <4⁰C

Instrument: Flame Atomic Absorption Spectrophotometer with HVG, Ar gas (99.999%) as carrier of sample. HCl 5M and 0.4% NaBH₄ as reagent for HVG. Sample flow rate 5 mL/min.

Reagents used: (i) KI (ii) Conc. HNO₃ (iii) Conc. HCl (iv) De-ionized water (DI water) (v) 1000 ppm standard solution of As (vi) NaBH₄ (vii) 5M HCl

Preparation of reagents

Preparation of NaBH₄ solution (0.4% w/v): 2.5 g Sodium Hydroxide and 2.0 g Sodium Borohydrate were dissolved in 500 mL volumetric flask and marked up to volume with distilled water.

Preparation of KI (20% w/v): 20 g KI was taken in 100 mL distilled water then dissolved in water and marked up to volume.

Preparation of 5M HCl: 200 mL distilled water was taken in a volumetric flask and then 208 mL of HCl (37%) was added and volume was marked up to with distilled water.

Preparation of calibration standard from 1000 ppm standard solution of As: 1 mL of As (1000 ppm standard solution) was taken in 100 mL volumetric flask and then mark up with distilled water. Then 1mL from 10 ppm solution was taken in 100 mL volumetric flask and marked up to volume with distilled water. Then dilutions were as follows from the 100 ppb solution:

0 mL= 100 mL water (0 ppb, blank)

2.5 mL= 100 mL water (2.5 ppb)

5 mL= 100 mL water (5 ppb)

10 mL= 100 mL water (10 ppb)

After that, 40 mL from each solution was taken in individual 50 mL volumetric flask and then 4 mL of 37% HCl and 2 mL of freshly prepared 20% (w/v) KI were added to each and left to dark for 15 minutes.

Preparation of blank: 40 mL distilled water was taken in 50 mL volumetric flask and 4 mL of 37% HCl and 2 mL of freshly prepared 20% (w/v) KI were added and left to dark for 15 minutes.

Treatment and preparation of sample: 1 g well-mixed sample was taken in a beaker. About 10 mL conc. HNO₃ was added. The sample was covered with a watch glass and heated on hot plate at 90⁰C to 95⁰C until the volume reduced to 15-20 mL. The beaker was removed and allowed to cooling. The beaker walls and watch glass were washed down with DI water when necessary filter or centrifuge the sample to remove silicates or other insoluble material. Then the final volume to 50 mL was with diluent. After that, 40 mL of this was taken in 50 mL volumetric flask and 4 mL of 37% HCl and 2 mL of freshly prepared 20% (w/v) KI were added and left to dark for 15 minutes.

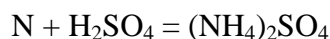
Procedure: For straw arsenic analysis, 1 mL of each samples were taken in a 100 mL conical flask and 50 mL of 0.5 mol/L NaHCO₃ solution was added. Then the whole materials were shaken for 1 hr in a “to and fro” horizontal shaker and after completion of shaking, the suspensions were filtered through Whatman filter paper No. 42. The filtered were collected for arsenic analysis with Atomic Absorption Spectrophotometer coupled with Hydride Vapor Generator (HVG) unit after reducing with 2 mL of 10% KI solution and 2 mL of 35% HCl, NaBH₄ solution and 4 mol/L HCl solution separately from three containers were allowed passing to a mixing manifold by a peristaltic pump. From the mixing manifold by argon (inert gas), carrier, AsH₃ (arsine) generated in the reaction loop. The arsenic was then atomized in a flame of air-acetylene and the direct arsenic concentrations in the sample were measured.

3.18.3 Determination of Nitrogen

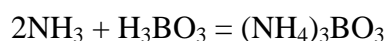
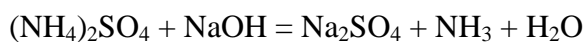
The Macro Kjeldahl method used to determine the total Nitrogen in straw of plant samples.

There were three steps in this method. These are as follows:-

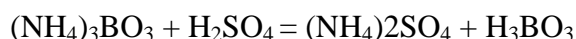
- A. Digestion: It is the first step of this method. Organic nitrogen was converted to ammonium sulphate by sulphuric acid and digestion accelerators in this step (Catalyst Mixture) at a temperature of 360⁰C-440° C.



- B. Distillation: The solution was made alkaline from the distillation of ammonia in this step. The distilled ammonia was received in boric acid solution.



- C. Titration: To determine the amount of NH₃, ammonium borate was titrated with standard sulfuric acid solution.



Reagents: 4% Boric Acid solution, Mixed indicator (Bromocresol green and Methyl red), 40% Sodium Hydroxide solution, Standard Sulphuric Acid solution 0.05 N and 0.05 N Na₂CO₃ solution.

Procedure: About 1.0g of oven dried sample was weighed with the help of electrical balance and then taken into a 250 mL Kjeldahl flask. Then 5g catalysts mixer (K₂SO₄:CuSO₄.5H₂O: Se=100:1:1) was added in to flask. After that about 25mL concentrated H₂SO₄ was also added o the flask. The flask was heated until the solution become clear and then allowed to cool at room temperature and then about 120 mL of distilled water was added and 5-6 glass bead into the flask. After digestion, 40% NaOH 125mL was added to the conical flask and attached quickly to the distillation set. Then the flask was heated continuously. In the meantime, 25mL of 4% boric acid solution and 2-4 drops of mixed indicator was taken in a 500mL receiver conical flask. After distillation, about 150ml distillate was collected into receiver conical flask. The distillate was then titrated with standard H₂SO₄ taken from a burette until the green color completely turns to pink color at the end point. The same

procedure was followed for a blank sample. The result was calculated using the following formula-

$$\%N = (T - B) \times N \times 1.4 / S$$

Where, T= Titration value for sample (mL), B= Titration value for blank (mL), N= Normality of H₂SO₄ (N), S= Weight of the sample (g), 1.4= conversion factor

3.18.4 Determination of Phosphorus

The amount of Phosphorus (P) was estimated from the plant extract by ascorbic acid blue color method with the help of a Spectrophotometer at 660 nm.

Reagents required

- A. Mixed reagent: 12.0 g ammonium molybdate (NH₄)₆Mo₇O₂₄·4H₂O was dissolved in 250 mL distilled water. About 0.2908 g antimony potassium tartarate K₂Sb₂(C₄H₂O₆)₂·3H₂O was dissolved in 1000 mL H₂SO₄. Two solutions were mixed together and volume was made up to 2000 mL with distilled water and stored in a pyrex bottle in a dark cool secure place.
- B. Color developing reagent: 0.53 g ascorbic acid was added to 100 mL of the mixed reagent.
- C. Standard Phosphorus solution (100 ppm): 0.439 g potassium dihydrogen phosphate (KH₂PO₄) was weighed into a 1L volumetric flask. About 500 mL distilled water was added and shaken the contents until the salt dissolved. Then the volume was made up to 1L with distilled water.

Procedure

- A. Color development: About 20 mL of the extract was pipetted out in a 100 mL volumetric flask. About 20 mL color developing reagent was added slowly and

carefully to prevent the loss of sample because of excessive foaming. After the evolution of CO₂ had ceased, the flask was shaken gently to mix the contents. The volume was made up to the mark with distilled water.

- B. Preparation of working standard P solution: About 20 mL of the standard P solutions (100 ppm) was pipetted to a 1L volumetric flask and volume was made up to the mark by distilled water. This solution contained 2 ppm P. About 0, 5, 10, 15, 20 and 25 mL aliquot were pipetted out from 2 ppm solution in 100 mL volumetric flask respectively. About 20 mL color developing reagent was added to each flask, mixed and volume was made with distilled water. These solutions gave 0, 0.1, 0.2, 0.3, 0.4 and 0.5 ppm of P solution respectively. The solution was allowed to stand for 15 minutes and then color intensity (% absorbance) was measured at 660 nm. A standard curve was prepared from the spectrophotometer reading and concentrations of plant samples were calculated from the curve.

3.18.5 Determination of Potassium

The amount of Potassium (K) in the plant extract was determined with the help of a Flame photometer.

Preparation of primary potassium standard solution (1000 ppm): 1.918 g potassium chloride was taken in a 1L volumetric flask. About 200-300 mL distilled water was added and the flask was shaken properly until a clear solution was obtained. The volume was made up to the mark with distilled water. Thus, 1000 ppm K solution was prepared

Preparation of secondary potassium solution (100 ppm and 10 ppm): About 10 mL of the 1000 ppm K solution was taken in a 100 mL volumetric flask. The volume was made up to mark with distilled water and shaken properly. In this way, 100 ppm K solution was prepared. From 100 ppm solution, 10 mL was taken in a 100 mL volumetric flask. The volume was made up to the mark with distilled water and shaken properly. Thus, 10 ppm solution was obtained.

Preparation of potassium standard series solution: A series of standard solution containing 1 ppm, 2 ppm, 3 ppm, 4 ppm, 5 ppm and 6 ppm were prepared by pipetting 10 mL, 20 mL, 30 mL, 40 mL, 50 mL and 60 mL of 10 ppm K solution in six different 100 mL volumetric flask respectively. The volume was made up to the mark by distilled water and

shaked properly. Then, the reading (% emission) were taken from flame emission spectrophotometer and a standard curve was prepared from the reading taken. Plant samples were taken in volumetric flask and volume was made up to the mark by distilled water. Then the samples reading were taken and concentrations were calculated from the standard curve.

3.19 Statistical Analysis

The data obtained for different characters were statistically analyzed by using “Statistics 10” computer package program to find out the significance of the difference for arsenic stress effects on yield and yield contributing characters of BRR1 dhan29. The mean differences among the treatments were adjusted by Duncan’s Multiple Range Test (DMRT) test at 5% level of significance.

CHAPTER IV

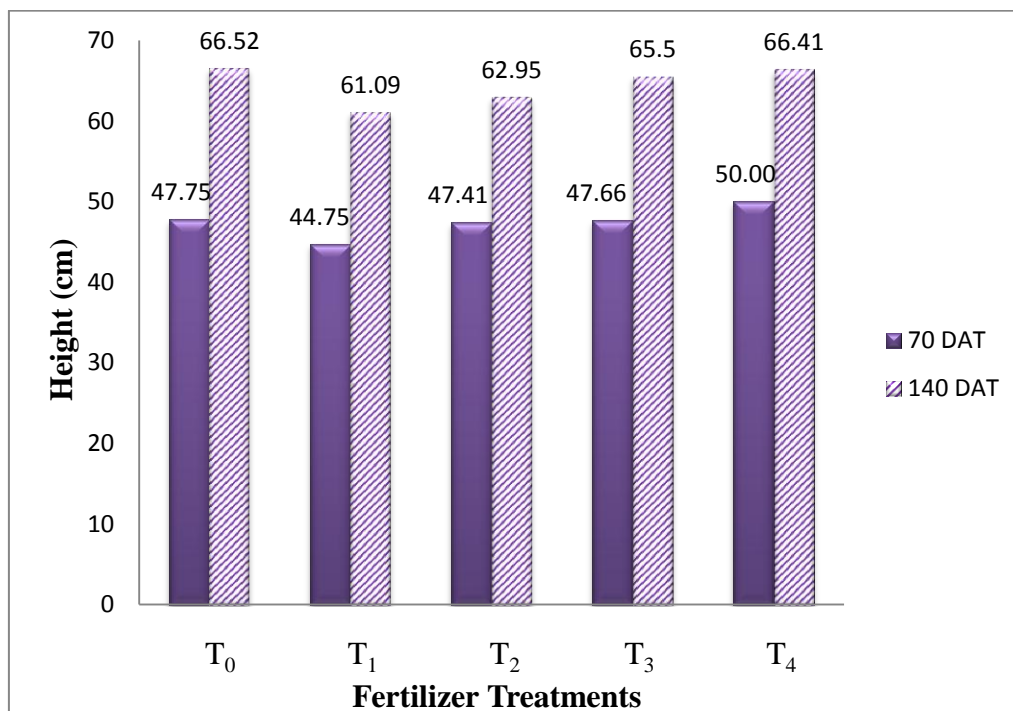
RESULTS AND DISCUSSION

A study was undertaken during the Boro season of December-June (2017-18) to evaluate the mitigation of arsenic stress in BRR1 dhan29 with cowdung and inorganic fertilizers. The results of the study regarding the effect of arsenic stress in BRR1 dhan29 under cowdung and inorganic fertilizers has been presented with the help of table, graphs and possible interpretations in the following headings:

4.1 Plant Height

Effect of fertilizer

The plant height (cm) of BRR1 dhan29 was significantly affected by the different doses of fertilizers (cowdung and inorganic) at 70 and 140 days after transplanting (DAT) (Figure 1 and Appendix IV). The result revealed that at 70 DAT, the treatment T₄ produced the tallest



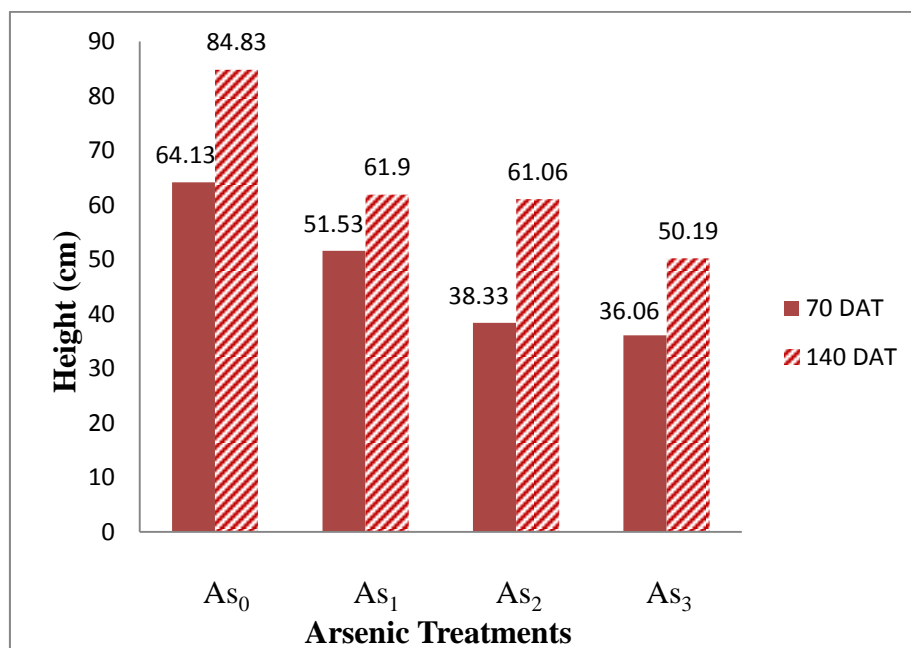
T₀ = Recommended doses of cowdung + inorganic fertilizers, T₁ = Recommended dose of cowdung without inorganic fertilizers, T₂ = Recommended dose of inorganic fertilizers without cowdung, T₃ = Reduction of 20% recommended dose of inorganic fertilizers + addition of 20% recommended dose of cowdung, T₄ = Reduction of 40% recommended dose of inorganic fertilizers + addition of 40% recommended dose of cowdung

Figure 1. Effect of different doses of fertilizers (cowdung and inorganic) on plant height at different days after transplanting

plant (50.00 cm) and at 140 DAT, the treatment T₀ produced the tallest plant (66.52 cm). At 70 and 140 DAT the treatment T₁ produced the shortest plant (44.75 cm, 61.09 cm respectively). Kobayashi *et al.* (1989) reported that the doses of organic manure had positive effect on plant height.

Effect of arsenic

Different levels of arsenic application showed distinct negative effect on the plant height of BRRI dhan29 at 70 and 140 DAT (Figure 2 and Appendix V). The result revealed that at 70 and 140 DAT, the highest plant height (64.13 cm and 84.83 cm respectively) were recorded from the treatment As₀ and, the lowest height (36.06 cm and 50.19 cm respectively) were recorded from the treatment As₃. Holmgren *et al.* (1993) and Das *et al.* (1997) stated that all the growth parameters in their experiment viz. plant height of rice plants were affected by the application of arsenic.



As₀ = No As applied, As₁= 20 ppm As, As₂= 40 ppm As, As₃= 60 ppm As on required water basis

Figure 2. Effect of different doses of arsenic on plant height at different days after transplanting

Interaction effect of fertilizer and arsenic

Interaction of different doses of fertilizers (cowdung and inorganic) and arsenic showed significant variation on plant height of BRRI dhan29 at 70 and 140 DAT (Table 1). At 70

DAT, the highest plant height (70.00 cm) was observed from the As₀T₄ treatment which was statistically similar with As₀T₃ (68.67 cm) whereas, the lowest plant height (31.66 cm) was observed from As₃T₁ treatment. At 140 DAT, the highest plant height (90.50 cm) was observed from the As₀T₄ and As₀T₃ treatment which was statistically similar with As₀T₀ (89.16 cm and 87.00 cm) whereas, the lowest plant height (47.76 cm) was observed from the treatment As₃T₃ which was statistically similar with As₃T₁ (48.30 cm). Hossain *et al.* (2008) found that plant height significantly varied with different concentrations of arsenic.

Table 1: Interaction effect of different doses of arsenic and different doses of fertilizers (cowdung and inorganic) on plant height at different days after transplanting

Treatments		Plant Height (cm)	
		70 DAT	140 DAT
As ₀	T ₀	63.00 ab	89.16 a
	T ₁	59.33 bc	75.66 b
	T ₂	59.68 bc	81.83 ab
	T ₃	68.67 a	87.00 a
	T ₄	70.00 a	90.50 a
As ₁	T ₀	59.33 bc	60.43 cd
	T ₁	48.00 de	57.93 cd
	T ₂	47.33 de	63.80 c
	T ₃	49.68 d	64.83 c
	T ₄	53.33 cd	62.53 c
As ₂	T ₀	34.00 gh	63.56 c
	T ₁	40.00 fg	62.46 c
	T ₂	41.66 ef	57.40 cde
	T ₃	35.00 fgh	62.43 c
	T ₄	41.00 efg	59.46 cd
As ₃	T ₀	34.66 fgh	52.93 def
	T ₁	31.66 h	48.30 f
	T ₂	41.00 efg	48.80 ef
	T ₃	37.33 fgh	47.76 f
	T ₄	35.66 fgh	53.16 def
S.E. (±)		1.9061	2.3570
CV (%)		4.91	4.48
Significant level		*	*

* - Significant at 5% level

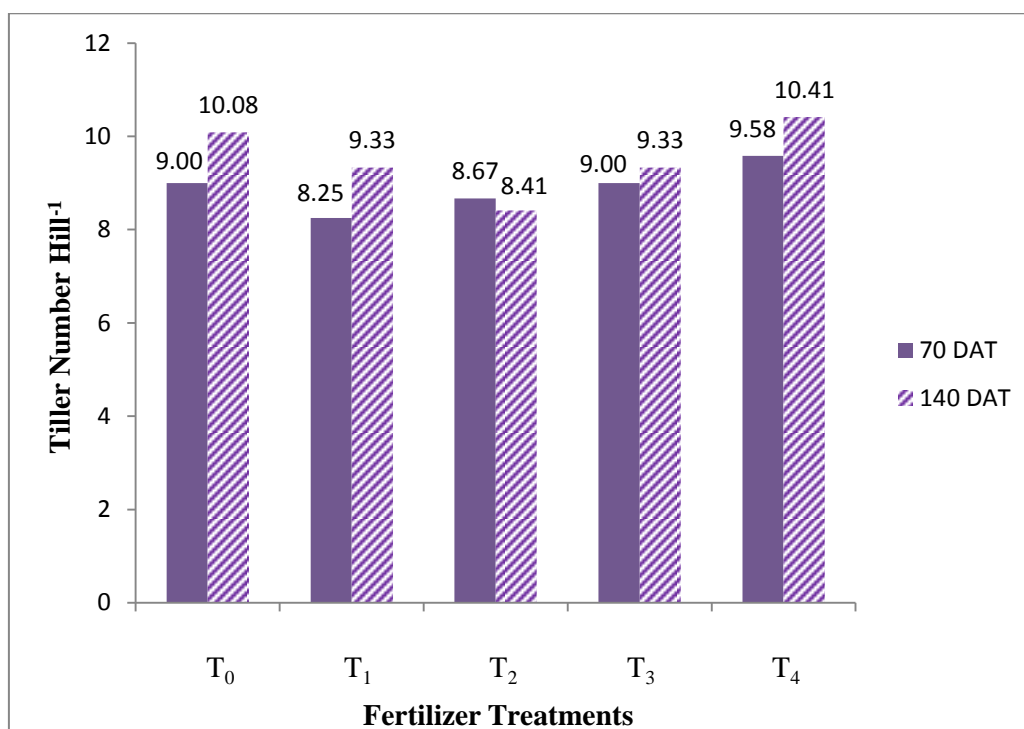
As₀ = No As applied, As₁= 20 ppm As, As₂= 40 ppm As, As₃= 60 ppm As on required water basis

T₀ = Recommended doses of cowdung + inorganic fertilizers, T₁ = Recommended dose of cowdung without inorganic fertilizers, T₂ = Recommended dose of inorganic fertilizers without cowdung, T₃ = Reduction of 20% recommended dose of inorganic fertilizers + addition of 20% recommended dose of cowdung, T₄ = Reduction of 40% recommended dose of inorganic fertilizers + addition of 40% recommended dose of cowdung

4.2 Number of tillers hill⁻¹

Effect of fertilizer

The number of tillers hill⁻¹ of BRR1 dhan29 was significantly affected by different doses of fertilizers (cowdung and inorganic fertilizers) at 70 and 140 DAT (Figure 3 and Appendix IV). The results revealed that at 70 and 140 DAT, the treatment T₄ produced the highest number of tillers hill⁻¹ (9.58 and 10.41 respectively). At 70 DAT the treatment T₁ produced the lowest number of tillers (8.25) hill⁻¹ and at 140 DAT the treatment T₂ produced the lowest number of tillers hill⁻¹ which was 8.41. This confirms the reports of Nayak *et al.* (2007) that a significant increase in number of tillers hill⁻¹ of rice plants due to the application of organic manure with chemical fertilizers.

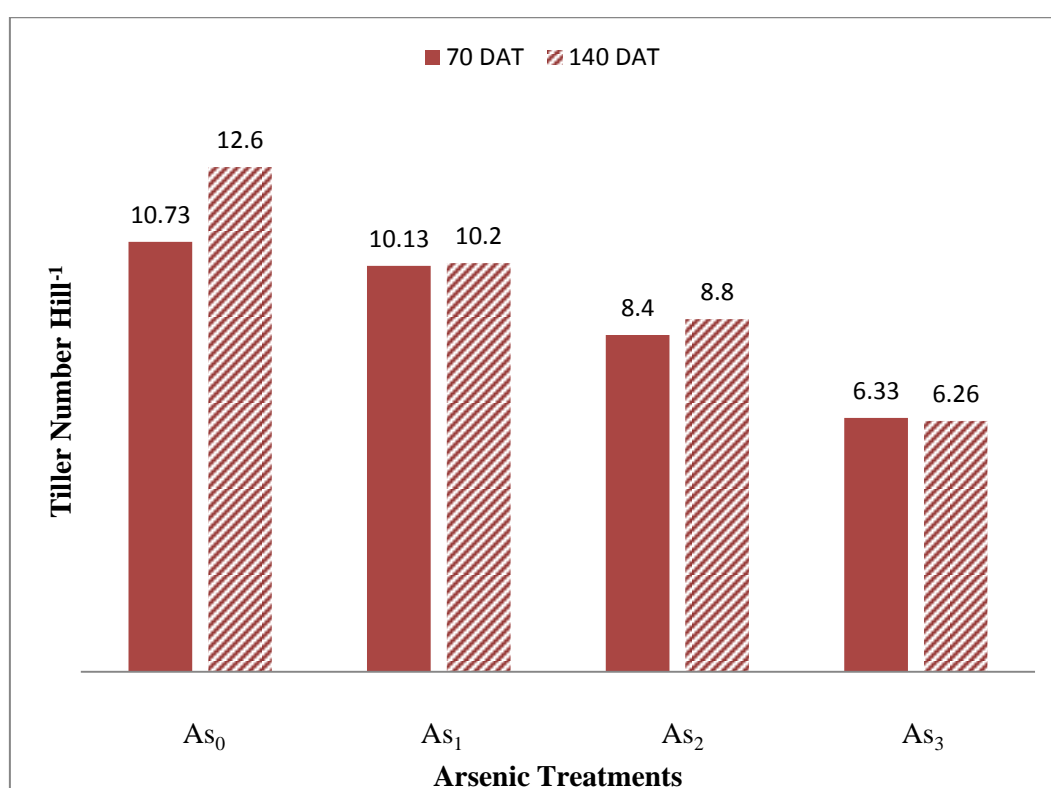


T₀ = Recommended doses of cowdung + inorganic fertilizers, T₁ = Recommended dose of cowdung without inorganic fertilizers, T₂ = Recommended dose of inorganic fertilizers without cowdung, T₃ = Reduction of 20% recommended dose of inorganic fertilizers + addition of 20% recommended dose of cowdung, T₄ = Reduction of 40% recommended dose of inorganic fertilizers + addition of 40% recommended dose of cowdung

Figure 3. Effect of different doses of fertilizers (cowdung and inorganic) on number of tillers hill⁻¹ at different days after transplanting

Effect of arsenic

Different doses of arsenic had significant effect on number of tillers hill⁻¹ of BRRI dhan29 at 70 and 140 DAT (Figure 4 and Appendix V). At 70 and 140 DAT, the highest number of tillers hill⁻¹ (10.73 and 12.60 respectively) were observed from the As₀ treatment and the lowest number of tillers hill⁻¹ (6.33 and 6.26 respectively) were observed from the As₃ treatment. Holmgren et al. (1993) and Das et al. (1997) reported that all the growth parameters tested in their experiment viz. tiller numbers of rice plants were affected by the application of arsenic.



As₀ = No As applied, As₁ = 20 ppm As, As₂ = 40 ppm As, As₃ = 60 ppm As on required water basis

Figure 4. Effect of different doses of arsenic on number of tillers hill⁻¹ at different days after transplanting

Interaction effect of fertilizer and arsenic

Interaction of different doses of fertilizers (cowdung and inorganic) and different doses of arsenic showed significant variation on number of tillers hill⁻¹ of BRRI dhan29 at 70 and 140 DAT (Table 2). At 70 DAT, the highest number of tillers hill⁻¹ (12.33) was observed from the

As₀T₀ treatment which was statistically similar with As₀T₄ (12.00) and As₀T₃ (11.00) and the lowest number of tillers hill⁻¹ (5.33) was observed from the As₃T₁ treatment which was statistically similar with As₃T₀ (5.34). At 140 DAT, the highest number of tillers hill⁻¹ (14.00) was observed from the As₀T₀ treatment which was statistically similar with As₀T₁ (13.66) and As₀T₄ (13.33) and the lowest number of tillers hill⁻¹ (5.33) was observed from As₃T₁ treatment which was statistically similar with As₃T₂ (5.66).

Table 2: Interaction effect of different doses of arsenic and different doses of fertilizers (cowdung and inorganic) on number of tillers hill⁻¹ at different days after transplanting

Treatments		Number of tillers hill ⁻¹	
		70 DAT	140 DAT
As ₀	T ₀	12.33 a	14.00 a
	T ₁	9.00 a-e	13.66 a
	T ₂	9.33 a-e	10.00 a-e
	T ₃	11.00 abc	12.00 abc
	T ₄	12.00 ab	13.33 ab
As ₁	T ₀	10.66 abc	10.66 a-d
	T ₁	10.00 a-d	9.00 b-f
	T ₂	9.33 a-e	9.66 a-f
	T ₃	10.33 a-d	10.00 a-e
	T ₄	10.33 a-d	11.66 abc
As ₂	T ₀	7.66 b-e	9.00 b-f
	T ₁	8.66 a-e	8.33 c-f
	T ₂	8.66 a-e	8.33 c-f
	T ₃	7.00 cde	8.66 c-f
	T ₄	10.00 a-d	9.66 a-f
As ₃	T ₀	5.34 e	6.66 def
	T ₁	5.33 e	5.33 f
	T ₂	7.33 cde	5.66 ef
	T ₃	7.66 b-e	6.66 def
	T ₄	6.00 de	7.00 def
S.E. (±)		1.2019	1.1450
CV (%)		16.54	14.81
Significant level		*	*

* - Significant at 5% level

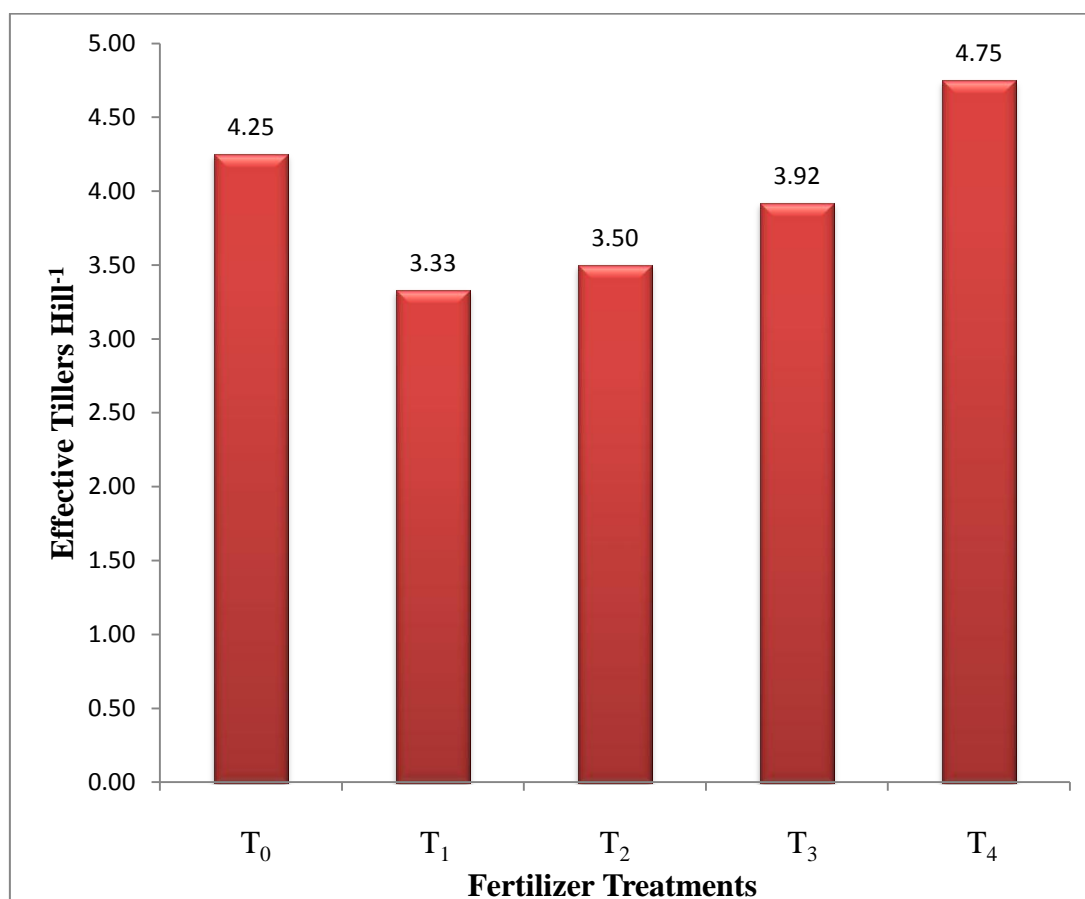
As₀ = No As applied, As₁ = 20 ppm As, As₂ = 40 ppm As, As₃ = 60 ppm As on required water basis

T₀ = Recommended doses of cowdung + inorganic fertilizers, T₁ = Recommended dose of cowdung without inorganic fertilizers, T₂ = Recommended dose of inorganic fertilizers without cowdung, T₃ = Reduction of 20% recommended dose of inorganic fertilizers + addition of 20% recommended dose of cowdung, T₄ = Reduction of 40% recommended dose of inorganic fertilizers + addition of 40% recommended dose of cowdung

4.3 Number of effective tillers hill⁻¹

Effect of fertilizer

The number of effective tillers hill⁻¹ of BRR1 dhan29 were significantly affected by different doses of fertilizers (cowdung and inorganic) (Figure 5 and Appendix VI). The results revealed that the treatment T₄ produced the highest number of effective tillers hill⁻¹ (4.75) and the treatment T₁ produced the lowest number of effective tillers hill⁻¹ (3.33). Nayak *et al.* (2007) reported that a significant increase in number of effective tillers hill⁻¹ of rice plants due to the application of organic manure with chemical fertilizers.

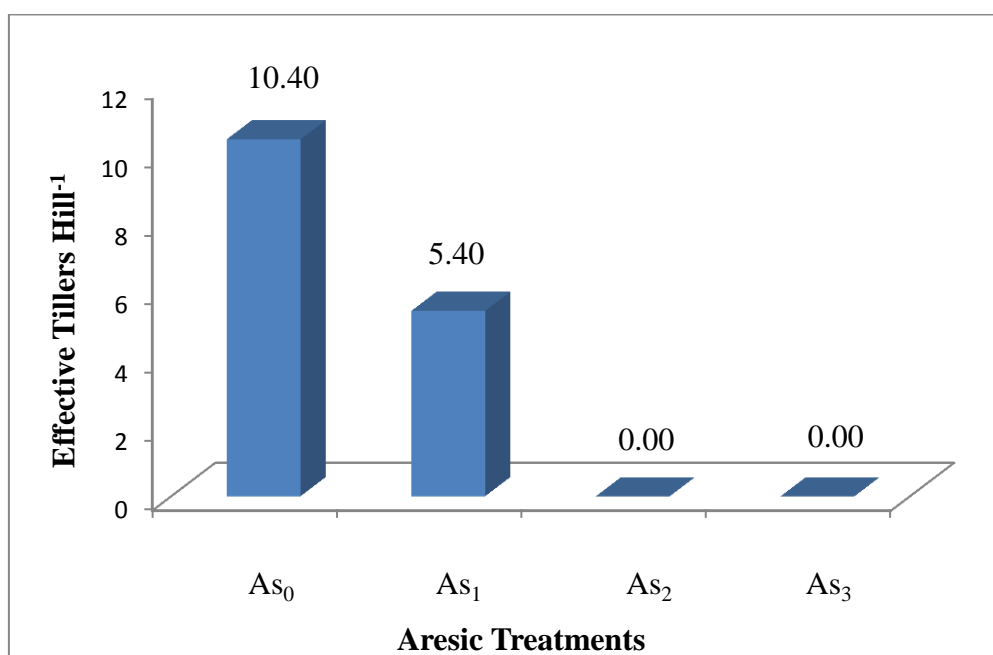


T₀ = Recommended doses of cowdung + inorganic fertilizers, T₁ = Recommended dose of cowdung without inorganic fertilizers, T₂ = Recommended dose of inorganic fertilizers without cowdung, T₃ = Reduction of 20% recommended dose of inorganic fertilizers + addition of 20% recommended dose of cowdung, T₄ = Reduction of 40% recommended dose of inorganic fertilizers + addition of 40% recommended dose of cowdung

Figure 5: Effect of different doses of fertilizers (cowdung and inorganic) on number of effective tillers hill⁻¹

Effect of arsenic

Different doses of arsenic had significant effect on the number of effective tillers hill⁻¹ of BRR1 dhan29 (Figure 6 and Appendix VII). The highest number of effective tillers hill⁻¹ (10.40) was observed from the As₀ treatment and the lowest (0.00) was observed from As₂ and As₃ treatment because arsenic is a poisonous and toxic heavy metal which exerts hampering and hindering effect on plant physiology. Kang *et al.* (1996) reported that when the level of arsenic increased, the number of effective tillers hill⁻¹ decreased.



As₀ = No As applied, As₁ = 20 ppm As, As₂ = 40 ppm As, As₃ = 60 ppm As on required water basis

Figure 6: Effect of different doses of arsenic on number of effective tillers hill⁻¹

Interaction effect of fertilizer and arsenic

Interaction of different doses of fertilizers (cowdung and inorganic) and different doses of arsenic showed significant variation on number of effective tillers hill⁻¹ of BRR1 dhan29 (Table 3). The highest number of effective tillers hill⁻¹ (12.00) was observed from the As₀T₄ treatment which was statistically similar with As₀T₀ (11.67). There is no effective tiller at 40 ppm and 60 ppm As treatments.

Table 3: Interaction effect of different doses of arsenic and different doses of fertilizers (cowdung and inorganic) on number of effective tillers hill⁻¹

Treatments		Number of effective tillers hill ⁻¹
As ₀	T ₀	11.67 a
	T ₁	9.00 bc
	T ₂	9.00 bc
	T ₃	10.33 ab
	T ₄	12.00 a
As ₁	T ₀	5.33 de
	T ₁	4.33 e
	T ₂	5.00 de
	T ₃	5.33 de
	T ₄	7.00 cd
As ₂	T ₀	0.00 f
	T ₁	0.00 f
	T ₂	0.00 f
	T ₃	0.00 f
	T ₄	0.00 f
As ₃	T ₀	0.00 f
	T ₁	0.00 f
	T ₂	0.00 f
	T ₃	0.00 f
	T ₄	0.00 f
S.E. (±)		0.5779
CV (%)		17.92
Significant level		*

* - Significant at 5% level

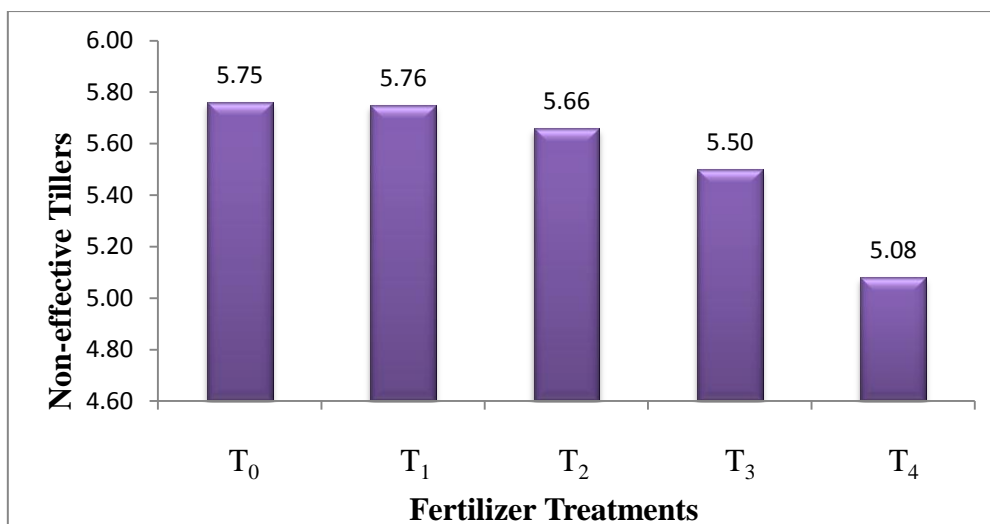
As₀ = No As applied, As₁= 20 ppm As, As₂= 40 ppm As, As₃= 60 ppm As on required water basis

T₀ = Recommended doses of cowdung + inorganic fertilizers, T₁ = Recommended dose of cowdung without inorganic fertilizers, T₂ = Recommended dose of inorganic fertilizers without cowdung, T₃ = Reduction of 20% recommended dose of inorganic fertilizers + addition of 20% recommended dose of cowdung, T₄ = Reduction of 40% recommended dose of inorganic fertilizers + addition of 40% recommended dose of cowdung

4.4 Number of non-effective tillers hill⁻¹

Effect of fertilizer

The number of non-effective tillers hill⁻¹ of BRR1 dhan29 were significantly affected by different doses of fertilizers (cowdung and inorganic) (Figure 7 and Appendix VI). The results revealed that the treatment T₁ produced the highest number of non-effective tillers per hill (5.76) and the treatment T₄ produced the lowest number of non-effective tillers (5.08).

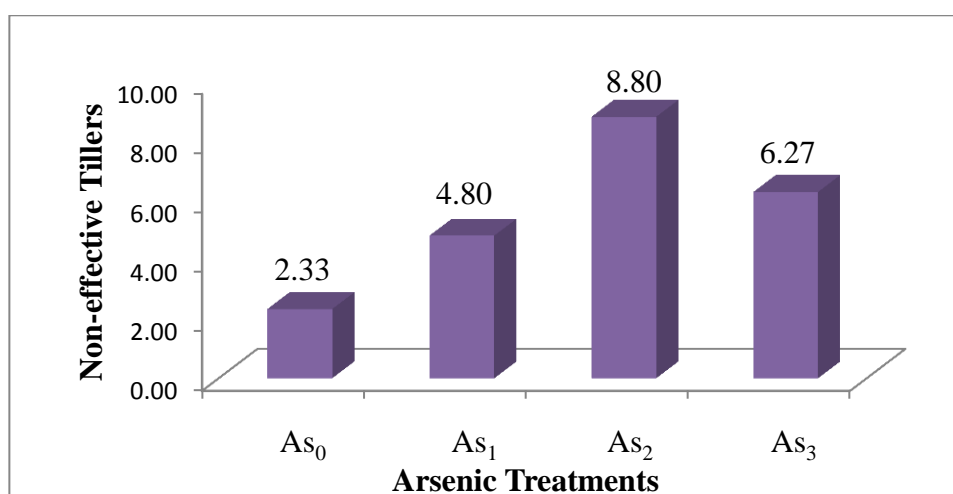


T₀ = Recommended doses of cowdung + inorganic fertilizers, T₁ = Recommended dose of cowdung without inorganic fertilizers, T₂ = Recommended dose of inorganic fertilizers without cowdung, T₃ = Reduction of 20% recommended dose of inorganic fertilizers + addition of 20% recommended dose of cowdung, T₄ = Reduction of 40% recommended dose of inorganic fertilizers + addition of 40% recommended dose of cowdung

Figure 7: Effect of different doses of fertilizers (cowdung and inorganic) on number of non-effective tillers hill⁻¹

Effect of arsenic

Number of non-effective tillers hill⁻¹ was significantly varied due to arsenic doses at all growth stages of rice plant (Figure 8 and Appendix VII). The highest number of non-effective tillers hill⁻¹ (8.80) was recorded from As₂ treatment because in the treatment As₂ total tillers number was more than As₃ but all tillers were non-effective. The lowest number of non-effective tillers hill⁻¹ (2.33) was recorded from As₀ treatment.



As₀ = No As applied, As₁ = 20 ppm As, As₂ = 40 ppm As, As₃ = 60 ppm As on required water basis

Figure 8: Effect of different doses of arsenic on number of non-effective tillers hill⁻¹

Interaction effect of fertilizer and arsenic

Interaction of different doses of fertilizers (cowdung and inorganic) and different doses of arsenic showed significant variation on number of effective tillers hill⁻¹ of BRR1 dhan29 (Table 4). The highest number of non-effective tillers hill⁻¹ (9.66) was recorded from As₂T₄ which was statistically similar with As₂T₀ (9.00) whereas, the lowest number of non-effective tillers hill⁻¹ (1.33) was recorded from As₀T₄ which was statistically similar with As₀T₃ (1.66) and As₀T₂ (1.67).

Table 4: Interaction effect of different doses of arsenic and different doses of fertilizers (cowdung and inorganic) on number of non-effective tillers hill⁻¹

Treatments		Number of non-effective tillers hill ⁻¹
As ₀	T ₀	2.33 ef
	T ₁	4.66 de
	T ₂	1.67 f
	T ₃	1.66 f
	T ₄	1.33 f
As ₁	T ₀	5.00 de
	T ₁	4.66 de
	T ₂	4.66 de
	T ₃	5.00 de
	T ₄	4.66 de
As ₂	T ₀	9.00 ab
	T ₁	8.33 abc
	T ₂	8.33 abc
	T ₃	8.66 ab
	T ₄	9.66 a
As ₃	T ₀	6.66 bcd
	T ₁	5.33 d
	T ₂	5.66 cd
	T ₃	6.66 bcd
	T ₄	7.00 a-d
S.E. (±)		0.7810
CV (%)		17.23
Significant level		*

* - Significant at 5% level

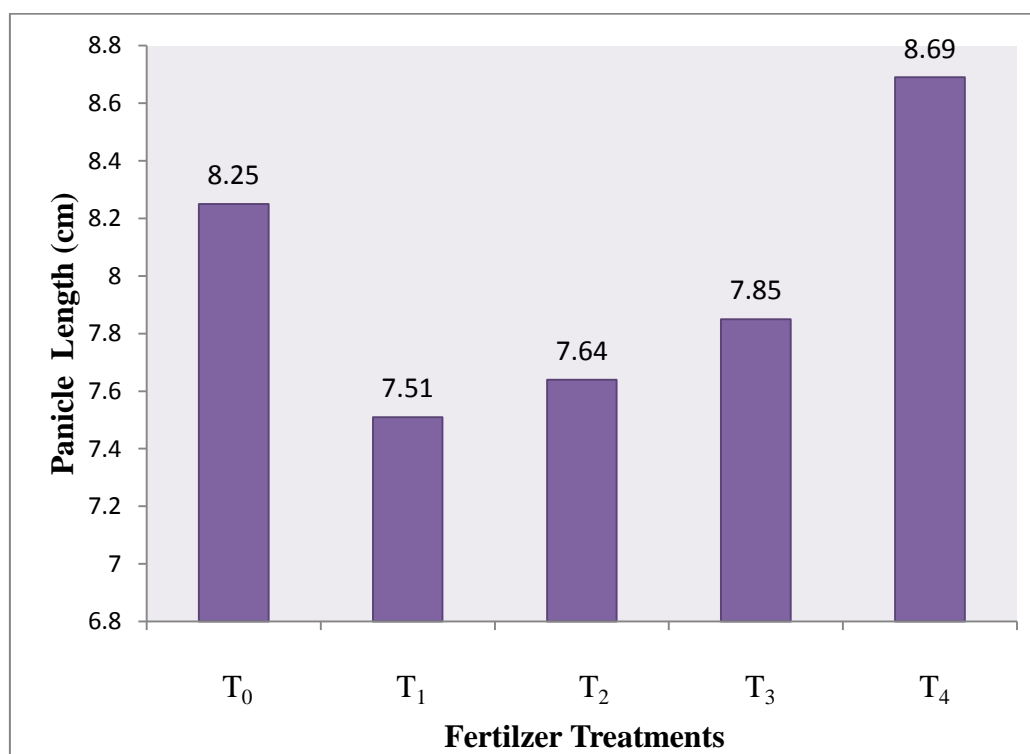
As₀ = No As applied, As₁= 20 ppm As, As₂= 40 ppm As, As₃= 60 ppm As on required water basis

T₀ = Recommended doses of cowdung + inorganic fertilizers, T₁ = Recommended dose of cowdung without inorganic fertilizers, T₂ = Recommended dose of inorganic fertilizers without cowdung, T₃ = Reduction of 20% recommended dose of inorganic fertilizers + addition of 20% recommended dose of cowdung, T₄ = Reduction of 40% recommended dose of inorganic fertilizers + addition of 40% recommended dose of cowdung

4.5 Panicle length (cm)

Effect of fertilizer

The panicle length of BRR1 dhan29 was significantly affected by different doses of fertilizers (cowdung and inorganic) (Figure 9 and Appendix VI). The results revealed that the treatment T₄ showed the highest panicle length (8.69 cm) which was statistically similar with T₀ (8.25 cm) and the lowest panicle length (7.51 cm) was recorded from the treatment T₁ which was statistically similar with T₂ (7.64 cm).

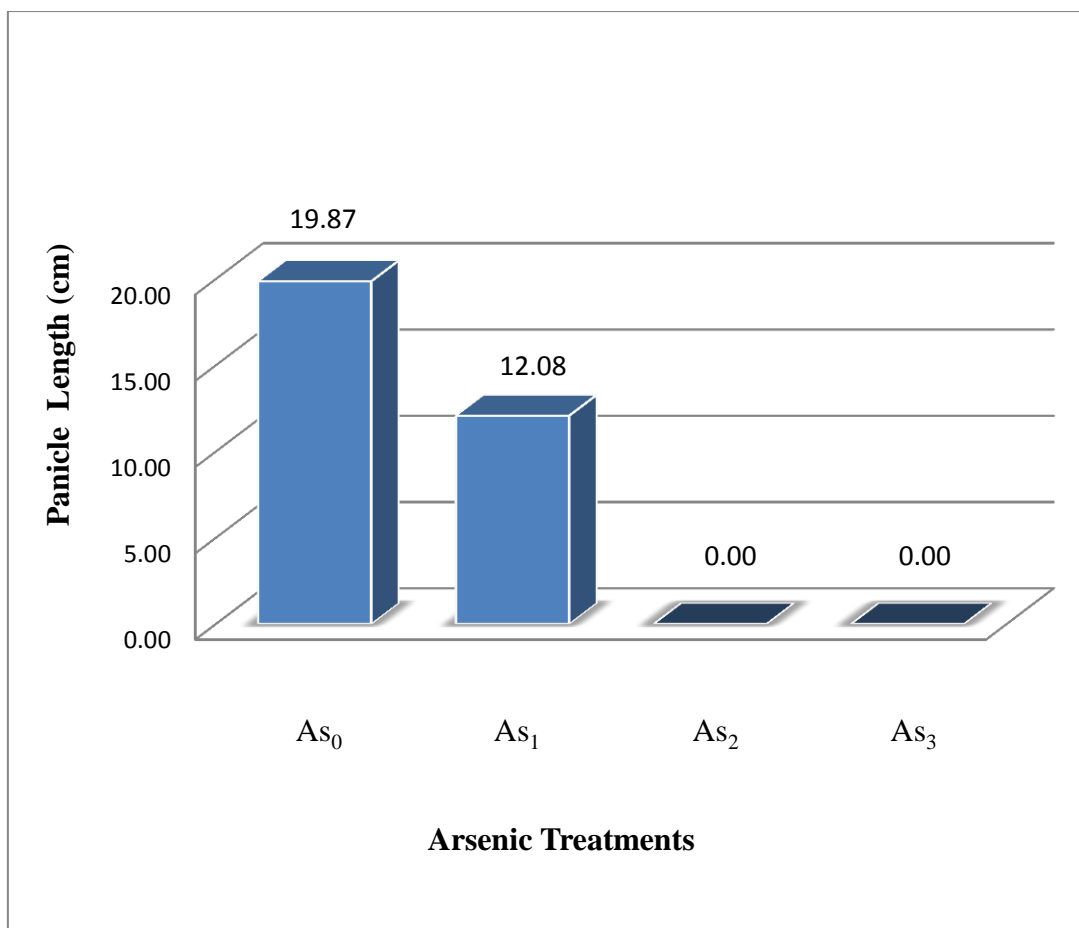


T₀ = Recommended doses of cowdung + inorganic fertilizers, T₁ = Recommended dose of cowdung without inorganic fertilizers, T₂ = Recommended dose of inorganic fertilizers without cowdung, T₃ = Reduction of 20% recommended dose of inorganic fertilizers + addition of 20% recommended dose of cowdung, T₄ = Reduction of 40% recommended dose of inorganic fertilizers + addition of 40% recommended dose of cowdung

Figure 9: Effect of different doses of fertilizers (cowdung and inorganic) on panicle length (cm)

Effect of arsenic

Different doses of arsenic had significant effect on the panicle length (Figure 10 and Appendix VII). The highest panicle length (19.87 cm) was observed in As₀ treatment and no panicle was observed in both As₂ and As₃ treatments, because the treatments As₂ and As₃ did not produce any panicle.



As₀ = No As applied, As₁= 20 ppm As, As₂= 40 ppm As, As₃= 60 ppm As on required water basis

Figure 10: Effect of different doses of arsenic on panicle length (cm)

Interaction effect of fertilizer and arsenic

Interaction of different doses of fertilizers (cowdung and inorganic) and different doses of arsenic showed significant effect on panicle length (Table 5). The highest panicle length (21.69 cm) was recorded from As₀T₄. The lowest panicle length (11.26 cm) showed in As₁T₁ treatment. The arsenic treatments 40 ppm and 60 ppm (As₂ and As₃) did not produce any panicle.

Table 5: Interaction effect of different doses of arsenic and different doses of fertilizers (cowdung and inorganic) on panicle length (cm)

Treatments		Panicle Length (cm)
As ₀	T ₀	20.52 b
	T ₁	18.77 c
	T ₂	19.04 c
	T ₃	19.32 c
	T ₄	21.69 a
As ₁	T ₀	12.48 de
	T ₁	11.26 f
	T ₂	11.52 ef
	T ₃	12.09 def
	T ₄	13.06 d
As ₂	T ₀	0.00 g
	T ₁	0.00 g
	T ₂	0.00 g
	T ₃	0.00 g
	T ₄	0.00 g
As ₃	T ₀	0.00 g
	T ₁	0.00 g
	T ₂	0.00 g
	T ₃	0.00 g
	T ₄	0.00 g
S.E. (±)		0.2637
CV (%)		4.04
Significant level		*

* - Significant at 5% level

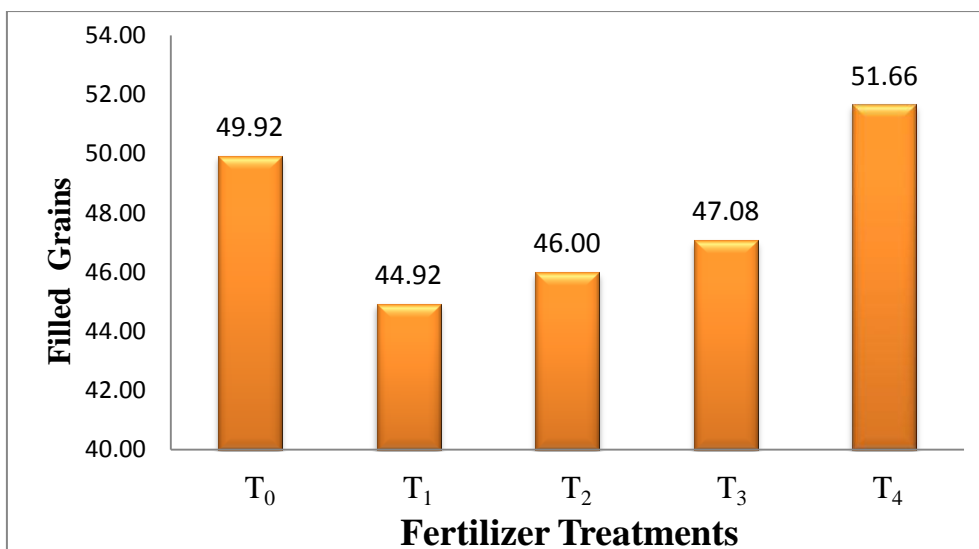
As₀ = No As applied, As₁= 20 ppm As, As₂= 40 ppm As, As₃= 60 ppm As on required water basis

T₀ = Recommended doses of cowdung + inorganic fertilizers, T₁ = Recommended dose of cowdung without inorganic fertilizers, T₂ = Recommended dose of inorganic fertilizers without cowdung, T₃ = Reduction of 20% recommended dose of inorganic fertilizers + addition of 20% recommended dose of cowdung, T₄ = Reduction of 40% recommended dose of inorganic fertilizers + addition of 40% recommended dose of cowdung

4.6 Number of filled grains panicle⁻¹

Effect of fertilizer

The number of filled grains panicle⁻¹ of BRRI dhan29 were significantly affected by different doses of fertilizers (cowdung and inorganic) (Figure 11 and Appendix VI). The results revealed that the treatment T₄ produced the highest number of filled grains (51.66) which was statistically similar with T₀ (49.92) and the lowest (44.92) was recorded from the treatment T₁.

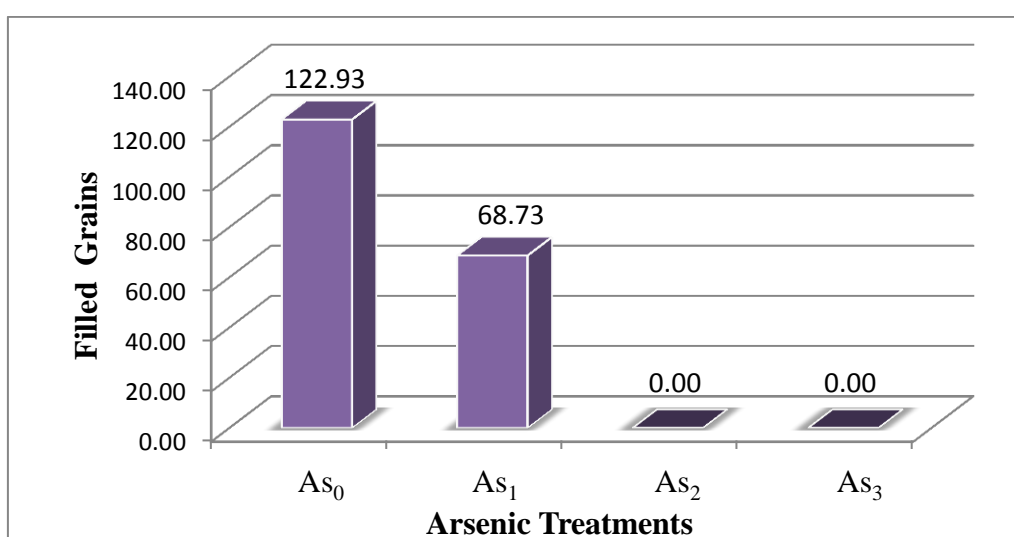


T₀ = Recommended doses of cowdung + inorganic fertilizers, T₁ = Recommended dose of cowdung without inorganic fertilizers, T₂ = Recommended dose of inorganic fertilizers without cowdung, T₃ = Reduction of 20% recommended dose of inorganic fertilizers + addition of 20% recommended dose of cowdung, T₄ = Reduction of 40% recommended dose of inorganic fertilizers + addition of 40% recommended dose of cowdung

Figure 11: Effect of different doses of fertilizers (cowdung and inorganic) on number of filled grains panicle⁻¹

Effect of arsenic

Different doses of arsenic had significantly affected the number of filled grains panicle⁻¹ (Figure 12 and Appendix VII). The highest number of filled grains (122.93) was observed in As₀ treatment and the lowest (0.00) was observed for both As₂ and As₃ treatment. The number of filled grains panicle⁻¹ is a growth contributing character, increase in the number of



As₀ = No As applied, As₁ = 20 ppm As, As₂ = 40 ppm As, As₃ = 60 ppm As on required water basis

Figure 12: Effect of different doses of arsenic on number of filled grains panicle⁻¹

filled grains panicle⁻¹ increase the yield. The yield of rice grain was highly affected by arsenic treatments. Similarly, Islam *et al.* (2004) reported that the irrigation water added arsenic up to 0.25 ppm enhanced unfilled grains panicle⁻¹ and finally the grain yield of Boro rice and the further doses of arsenic depressed the plant growth, yield and yield components.

Interaction effect of fertilizer and arsenic

Interaction of different doses of fertilizers (cowdung and inorganic) and different doses of arsenic showed significant effect on number of filled grains panicle⁻¹. In respect of the

Table 6: Interaction effect of different doses of arsenic and different doses of fertilizers (cowdung and inorganic) on number of filled grains panicle⁻¹

Treatments		Number of filled grains panicle ⁻¹
As ₀	T ₀	129.33 a
	T ₁	115.67 b
	T ₂	116.33 b
	T ₃	119.33 b
	T ₄	134.00 a
As ₁	T ₀	70.33 c
	T ₁	64.00 d
	T ₂	67.66 cd
	T ₃	69.00 c
	T ₄	72.66 c
As ₂	T ₀	0.00 e
	T ₁	0.00 e
	T ₂	0.00 e
	T ₃	0.00 e
	T ₄	0.00 e
As ₃	T ₀	0.00 e
	T ₁	0.00 e
	T ₂	0.00 e
	T ₃	0.00 e
	T ₄	0.00 e
S.E. (±)		1.5612
CV (%)		3.99
Significant level		*

* - Significant at 5% level

As₀ = No As applied, As₁ = 20 ppm As, As₂ = 40 ppm As, As₃ = 60 ppm As on required water basis

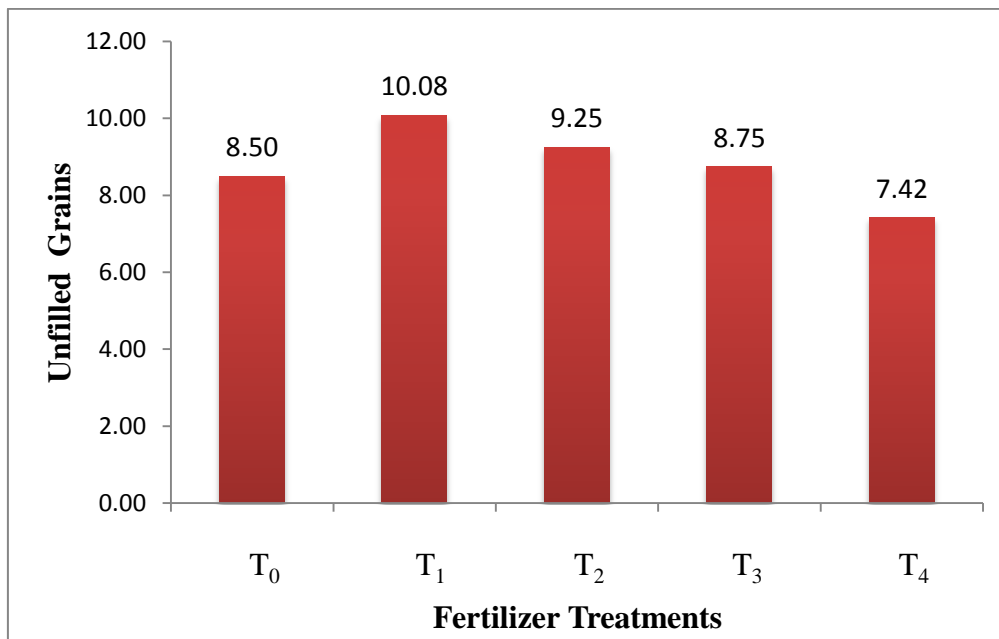
T₀ = Recommended doses of cowdung + inorganic fertilizers, T₁ = Recommended dose of cowdung without inorganic fertilizers, T₂ = Recommended dose of inorganic fertilizers without cowdung, T₃ = Reduction of 20% recommended dose of inorganic fertilizers + addition of 20% recommended dose of cowdung, T₄ = Reduction of 40% recommended dose of inorganic fertilizers + addition of 40% recommended dose of cowdung

number of filled grains, the highest number (134.00) of filled grains was recorded from As_0T_4 which was statistically similar with As_0T_0 (129.33) whereas, no grain was found in As_2 and As_3 arsenic treatments (Table 6). This result agreed with Hossain *et al.* (2008) who reported that the number of filled grains panicle⁻¹ decreased with increasing the concentration of arsenic.

4.7 Number of unfilled grains panicle⁻¹

Effect of fertilizer

The number of unfilled grains panicle⁻¹ of BRR dhan29 were significantly affected by different doses of fertilizers (cowdung and inorganic) (Figure 13 and Appendix VI). The results revealed that the treatment T_1 produced the highest number of unfilled grains (10.08) which was statistically similar with T_2 (9.25) and the lowest (7.42) was recorded from the treatment T_4 .

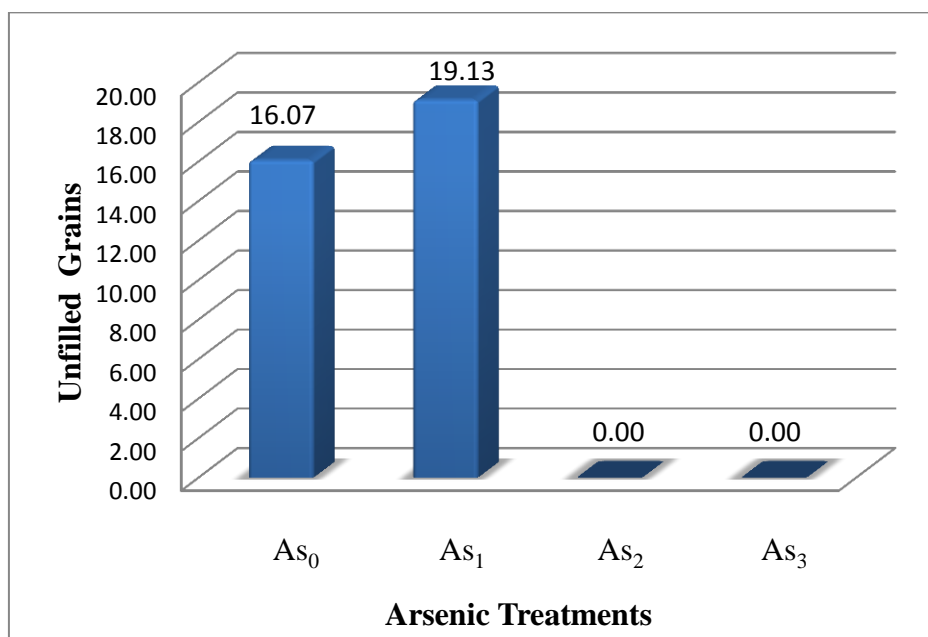


T_0 = Recommended doses of cowdung + inorganic fertilizers, T_1 = Recommended dose of cowdung without inorganic fertilizers, T_2 = Recommended dose of inorganic fertilizers without cowdung, T_3 = Reduction of 20% recommended dose of inorganic fertilizers + addition of 20% recommended dose of cowdung, T_4 = Reduction of 40% recommended dose of inorganic fertilizers + addition of 40% recommended dose of cowdung

Figure 13: Effect of different doses of fertilizers (cowdung and inorganic) on number of unfilled grains panicle⁻¹

Effect of arsenic

Different doses of arsenic had significant effect on the number of unfilled grains panicle⁻¹ (Figure 14 and Appendix VII). The highest number of unfilled grains (19.14) was observed in As₁ treatment and the lowest (0.00) was observed in both As₂ and As₃ treatments, because the treatments As₂ and As₃ did not produce any grain.



As₀ = No As applied, As₁ = 20 ppm As, As₂ = 40 ppm As, As₃ = 60 ppm As on required water basis

Figure 14: Effect of different doses of arsenic on number of unfilled grains panicle⁻¹

Interaction effect of fertilizer and arsenic

Interaction of different doses of fertilizers (cowdung and inorganic) and different doses of arsenic showed significant effect on number of unfilled grains panicle⁻¹ (Table 7). The highest number of unfilled grains panicle⁻¹ (21.66) was recorded from As₁T₁. The lowest unfilled grains panicle⁻¹ (0.00) showed all As₂T₀, As₂T₁, As₂T₂, As₂T₃, As₂T₄ and As₃T₀, As₃T₁, As₃T₂, As₃T₃, As₃T₄ treatments, because those treatments did not produce any grain.

Table 7: Interaction effect of different doses of arsenic and different doses of fertilizers (cowdung and inorganic) on number of unfilled grains panicle⁻¹

Treatments		Number of unfilled grains panicle ⁻¹
As ₀	T ₀	15.33 cd
	T ₁	18.66 abc
	T ₂	17.00 bcd
	T ₃	16.00 cd
	T ₄	13.33 d
As ₁	T ₀	18.66 abc
	T ₁	21.66 a
	T ₂	20.00 ab
	T ₃	19.00 abc
	T ₄	16.33 bcd
As ₂	T ₀	0.00 e
	T ₁	0.00 e
	T ₂	0.00 e
	T ₃	0.00 e
	T ₄	0.00 e
As ₃	T ₀	0.00 e
	T ₁	0.00 e
	T ₂	0.00 e
	T ₃	0.00 e
	T ₄	0.00 e
S.E. (±)		1.0061
CV (%)		14.00
Significant level		*

* - Significant at 5% level

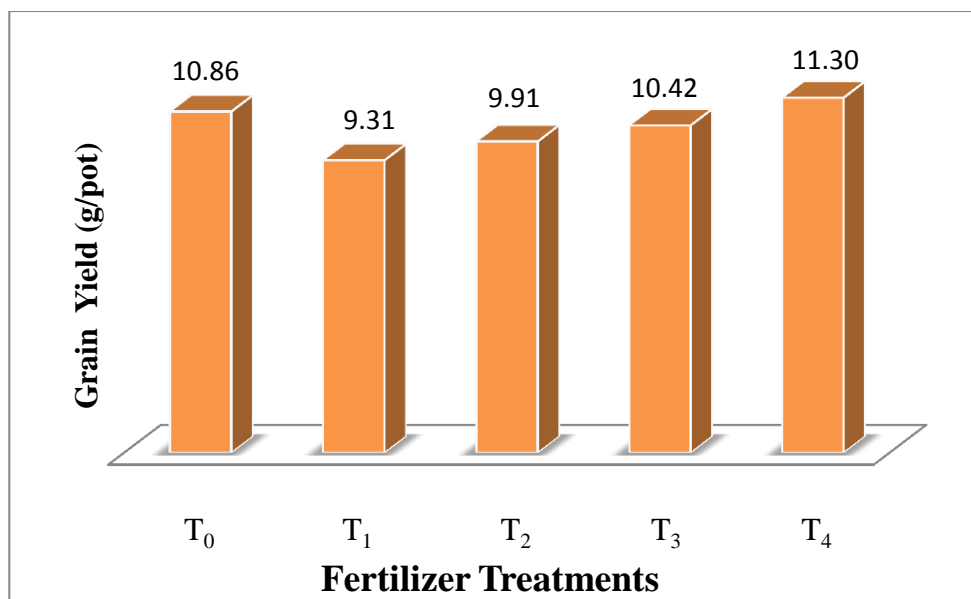
As₀ = No As applied, As₁= 20 ppm As, As₂= 40 ppm As, As₃= 60 ppm As on required water basis

T₀ = Recommended doses of cowdung + inorganic fertilizers, T₁ = Recommended dose of cowdung without inorganic fertilizers, T₂ = Recommended dose of inorganic fertilizers without cowdung, T₃ = Reduction of 20% recommended dose of inorganic fertilizers + addition of 20% recommended dose of cowdung, T₄ = Reduction of 40% recommended dose of inorganic fertilizers + addition of 40% recommended dose of cowdung

4.8 Grain yield (g/pot)

Effect of fertilizer

Grain yield (g/pot) of BRRI dhan29 was significantly affected by different doses of fertilizers (cowdung and inorganic) (Figure 15 and Appendix VIII). The highest grain yield (11.30 g/pot) was produced from T₄ treatment and the lowest (9.31 g/pot) was recorded from T₁. Rahman *et al.* (2009) reported that the application of organic manure and chemical fertilizers increased the grain yield of rice.

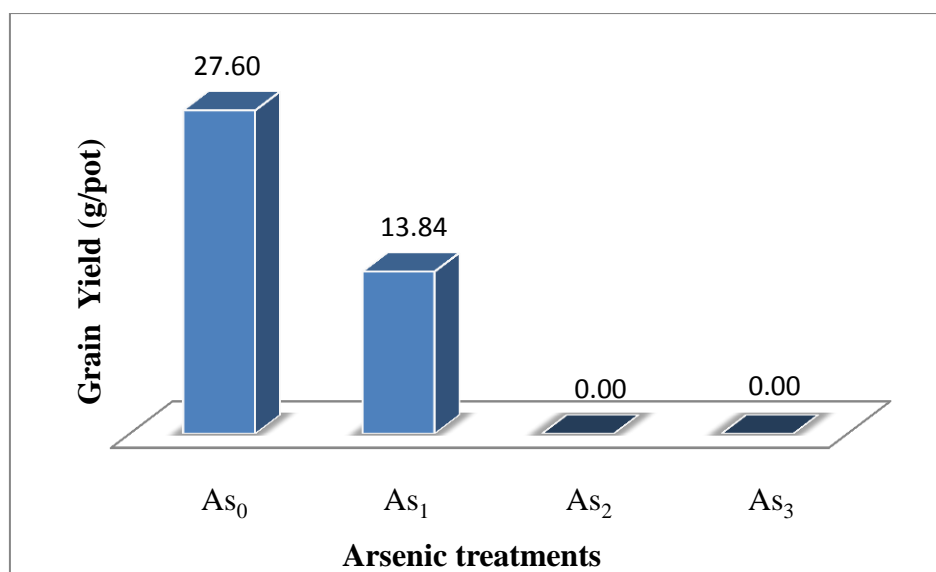


T₀ = Recommended doses of cowdung + inorganic fertilizers, T₁ = Recommended dose of cowdung without inorganic fertilizers, T₂ = Recommended dose of inorganic fertilizers without cowdung, T₃ = Reduction of 20% recommended dose of inorganic fertilizers + addition of 20% recommended dose of cowdung, T₄ = Reduction of 40% recommended dose of inorganic fertilizers + addition of 40% recommended dose of cowdung

Figure 15: Effect of different doses of fertilizers (cowdung and inorganic) on grain yield (g/pot)

Effect of arsenic

Different doses of arsenic had significant effect on grain yield (Figure 16 and Appendix IX). The highest grain yield (27.60 g/pot) was obtained from As₀ treatment and no yield was obtained from both As₂ and As₃ treatments.



As₀ = No As applied, As₁ = 20 ppm As, As₂ = 40 ppm As, As₃ = 60 ppm As on required water basis

Figure 16: Effect of different doses of arsenic on grain yield (g/pot)

Interaction effect of fertilizer and arsenic

Grain yield of BRRI dhan29 was significantly affected by the interaction of different doses of fertilizers (cowdung and inorganic) and different doses of arsenic (Table 8). The highest grain yield (29.30 g/pot) was recorded from As₀T₄ which was statistically similar with As₀T₀ (28.49 g/pot) and the lowest yield (11.55 g/pot) was recorded in As₁T₁ treatment. The arsenic treatments 40 ppm and 60 ppm (As₂ and As₃) did not produce any grain.

Table 8: Interaction effect of different doses of arsenic and different doses of fertilizers (cowdung and inorganic) on grain yield (g/pot)

Treatments		Grain yield (g/pot)
As ₀	T ₀	28.49 ab
	T ₁	25.69 d
	T ₂	26.88 c
	T ₃	27.63 bc
	T ₄	29.30 a
As ₁	T ₀	14.94 f
	T ₁	11.55 i
	T ₂	12.76 h
	T ₃	14.04 g
	T ₄	15.92 e
As ₂	T ₀	0.00 j
	T ₁	0.00 j
	T ₂	0.00 j
	T ₃	0.00 j
	T ₄	0.00 j
As ₃	T ₀	0.00 j
	T ₁	0.00 j
	T ₂	0.00 j
	T ₃	0.00 j
	T ₄	0.00 j
S.E. (±)		0.2333
CV (%)		2.76
Significant level		*

* - Significant at 5% level

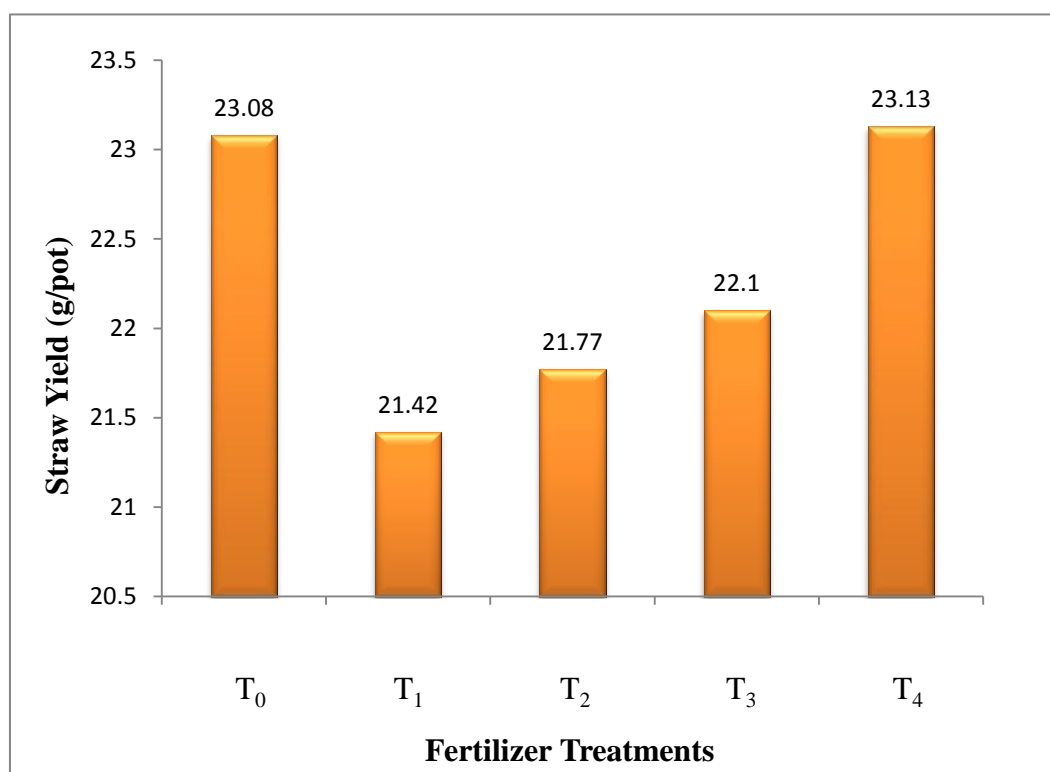
As₀ = No As applied, As₁= 20 ppm As, As₂= 40 ppm As, As₃= 60 ppm As on required water basis

T₀ = Recommended doses of cowdung + inorganic fertilizers, T₁ = Recommended dose of cowdung without inorganic fertilizers, T₂ = Recommended dose of inorganic fertilizers without cowdung, T₃ = Reduction of 20% recommended dose of inorganic fertilizers + addition of 20% recommended dose of cowdung, T₄ = Reduction of 40% recommended dose of inorganic fertilizers + addition of 40% recommended dose of cowdung

4.9 Straw yield (g/pot)

Effect of fertilizer

Straw yield (g/pot) of BRR1 dhan29 was significantly affected by different doses of fertilizers (cowdung and inorganic) (Figure 17 and Appendix VI). The highest straw yield (23.13 g/pot) was recorded from T₄ and the lowest (21.42 g/pot) was recorded from the treatment T₁.



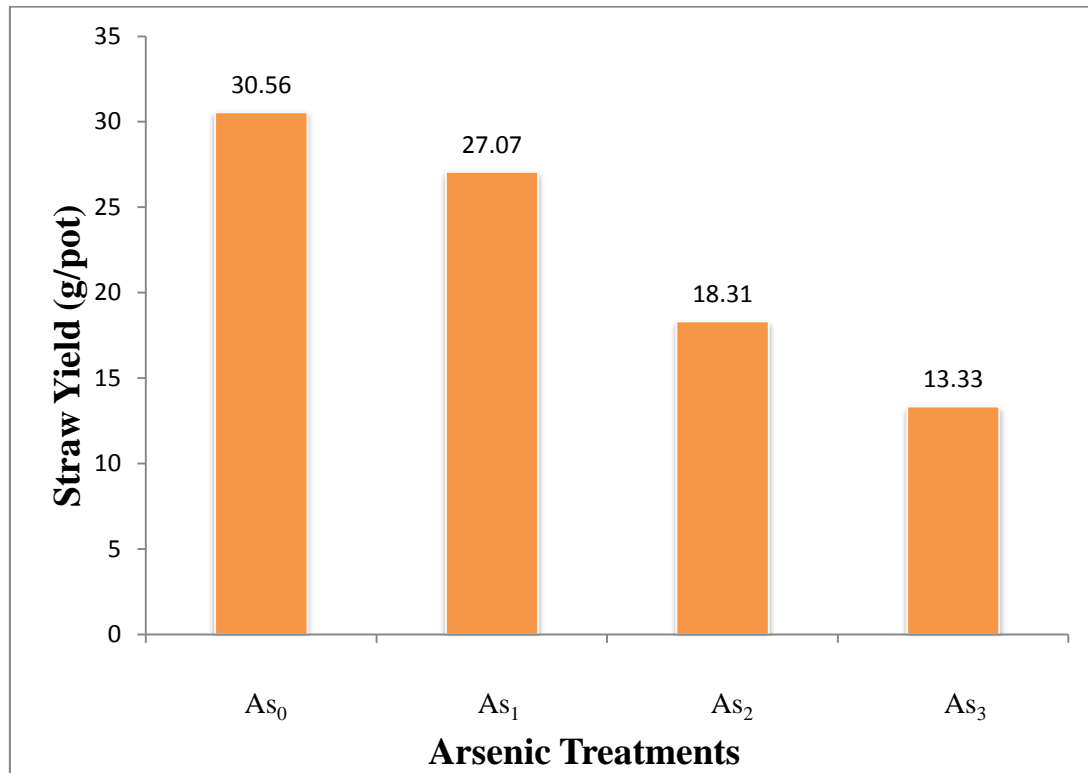
T₀ = Recommended doses of cowdung + inorganic fertilizers, T₁ = Recommended dose of cowdung without inorganic fertilizers, T₂ = Recommended dose of inorganic fertilizers without cowdung, T₃ = Reduction of 20% recommended dose of inorganic fertilizers + addition of 20% recommended dose of cowdung, T₄ = Reduction of 40% recommended dose of inorganic fertilizers + addition of 40% recommended dose of cowdung

Figure 17: Effect of different doses of fertilizers (cowdung and inorganic) on straw yield (g/pot)

Effect of arsenic

Different arsenic doses had significant effect on straw yield (Figure 18 and Appendix VII). The highest straw yield (30.56 g/pot) was obtained from As₀ treatment and the lowest (13.33 g/pot) was recorded from As₃ treatment. Results showed that higher doses of arsenic gave

lower yield. This may be due to toxic effect of arsenic. Begum *et al.* (2008) showed that the straw yield of Boro rice was reduced by 21.0 % for 15 ppm As treatment and 65.2 % due to 30 ppm As.



As₀ = No As applied, As₁= 20 ppm As, As₂= 40 ppm As, As₃= 60 ppm As on required water basis

Figure 18: Effect of different doses of arsenic on straw yield (g/pot)

Interaction effect of fertilizer and arsenic

Straw yield of BRRI dhan29 rice was significantly affected by the interaction of different doses of fertilizers (cowdung and inorganic) and different doses of arsenic (Table 9). The highest straw yield (32.15 g/pot) was recorded from the treatment As₀T₄ which was statistically similar with As₀T₀ (31.19 g/pot) and As₀T₁ (31.05 g/pot) and the lowest yield (11.31 g/pot) was recorded from the treatment As₃T₁ which was statistically similar with As₃T₃ (11.64 g/pot).

Table 9: Interaction effect of different doses of arsenic and different doses of fertilizers (cowdung and inorganic) on straw yield (g/pot)

Treatments		Straw yield (g/pot)
As ₀	T ₀	31.19 ab
	T ₁	31.05 ab
	T ₂	29.32 abc
	T ₃	29.09 abc
	T ₄	32.15 a
As ₁	T ₀	27.25 bcd
	T ₁	26.00 cd
	T ₂	23.64 d
	T ₃	28.91 abc
	T ₄	29.22 abc
As ₂	T ₀	18.09 e
	T ₁	17.31 e
	T ₂	18.65 e
	T ₃	18.77 e
	T ₄	18.74 e
As ₃	T ₀	15.81 ef
	T ₁	11.31 g
	T ₂	15.50 efg
	T ₃	11.64 fg
	T ₄	12.41 fg
S.E. (±)		1.1500
CV (%)		6.31
Significant level		*

* - Significant at 5% level

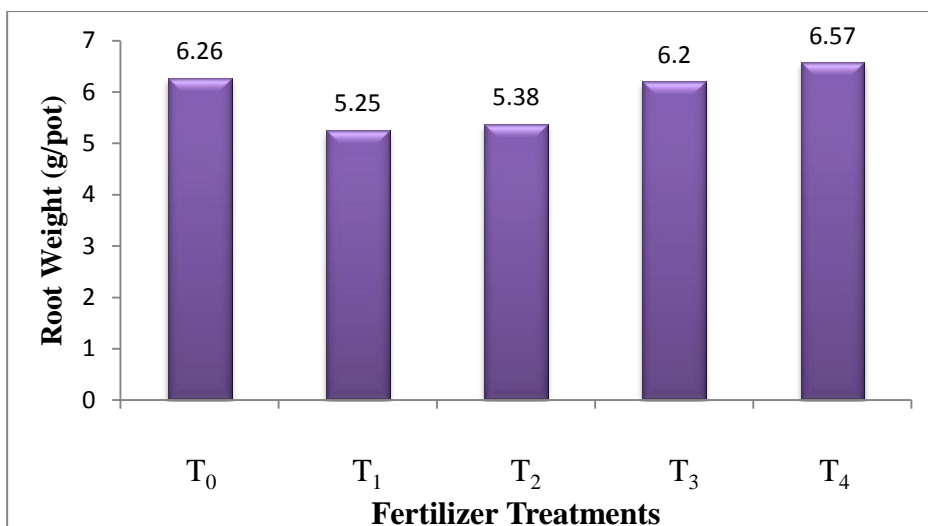
As₀ = No As applied, As₁ = 20 ppm As, As₂ = 40 ppm As, As₃ = 60 ppm As on required water basis

T₀ = Recommended doses of cowdung + inorganic fertilizers, T₁ = Recommended dose of cowdung without inorganic fertilizers, T₂ = Recommended dose of inorganic fertilizers without cowdung, T₃ = Reduction of 20% recommended dose of inorganic fertilizers + addition of 20% recommended dose of cowdung, T₄ = Reduction of 40% recommended dose of inorganic fertilizers + addition of 40% recommended dose of cowdung

4.10 Root Weight (g/pot)

Effect of fertilizer

Root weight (g/pot) of BRR1 dhan29 was significantly affected by different doses of fertilizers (cowdung and inorganic) (Figure 19 and Appendix VI). The highest root weight (6.57 g/pot) was recorded from T₄ and the lowest (5.25 g/pot) was recorded from the treatment T₁.

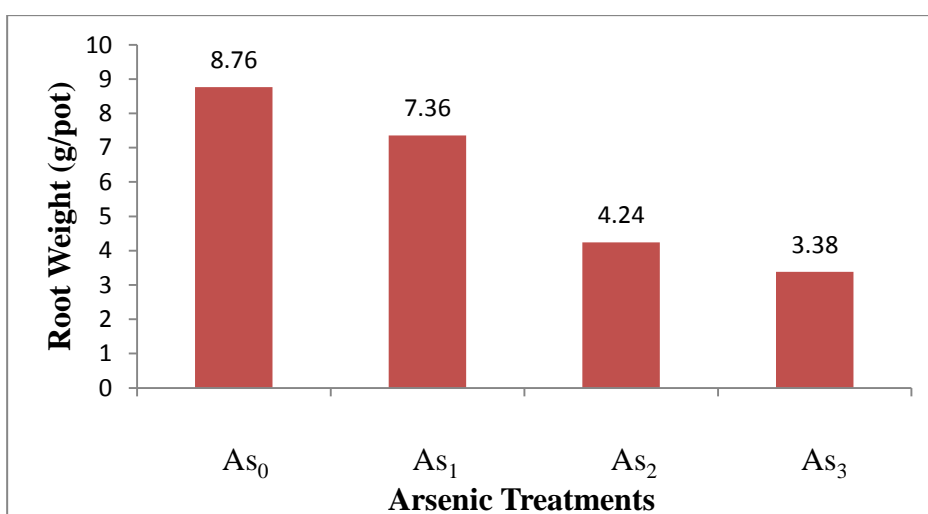


T₀ = Recommended doses of cowdung + inorganic fertilizers, T₁ = Recommended dose of cowdung without inorganic fertilizers, T₂ = Recommended dose of inorganic fertilizers without cowdung, T₃ = Reduction of 20% recommended dose of inorganic fertilizers + addition of 20% recommended dose of cowdung, T₄ = Reduction of 40% recommended dose of inorganic fertilizers + addition of 40% recommended dose of cowdung

Figure 19: Effect of different doses of fertilizers (cowdung and inorganic) on root weight (g/pot)

Effect of arsenic

Different arsenic doses had significant effect on root weight (Figure 20 and Appendix VII). The highest root weight (8.76 g/pot) was obtained from As₀ treatment and the lowest (3.38 g/pot) was recorded from As₃ treatment. Results showed that higher doses of arsenic gave lower yield. This may be due to toxic effect of arsenic.



As₀ = No As applied, As₁ = 20 ppm As, As₂ = 40 ppm As, As₃ = 60 ppm As on required water basis

Figure 20: Effect of different doses of arsenic on root weight (g/pot)

Interaction effect of fertilizer and arsenic

Root weight of BRR1 dhan29 rice was significantly affected by the interaction of different doses of fertilizers (cowdung and inorganic) and different doses of arsenic (Table 9). The highest root weight (9.50 g/pot) was recorded from the treatment As₀T₄ which was statistically similar with As₀T₀ (9.20 g/pot) and the lowest yield (2.43 g/pot) was recorded from the treatment As₃T₁ which was statistically similar with As₃T₂ (2.92 g/pot).

Table 10: Interaction effect of different doses of arsenic and different doses of fertilizers (cowdung and inorganic) root weight (g/pot)

Treatments		Root Weight (g/pot)
As ₀	T ₀	9.20 ab
	T ₁	8.31 abc
	T ₂	7.80 abc
	T ₃	8.95 ab
	T ₄	9.50 a
As ₁	T ₀	7.51 bc
	T ₁	6.93 cd
	T ₂	6.94 cd
	T ₃	7.80 abc
	T ₄	7.63 bc
As ₂	T ₀	4.57 ef
	T ₁	3.03 fg
	T ₂	3.84 efg
	T ₃	4.22 ef
	T ₄	5.24 de
As ₃	T ₀	3.77 efg
	T ₁	2.43 g
	T ₂	2.92 fg
	T ₃	3.82 efg
	T ₄	3.91 efg
S.E. (±)		0.4576
CV (%)		9.45
Significant level		*

* - Significant at 5% level

As₀ = No As applied, As₁ = 20 ppm As, As₂ = 40 ppm As, As₃ = 60 ppm As on required water basis

T₀ = Recommended doses of cowdung + inorganic fertilizers, T₁ = Recommended dose of cowdung without inorganic fertilizers, T₂ = Recommended dose of inorganic fertilizers without cowdung, T₃ = Reduction of 20% recommended dose of inorganic fertilizers + addition of 20% recommended dose of cowdung, T₄ = Reduction of 40% recommended dose of inorganic fertilizers + addition of 40% recommended dose of cowdung

4.11 Chemical Composition

4.11.1 Nitrogen (N) content in straw

Effect of fertilizer

Nitrogen (N) content in straw showed statistically significant difference due to different doses of fertilizers (cowdung and inorganic) (Table 11). The highest N content (0.955 %) was observed in straw from the treatment T₄ and the lowest amount of N (0.745 %) found in straw for the treatment T₁.

Table 11: Effect of different doses of fertilizers (cowdung and inorganic) on N, P, K and As content in straw

Treatments	Straw			
	N (%)	P (%)	K (%)	As (ppm)
T ₀	0.900 b	0.500 b	1.115 b	2.595 d
T ₁	0.745 e	0.365 d	0.933 e	3.557 a
T ₂	0.803 d	0.400 d	1.003 d	3.423 b
T ₃	0.843 c	0.455 c	1.053 c	2.756 c
T ₄	0.955 a	0.548 a	1.173 a	2.465 e
S.E. (±)	0.0311	0.0111	0.0112	0.2144
CV (%)	1.31	3.47	0.21	6.94
Significant level	*	*	*	*

* - Significant at 5% level

T₀ = Recommended doses of cowdung + inorganic fertilizers, T₁ = Recommended dose of cowdung without inorganic fertilizers, T₂ = Recommended dose of inorganic fertilizers without cowdung, T₃ = Reduction of 20% recommended dose of inorganic fertilizers + addition of 20% recommended dose of cowdung, T₄ = Reduction of 40% recommended dose of inorganic fertilizers + addition of 40% recommended dose of cowdung

Effect of arsenic

Nitrogen (N) content in straw showed statistically significant difference due to different doses of arsenic (Table 12). The highest N content (1.260 %) was observed in straw from the treatment As₀ and the lowest amount of N (0.454 %) found in straw from the treatment As₃.

Table 12. Effect of different doses of arsenic on N, P, K and As content in straw

Treatments	Straw			
	N (%)	P (%)	K (%)	As (ppm)
As ₀	1.260 a	0.834 a	1.592 a	0.00 d
As ₁	0.984 b	0.560 b	1.242 b	2.498 c
As ₂	0.698 c	0.324 c	0.842 c	3.888 b
As ₃	0.454 d	0.096 d	0.544 d	5.450 a
S.E. (±)	0.0292	0.0102	0.0103	0.1948
CV (%)	1.31	3.47	0.21	6.94
Significant level	*	*	*	*

* - Significant at 5% level

As₀ = No As applied, As₁= 20 ppm As, As₂= 40 ppm As, As₃= 60 ppm As on required water basis**Interaction effect of fertilizer and arsenic**

Interaction of different doses of fertilizers (cowdung and inorganic) and different doses of arsenic had significant effect on Nitrogen (N) content in straw (Table 13). The highest N (1.370 %) in straw was observed from the treatment As₀T₄ which was statistically similar with As₀T₀ (1.320 %) and the lowest N (0.370 %) in straw was observed from As₃T₁ which was statistically similar with As₃T₂ (0.410 %) and As₃T₃ (0.440 %).

4.11.2 Phosphorus (P) content in straw**Effect of fertilizer**

Phosphorus (P) content in straw showed statistically significant difference due to different doses of fertilizers (cowdung and inorganic) (Table 11). The highest P content (0.548 %) was observed in straw from the treatment T₄ and the lowest amount of P (0.365 %) was found in straw for the treatment T₁.

Effect of arsenic

Phosphorus (P) content in straw showed statistically significant difference due to different doses of arsenic (Table 12). The highest P content (0.834 %) was observed in straw from the treatment As₀ and the lowest amount of P (0.096 %) was found in straw from the treatment As₃.

Interaction effect of fertilizer and arsenic

Interaction of different doses of fertilizers (cowdung and inorganic) and different doses of arsenic had significant influence on Phosphorus (P) content in straw (Table 13). The highest

P (0.930 %) in straw was observed from the treatment As₀T₄ and the lowest P (0.030 %) in straw was observed from As₃T₁.

Table 13: Interaction effect of different doses of arsenic and different doses of fertilizers (cowdung and inorganic) on N, P, K and As content in grain

Treatments		Straw			
		N (%)	P (%)	K (%)	As (ppm)
As ₀	T ₀	1.320 ab	0.890 ab	1.650 b	0.000 n
	T ₁	1.140 de	0.730 c	1.470 d	0.000 n
	T ₂	1.220 cd	0.780 c	1.540 c	0.000 n
	T ₃	1.250 bc	0.840 b	1.590 c	0.000 n
	T ₄	1.370 a	0.930 a	1.710 a	0.000 n
As ₁	T ₀	1.030 fg	0.610 e	1.300 f	1.940 l
	T ₁	0.870 hi	0.470 fg	1.120 h	3.420 h
	T ₂	0.940 gh	0.480 f	1.190 g	3.210 j
	T ₃	0.980 g	0.570 e	1.240 g	2.360 k
	T ₄	1.100 ef	0.670 d	1.360 e	1.560 m
As ₂	T ₀	0.750 jk	0.370 hi	0.900 j	3.330 i
	T ₁	0.600 m	0.230 k	0.720 l	4.780 f
	T ₂	0.640 lm	0.280 jk	0.790 k	4.620 g
	T ₃	0.700 kl	0.320 ij	0.840 k	3.440 h
	T ₄	0.800 ij	0.420 gh	0.960 i	3.270 i
As ₃	T ₀	0.500 no	0.130 lm	0.610 m	5.110 d
	T ₁	0.370 p	0.030 o	0.420 o	6.030 a
	T ₂	0.410 op	0.060 no	0.490 n	5.860 b
	T ₃	0.440 op	0.090 mn	0.540 n	5.220 c
	T ₄	0.550 mn	0.170 l	0.660 m	5.030 e
S.E. (±)		0.0623	0.0222	0.0224	0.4288
CV (%)		1.31	3.47	0.21	6.94
Significant level		*	*	*	*

* - Significant at 5% level

As₀ = No As applied, As₁= 20 ppm As, As₂= 40 ppm As, As₃= 60 ppm As on required water basis

T₀ = Recommended doses of cowdung + inorganic fertilizers, T₁ = Recommended dose of cowdung without inorganic fertilizers, T₂ = Recommended dose of inorganic fertilizers without cowdung, T₃ = Reduction of 20% recommended dose of inorganic fertilizers + addition of 20% recommended dose of cowdung, T₄ = Reduction of 40% recommended dose of inorganic fertilizers + addition of 40% recommended dose of cowdung

4.10.3 Potassium (K) content in straw

Effect of fertilizer

Potassium (K) content in straw showed statistically significant difference due to different doses of fertilizers (cowdung and inorganic) (Table 11). The highest K content (1.173 %) was

observed in straw from the treatment T₄ and the lowest amount of K (0.933 %) was found in straw for the treatment T₁.

Effect of arsenic

Potassium (K) content in straw showed statistically significant difference due to different doses of arsenic (Table 12). The highest K content (1.592 %) was observed in straw from the treatment As₀ and the lowest amount of K (0.544 %) was found in straw from the treatment As₃.

Interaction effect of fertilizer and arsenic

Interaction of different doses of fertilizers (cowdung and inorganic) and different doses of arsenic had significant influence on potassium (K) content in straw (Table 13). The highest K (1.710 %) in straw was observed from the treatment As₀T₄ and the lowest K (0.420 %) in straw was observed from As₃T₁.

4.10.4 Arsenic (As) content in straw

Effect of fertilizer

Arsenic (As) content in straw showed statistically significant difference due to different doses of fertilizers (cowdung and inorganic) (Table 11). The highest As content (3.557 ppm) was observed in straw from the treatment T₁ and the lowest amount of As (2.465 ppm) was found in straw for the treatment T₄.

Effect of arsenic

Arsenic (As) content in straw showed statistically significant difference due to different doses of arsenic (Table 12). The highest As content (5.450 ppm) was observed in straw from the treatment As₃ and the lowest amount of As (0 ppm) was found in straw from the treatment As₀.

Interaction effect of fertilizer and arsenic

Interaction of different doses of fertilizers (cowdung and inorganic) and different doses of arsenic had significant influence on arsenic (As) content in straw (Table 13). The highest As (6.030 ppm) in straw was observed from the treatment As₃T₁ and the lowest As (0 ppm) in straw was observed from As₀T₀ which was statistically similar with As₀T₁ (0 ppm), As₀T₂ (0 ppm), As₀T₃ (0 ppm) and As₀T₄ (0 ppm).

CHAPTER V

SUMMARY AND CONCLUSION

The experiment was conducted in the net house and the agro-environmental chemistry laboratory of the Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Dhaka-1207 under pot-culture during the Boro season of the year 2017-18 to evaluate the mitigation of arsenic stress in BRRI dhan29 with cowdung and inorganic fertilizers. The two factorials experiment was laid out in a Completely Randomized Design (CRD) with three replications. Factor A: different doses of arsenic on required water basis [As_0 =No arsenic applied, As_1 = 20 ppm arsenic, As_2 = 40 ppm arsenic, As_3 = 60 ppm arsenic] and Factor B: different doses of cowdung and inorganic fertilizers [T_0 = Recommended dose of cowdung + inorganic fertilizer, T_1 = Recommended dose of cowdung without inorganic fertilizer, T_2 = Recommended dose of inorganic fertilizer without cowdung, T_3 = Reduction of 20% recommended dose of inorganic fertilizer + addition of 20% recommended dose of cowdung, T_4 = Reduction of 40% recommended dose of inorganic fertilizer + addition of 40% recommended dose of cowdung].

Different growth and yield parameters varied significantly due to difference in the doses of fertilizers (cowdung and inorganic). At 70 DAT the treatment T_4 produced the tallest plant (50.00 cm) but at 140 DAT the treatment T_0 produced the tallest plant (66.52 cm) and at 70 and 140 DAT the treatment T_1 produced the shortest plant (44.75 cm, 61.09 cm respectively). At 70 and 90 DAT, the treatment T_4 produced the highest number of tillers hill⁻¹ (9.58 and 10.41 respectively) and the treatment T_1 and T_2 produced the lowest number of tillers hill⁻¹ 8.25 and 8.41 respectively due to arsenic affect. Among the treatments, the treatment T_4 produced the highest number of effective tillers hill⁻¹ (4.75) and the treatment T_1 produced the lowest number of effective tillers (3.33) whereas, the treatment T_1 produced the highest number of non-effective tillers hill⁻¹ (5.76) and the treatment T_4 produced the lowest number of non-effective tillers hill⁻¹ (5.08). The highest number of filled grains panicle⁻¹ (51.66) produced by the treatment T_4 and the lowest number (44.92) produced by T_1 . The maximum number of unfilled grains panicle⁻¹ (10.08) produced by the treatment T_1 and the lowest number (7.42) produced by T_4 . The maximum panicle length (8.69 cm) produced by T_4 and the lowest panicle length (7.51 cm) produced by T_1 . The maximum grain yield (11.30 g/pot) was recorded from the treatment T_4 and the minimum (9.31 g/pot) from the T_1 . The highest

straw yield (23.13 g/pot) was found in the treatment T₄ and the lowest (21.42 g/pot) from the T₁. The highest root weight (6.57 g/pot) was found in the treatment T₄ and the lowest (5.25 g/pot) from the T₁. The highest N (0.955 %) in straw was recorded from T₄ and the lowest (0.745 %) from T₁. The highest P (0.548 %) in straw was recorded from T₄ and the lowest (0.365 %) from T₁. The maximum value (1.173 %) of K in straw was recorded from T₄ and the lowest (0.933 %) from T₁. The highest As content (3.557 ppm) in straw was recorded from T₁ and the lowest (2.465 ppm) from T₄.

Different doses of arsenic had significant effect on growth and yield of BRRI dhan29. At 70 and 140 DAT, the treatment As₀ showed the highest plant height (64.13 cm and 84.83 cm respectively) and As₃ showed the lowest (36.06 cm and 50.19 cm respectively). At 70 and 140 DAT, the treatment As₀ produced the highest number of tillers hill⁻¹ (10.73 and 12.60 respectively) and the treatment As₃ produced the lowest number of tillers hill⁻¹ (6.33 and 6.26 respectively). The highest number of effective tillers hill⁻¹ (10.40) was observed from the As₀ treatment and the lowest (0.00) was observed from both As₂ and As₃ treatment. The highest number of non-effective tillers hill⁻¹ (8.80) was recorded from As₂ treatment and the lowest number of non-effective tillers hill⁻¹ (2.33) was recorded from As₀ treatment. The highest number of filled grains panicle⁻¹ (122.93) was observed from the As₀ treatment and the lowest (0.00) was observed from both As₂ and As₃ treatment. The highest number of unfilled grains panicle⁻¹ (19.14) was observed in As₁ treatment and the lowest (0.00) in both As₂ and As₃ treatment. The maximum panicle length (19.87 cm) produced by As₀ and the lowest panicle length (0.00 cm) produced by both As₂ and As₃ treatments. The maximum grain yield (27.60 g/pot) was recorded from the treatment As₀ whereas the minimum (0.00 g/pot) from the both As₂ and As₃. The highest straw yield (30.56 g/pot) was found in the treatment As₀ and the lowest (13.33 g/pot) from the As₃. The highest root weight (8.76 g/pot) was found in the treatment As₀ and the lowest (3.38 g/pot) from the As₃. The highest N (1.260 %) in straw was recorded from As₀ and the lowest (0.454 %) from As₃. The highest P (0.834 %) in straw was recorded from As₀ and the lowest (0.096 %) from As₃. The maximum value (1.592 %) of K in straw was recorded from As₀ and the lowest (0.544 %) from As₃. The highest As content (5.450 ppm) in straw was recorded from As₃ and the lowest (0 ppm) from As₀.

In case of interaction effect of different doses of fertilizers (cowdung and inorganic) and arsenic show that, the highest plant height (70.00 cm and 90.50 cm) were observed from the As_0T_4 treatment at 70 DAT and 140 DAT respectively and at 70 DAT the treatment As_3T_1 showed the lowest plant height (31.66 cm) and at 140 DAT the treatment As_3T_3 showed the lowest plant height (47.76 cm). At 90 DAT, the highest number of tillers hill⁻¹ (12.33) was observed from the As_0T_0 treatment and the lowest number of tillers hill⁻¹ (5.33) was observed from the As_3T_1 treatment. At 140 DAT, the highest number of tillers hill⁻¹ (14.00) was observed from the As_0T_0 treatment and the lowest number of tillers hill⁻¹ (5.33) was observed from As_3T_1 treatment. The highest number of effective tillers hill⁻¹ (12.00) was observed from the As_0T_4 treatment and the lowest tillers hill⁻¹ (0.00) were observed from all As_2T_0 , As_2T_1 , As_2T_2 , As_2T_3 , As_2T_4 and As_3T_0 , As_3T_1 , As_3T_2 , As_3T_3 , As_3T_4 treatments. The highest number of non-effective tillers hill⁻¹ (9.66) was recorded from As_2T_4 treatment and the lowest number of non-effective tillers hill⁻¹ (1.33) was recorded from As_0T_4 treatment. The highest number of filled grains panicle⁻¹ (134.00) was observed from the As_0T_4 treatment and the lowest (0.00) were observed from all As_2 and As_3 with fertilizer treatments. The highest number of unfilled grains panicle⁻¹ (21.66) was observed in As_1T_1 treatment and the lowest (0.00) were observed from all As_2 and As_3 with fertilizer treatments. The maximum panicle length (21.69 cm) produced by As_0T_4 and the lowest panicle length (0.00 cm) produced by both As_2 and As_3 with fertilizer treatments. The maximum grain yield (29.30 g/pot) was recorded from the treatment As_0T_4 whereas the minimum (0.00 g/pot) from the both As_2 and As_3 with fertilizer treatments. The highest straw yield (32.15 g/pot) was found in the treatment As_0T_4 and the lowest (11.31 g/pot) from the As_3T_1 . The highest root weight (9.50 g/pot) was found in the treatment As_0T_4 and the lowest (2.43 g/pot) from the As_3T_1 . The highest N (1.370 %) in straw was recorded from As_0T_4 and the lowest (0.370 %) from As_3T_1 . The highest P (0.930 %) in straw was recorded from As_0T_4 and the lowest (0.030 %) from As_3T_1 . The maximum value (1.710 %) of K in straw was recorded from As_0T_4 and the lowest (0.420 %) from As_3T_1 . The highest As content (6.030 ppm) in straw was recorded from As_3T_1 and the lowest (0 ppm) from As_0T_0 .

From the above results it can be concluded that,

- Arsenic toxicity negatively affects all the growth and yield related attributes of BRRIdhan29.
- Treatment As₀T₄ gave the better yield and yield contributing characters among all the combinations of cowdung and inorganic fertilizers.
- Treatment T₄ has lower arsenic accumulation in straw.
- Use of cowdung with inorganic fertilizers decreased the adverse effects of high arsenic toxicity on rice plant and improved all the traits mentioned above

From the above conclusions, the following recommendations can be made:

- Farmers can adopt the use of cowdung to reduce the toxicity affect of arsenic.
- Such studies should be carried out to different arsenic prone areas of the country.

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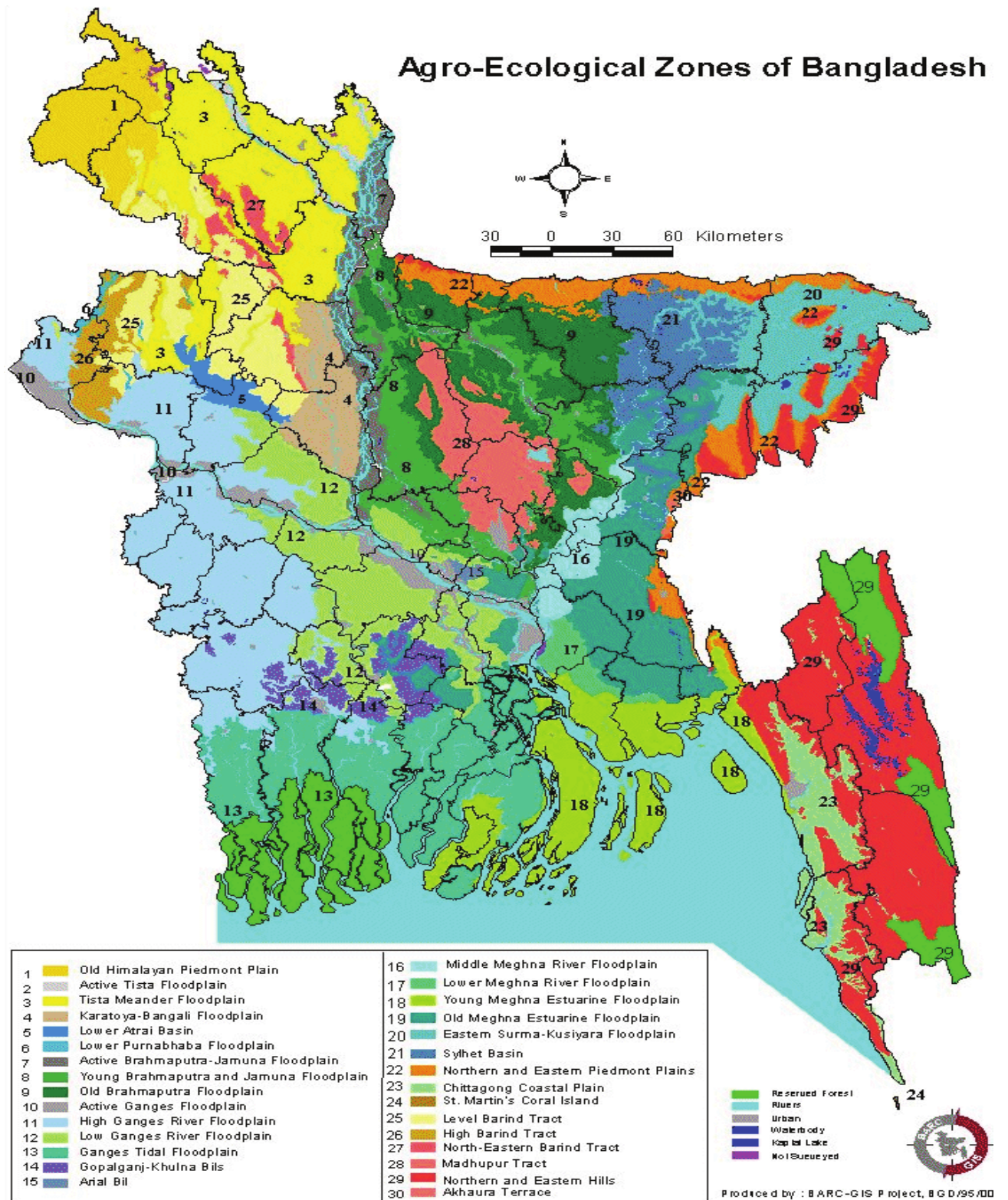
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APPENDICES

Appendix I. Experimental location on the map of Agro-ecological Zones of Bangladesh



Appendix II. Morphological characteristics of the experimental field

Morphology	Characteristics
Location	SAU Farm, Dhaka
Agro-ecological zone	Madhupur Tract (AEZ- 28)
General Soil Type	Deep Red Brown Terrace Soil
Parent material	Madhupur Terrace
Topography	Fairly level
Drainage	Well drained
Flood level	Above flood level

(SAU Farm, Dhaka)

Appendix III. Initial physical and chemical characteristics of the soil

Characteristics	Value
Mechanical fractions:	
% Sand (2.0-0.02 mm)	22.26
% Silt (0.02-0.002 mm)	56.72
% Clay (<0.002 mm)	20.75
Textural class	Silt Loam
pH (1: 2.5 soil- water)	5.9
Organic Matter (%)	1.09
Total N (%)	0.028
Available K (ppm)	15.625
Available P (ppm)	7.988
Available S (ppm)	2.066

(SAU Farm, Dhaka)

Appendix IV. Effect of different doses of fertilizers (cowdung and inorganic) on plant height and number of tillers hill⁻¹ at different days after transplanting

Treatments	Plant Height (cm)		Number of tillers hill ⁻¹	
	70 DAT	140 DAT	70 DAT	140 DAT
T ₀	47.75 a	66.52 a	9.00 a	10.08 a
T ₁	44.75 b	61.09 c	8.25 a	9.33 ab
T ₂	47.41 ab	62.95 bc	8.66 a	8.41 b
T ₃	47.66 a	65.50 ab	9.00 a	9.33 ab
T ₄	50.00 a	66.41 a	9.58 a	10.41 a
S.E. (±)	0.9531	1.1785	0.6009	0.5725
CV (%)	4.91	4.48	16.54	14.81
Significant level	*	*	*	*

* - Significant at 5% level

T₀ = Recommended doses of cowdung + inorganic fertilizers, T₁ = Recommended dose of cowdung without inorganic fertilizers, T₂ = Recommended dose of inorganic fertilizers without cowdung, T₃ = Reduction of 20% recommended dose of inorganic fertilizers + addition of 20% recommended dose of cowdung, T₄ = Reduction of 40% recommended dose of inorganic fertilizers + addition of 40% recommended dose of cowdung

Appendix V. Effect of different doses of arsenic on plant height and number of tillers hill⁻¹ at different days after transplanting

Treatments	Plant Height (cm)		Number of tillers hill ⁻¹	
	70 DAT	140 DAT	70 DAT	140 DAT
As ₀	64.13 a	84.83 a	10.73 a	12.60 a
As ₁	51.53 b	61.90 b	10.13 a	10.20 b
As ₂	38.33 c	61.06 b	8.40 b	8.80 c
As ₃	36.06 c	50.19 c	6.33 c	6.26 d
S.E. (±)	0.8524	1.0541	0.5375	0.5121
CV (%)	4.91	4.48	16.54	14.81
Significant level	*	*	*	*

* - Significant at 5% level

As₀ = No As applied, As₁ = 20 ppm As, As₂ = 40 ppm As, As₃ = 60 ppm As on required water basis

Appendix VI. Effect of different doses of fertilizers (cowdung and inorganic) on number of effective tillers hill⁻¹, non-effective tillers hill⁻¹, number of filled grains panicle⁻¹, number of unfilled grains panicle⁻¹, panicle length (cm), straw yield (g/pot) and root weight (g/pot)

Treatments	Number of effective tillers hill ⁻¹	Number of non-effective tillers hill ⁻¹	Number of filled grains panicle ⁻¹	Number of unfilled grains panicle ⁻¹	Panicle length (cm)	Straw yield (g/pot)	Root Weight (g/pot)
T ₀	4.25 ab	5.75 a	49.92 a	8.50 bc	8.25 b	23.08 a	6.26 a
T ₁	3.33 c	5.76 a	44.92 b	10.08 a	7.51 c	21.42 b	5.25 b
T ₂	3.50 bc	5.66 a	46.00 b	9.25 ab	7.64 c	21.77 ab	5.38 b
T ₃	3.92 bc	5.50 a	47.08 b	8.75 abc	7.85 c	22.10 ab	6.20 a
T ₄	4.75 a	5.08 a	51.66 a	7.42 c	8.69 a	23.13 a	6.57 a
S.E. (±)	0.2889	0.3905	0.7806	0.5031	0.1319	0.5750	0.2288
CV (%)	17.92	17.23	3.99	14.00	4.04	6.31	9.45
Significant level	*	*	*	*	*	*	*

* - Significant at 5% level

T₀ = Recommended doses of cowdung + inorganic fertilizers, T₁ = Recommended dose of cowdung without inorganic fertilizers, T₂ = Recommended dose of inorganic fertilizers without cowdung, T₃ = Reduction of 20% recommended dose of inorganic fertilizers + addition of 20% recommended dose of cowdung, T₄ = Reduction of 40% recommended dose of inorganic fertilizers + addition of 40% recommended dose of cowdung

Appendix VII. Effect of different doses of arsenic on number of effective tillers hill⁻¹, non-effective tillers hill⁻¹, number of filled grains panicle⁻¹, number of unfilled grains panicle⁻¹, panicle length (cm), straw yield (g/pot) and root weight (g/pot)

Treatments	Number of effective tillers hill ⁻¹	Number of non-effective tillers hill ⁻¹	Number of filled grains panicle ⁻¹	Number of unfilled grains panicle ⁻¹	Panicle length (cm)	Straw yield (g/pot)	Root Weight (g/pot)
As ₀	10.40 a	2.33 d	122.93 a	16.07 b	19.87 a	30.56 a	8.76 a
As ₁	5.40 b	4.80 c	68.73 b	19.13 a	12.08 b	27.07 b	7.36 b
As ₂	0.00 c	8.80 a	0.00 c	0.00 c	0.00 c	18.31 c	4.24 c
As ₃	0.00 c	6.27 b	0.00 c	0.00 c	0.00 c	13.33 d	3.38 d
S.E. (±)	0.2584	0.3493	0.6982	0.4500	0.1179	0.5143	0.2047
CV (%)	17.92	17.23	3.99	14.00	4.04	6.31	9.45
Significant level	*	*	*	*	*	*	*

* - Significant at 5% level

As₀ = No As applied, As₁ = 20 ppm As, As₂ = 40 ppm As, As₃ = 60 ppm As on required water basis

Appendix VIII. Effect of different doses of fertilizers (cowdung and inorganic) on grain yield (g/pot)

Treatments	Grain yield (g/pot)
T ₀	10.86 b
T ₁	9.31 e
T ₂	9.91 d
T ₃	10.42 c
T ₄	11.30 a
S.E. (±)	0.1166
CV (%)	2.76
Significant level	*

* - Significant at 5% level

T₀ = Recommended doses of cowdung + inorganic fertilizers, T₁ = Recommended dose of cowdung without inorganic fertilizers, T₂ = Recommended dose of inorganic fertilizers without cowdung, T₃ = Reduction of 20% recommended dose of inorganic fertilizers + addition of 20% recommended dose of cowdung, T₄ = Reduction of 40% recommended dose of inorganic fertilizers + addition of 40% recommended dose of cowdung

Appendix IX. Effect of different doses of arsenic on grain yield (g/pot)

Treatments	Grain yield (g/pot)
As ₀	27.60 a
As ₁	13.84 b
As ₂	0.00 c
As ₃	0.00 c
S.E. (±)	0.1043
CV (%)	2.76
Significant level	*

* - Significant at 5% level

As₀ = No As applied, As₁= 20 ppm As, As₂= 40 ppm As, As₃= 60 ppm As on required water basis