# ROLE OF ORGANIC AMENDMENTS ON MORPHO-PHYSIOLOGY OF RICE UNDER ARSENIC STRESS

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## *CERTIFICATE*

*This is to certify that the thesis entitled* ***"ROLE OF ORGANIC AMENDMENTS ON MORPHO-PHYSIOLOGY OF RICE UNDER ARSENIC STRESS"*** *submitted to the Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE (MS) in AGRONOMY****, embodies the result of a piece of bonafide research work carried out by* ***MST. MAHBUBA KHATUN,*** *Registration No.* ***20-11085*** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

**Dated:**
**Place: Dhaka, Bangladesh**

**Dr. Md. Mahabub Alam**
**Supervisor**

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*The author*

# ROLE OF ORGANIC AMENDMENTS ON MORPHO-PHYSIOLOGY OF RICE UNDER ARSENIC STRESS

## ABSTRACT

Our study investigated the arsenic induced stress of morpho-physiology in rice plant and the role of organic amendments; cowdung, vermicompost, biochar to mitigate the damages. Rice seedling was grown in 18L pot with 4 arsenic treatment at 21DAT, 32DAT, 42DAT and 52DAT. Sodium Arsenate (0.5Nm, $Na_2HAsO_4$) considered as $As_1$ and Sodium (meta) arsenite (0.5Mm, $NaAsO_2$) considered as $As_2$ and 1.5kg cow-dung, 600g vermi-compost and 500g biochar used as protectant. Plant height, leaf number, leaf area, tiller no. hill$^{-1}$, chlorophyll content data are collected from 35DAT to 49 DAT with 7 or 15days interval. Relative water content, shoot and root fresh weight and dry weight data are recorded at 42DAT. The highest plant height was found at control condition at 35DAT, 42DAT and 49DAT. In case of $As_1$ at all the DAT, vermicompost shows the highest result of plant height and in $As_2$ condition biochar shows the highest plant height. Also these amendments increased the fresh and dry weight of shoot and root of rice plants. All parameters decreased significantly at 0.5 mM of arsenic stress. For both $As_1$ and $As_2$ treated plants combined with vermicompost and biochar shows an increasing percentage at all the yield contributing data parameters compare to $As_1$ and $As_2$ treated plant only. Our experiment concludes that rice plant under arsenic stress, vermicompost shows the better percentage of increasing result when plant expost to $As_1$ ($As_1$ (0.5 mM; Sodium arsenate, $Na_2HAsO_4$**)** and in case of $As_2$ (0.5 mM; Sodium meta arsenite, $NaAsO_2$) biochar shows an increasing result compare to all the treatments.

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# ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| FAO | Food and Agricultural Organization |
| IRRI | International Rice Research Institute |
| BRRI | Bangladesh Rice Research Institute |
| ROS | Reactive Oxygen Species |
| CAT | Catalase |
| SOD | Superoxide Dismutase |
| POD | Peroxidase |
| GR | Glutathione Reductase |
| GPX | Glutathione Peroxidase, |
| HM | Heavy Metal |
| MAPK | Mitogen Activated Protein Kinases |
| MMA | Monomethyl Arsenate |
| DMA | Dimethyl Arsenate |
| TMAsO | Trimethyl Arsine Oxide |
| TF | Translocation Factor |
| EPA | Environmental Protection Agency |
| IARC | International Agency for Research on Cancer |
| MDHAR | Monodehydroascorbate Reductase |
| DHAR | Dehydroascorbate Reductase |
| GST | Glutathione S-transferase |
| AOX | Alternative Oxidase |
| PRX | Peroxiredoxins |
| TRXs | Thioredoxins |
| iAs | Inorganic arsenic |
| SOB | Sulphur oxidized bacteria |
| AsA | Ascorbic Acid |
| GSH | Glutathione |
| MAPK | Mitogen-activated Protein Kinase |
| PCD | Programmed Cell Death |
| As(III) | Arsenite |
| As(V) | Arsenate |

| | |
|---|---|
| MAs(V) | Methylarsenate |
| MAs(III) | Methylarsenite |
| DMAs(V) | Dimethylarsenate |
| DMAs(III) | Dimethylarsenite |
| TMAs(III) | Trimethylarsine |
| TMAs(V)O | Trimethylarsineoxide |
| OM | Organic matter |
| ATSDR | Agency for Toxic Substances and Disease Registry |

# CHAPTER I

# INTRODUCTION

Plants encounter various abiotic stresses due to their sessile nature which include heavy metals, salt, drought, nutrient deficiency, light intensity, pesticide contamination, as well as extreme temperatures. These stresses impose major constraints limiting crop production and food security worldwide. Most of the abiotic stresses reduce the availability of $CO_2$ and hinder carbon fixation and contribute to successive reduction of molecular oxygen, which yields excess ROS and impairs the performance of chloroplasts, thus disturbing photosynthetic process (Gill and Tuteja, 2010). ROS damage molecular and cellular components due to the oxidation of biomolecules (lipid, carbohydrates, proteins, enzymes, DNA) and cause plant death (Maurya, 2020). To avert the damages, plants tightly regulate ROS production via the recruitment of enzymatic and non-enzymatic antioxidants. The enzymatic antioxidant system comprising superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), peroxidase (POX), etc. and non-enzymatic antioxidants such as vitamins, flavonoids, stilbenes, and carotenoids quench the excess ROS, thereby providing a shield against oxidative stress (Hasanuzzaman *et al.*, 2020).

Arsenic (As) is a ubiquitous metalloid contaminant. It enters the soil–plant system via natural (geochemical processes) and anthropogenic (mining activity, pesticides containing arsenic, and industrial waste) routes (Bhattacharyya *et al.,* 2003). Inorganic As (iAs) is a recognized human carcinogen, and the intake of large amounts of iAs through rice consumption can pose a serious threat to human health. Unfortunately, arsenic is more readily accumulated in rice grains than in other cereal crops, because of the high mobility and bioavailability of As under submerged conditions such as in rice paddy fields (Su *et al.,* 2010). In addition, the high concentrations of As existing in soils can result in phytotoxicity and reduction of grain yields (Syu *et al.,* 2015). Arsenic (As) pollution in agricultural soils and waters which is rapidly increasing worldwide due to industrialization and urbanization, causes serious environmental issues and adversely influences crop yield and quality (Kim *et al.,* 2016). In plants, As disturbs protein functions due to its high affinity to their sulfhydryl group. Moreover, it disrupts the cell membrane through intracellular lipid peroxidation by generating reactive oxygen species (ROS), such as superoxide anion radical ($O_2^{\bullet-}$), hydrogen peroxide ($H_2O_2$), and hydroxyl radicals ($OH^{\bullet}$), leading to apoptosis (Farooq *et al*., 2016). The ROS generated by As toxicity accumulates in the plant cells. This can severely deteriorate the normal biological functions or processes not only by inducing a negative effect on the maintenance of redox homeostasis but also by impairing the biosynthesis of basic substances that support plant growth, such as carbohydrates, proteins, fats, and nucleic acids (Petrov *et al.,* 2015). However, plants attempt to overcome As toxicity by activating antioxidant scavenging systems that can alleviate the oxidative stress caused by excessive absorption and accumulation of As (Sharma *et al.,* 2017). The alleviation of As-induced oxidative stress and maintenance of redox homeostasis are mostly achieved by two biochemical scavenging systems. The first is an enzymatic antioxidant scavenging system based on several identified enzymes, including superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate (AsA) POX (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate (DHA) reductase (DHAR), and glutathione (GSH) reductase (GR). The second is a non-enzymatic antioxidant scavenging system, such as AsA and GSH, which is reactivated and regulated to scavenge excessive ROS accumulated in the cells.

Rice (*Oryza sativa* L.) is an important cereal which is consumed as staple food by more than half of world's population (Ma *et al*., 2008). The world population is increasing day by day but the crop production is decreasing at an alarming rate. Rice which is the largest cereal crop and staple food in Bangladesh is dominant over all other crops in respect of economic and social significance. Rice belongs to the poaceae family and acts as the main source of calories for almost 40% of the world population. Rice is the staple food for nearly half the world's population, and its continued increased production to meet the enhanced demand due to ever-increasing population faces many challenges. Per capita availability of land and water is increasing at a fast rate, and there is worldwide mass rural-to-urban movement of youth in search of better livelihood reducing the availability of farm labor. Thus, efforts aimed at decreasing As bioavailability in paddy soils and reducing As uptake by rice plants have received much concern in recent years. It has been established that rice accumulates high concentrations of arsenic in its grain compared to other cereal crops (Williams *et al.,* 2007). The arsenic in rice grains is present primarily as inorganic arsenic (arsenite and arsenate) and dimethylarsinic acid (DMA) (Meharg *et al.,* 2008). Traces of monomethylarsinic acid (MMA) and tetramethylarsonium have also been identified

(Hansen *et al.,* 2011). The accumulation of inorganic arsenic is of concern as it is a nonthreshold, class 1 carcinogen (NRC, 2001). It has been proposed that rice accumulates higher concentrations of arsenic due to its cultivation in anaerobic conditions, where arsenic is more available (Xu *et al.,* 2008). Not only is the accumulation of arsenic in rice grains a major concern, but rice growing in arsenic contaminated environments can have reduced yields (Panaullah *et al.,* 2009). The addition of organic matter to soil has many important roles. For example, it can improve the soil structure as well as being a nutrient supply of key elements such as nitrogen, phosphorus and sulphur (Batey, 1988).

Organic matter has a major role in the mobilisation of arsenic from paddy fields (Williams *et al.,* 2011). This is because microbes utilising the organic matter consume oxygen that leads to a decrease in redox potential, which in turn leads to arsenic dissolution from FeOOH (Rowland *et al.,* 2009). Organic matter may also have two other roles in arsenic availability in soils: by desorbing arsenic species from soil surface exchange sites (Weng *et al*., 2009), and dissolved organic matter (DOM) complexing arsenic species (Williams *et al.,* 2011). In order to regain soil fertility by improving its texture and activities, the use of organic residues is being advocated.

Cow dung is being used for different purposes from the ancient time and has a significant role in crop growth because of the content in humic compounds and fertilizing bio-elements available in it. The application of acidified cow dung slurry along with sulfur-oxidizing bacteria (SOB) and molasses led to a tremendous decline in antioxidant enzymes activities, and this might be due to the greater improvement in nutrient uptake under low pH conditions, which might have diluted the heavy metal stress to the plants, and relatively less activity of antioxidant enzymes was observed (Ashraf *et al.,* 2022). The lower production of antioxidant enzymes is an index of less oxidative stress to the plant.

Vermicompost is a product transformed by organic residues using earthworms (Blouin *et al.,* 2019). A previous study showed that vermicompost is an excellent organic fertilizer, with physical, chemical, and biological properties that could improve soil fertility and control crop diseases (Patnaik *et al.,* 2020). The study of (Fernandaez-Gomez *et al.,* 2011) showed that vermicompost has high microbial functional diversity and the potential to be used for the treatment of pesticide pollution in agricultural production. Jahanbakhshi and Kheiralipour *(*2019), demonstrated that vermicompost was a good organic fertilizer with an appropriate carbon and nitrogen ratio, acidity, as well as salinity. Al Jaonui *et al. (*2019) also indicated that vermicompost can improve the chemical composition of jujube coconut fruit and increase the nutritional and medicinal value. In consideration of the multiple benefits of vermicompost on crop growth and development, we wonder whether the incorporation of vermicompost into rice cultivation can promote the rice seedlings' performance to cope up against heavy metal stress such as arsenic stress.

Biochar (BC) soil amendment has been widely reported for the reduction of As uptake and toxicity in plants. Under As stress, BC application increases plant growth, biomass, photosynthetic pigments, grain yield, and quality (Rizwan *et al.,* 2016). Qiao *et al.* (2018) found that biochar can enhance As reduction and release in flooded paddy soils through stimulation of the active arsenate-respiring bacteria Geobacter species, which probably increases the bioavailability of As to rice plants Similarly, As-induced oxidative stress (generation of $H_2O_2$ and lipid peroxidation) in plants is reduced – significantly by arbuscular mycorrhizal fungi (AMF) inoculation. The alleviation potential of AMF is more evident with the increase in severity of As stress. Colonization of AMF in food crops results in higher activity of the antioxidant enzymes [superoxide dismutase (SOD), CAT, and guaiacol peroxidase] and increases the concentrations of antioxidant molecules (carotenoids, proline, and α-tocopherol) (Sharma *et al.,* 2017).

Bangladesh has a long history of rice cultivation. Rice is the staple food for about 156 million people of the country. The population growth rate is 2 million per year, and if the population increases at this rate, the total population will reach 238 million by 2050. An increase in total rice production is required to feed this ever-increasing population. According to WHO recommendation the permissible limit of arsenic in rice is 1mgkg$^{-1}$. The As contaminated areas in Bangladesh have shown as more than 20 mg Askg$^{-1}$ soil (Ali *et al*., 2003). A number of mitigation approaches have been tried to control arsenic accumulation in plants like phyto-remediation that the removal of contaminants with the help of green plants includes the mitigation of arsenic accumulation in plants which is eco-friendly and available to farmers. Incorporation of organic manures significantly reduced the arsenic uptake by different plant parts of rice is more pronounced and consistent with FYM (Sinha and Bhattacharyya, 2011). Therefore, the use of organic soil amendment such as cowdung, vermicompost and biochar could be an approach to alleviate the accumulation of As of rice plants. Considering these facts this study has been designed with the following objectives:

I. To investigate the arsenic stress-induced morpho-physiological damages in rice plant.
II. To know the role of organic amendments on rice to mitigate the arsenic stress.

## CHAPTER II

# REVIEW OF LITERATURE

### 2.1 Rice

Rice (*Oryza sativa* L.) is one of the most important basic foods on earth. It is the staple food of more than three and a half billion people worldwide. Rice belongs to the Poaceae family and acts as the main source of calories for almost 40% of the world population. Rice has been cultivated for centuries, even though it is very labor-intensive and requires substantial water and warm, humid weather conditions to grow. Most rice is produced in Asia, where some 90 per cent of the world's rice is grown on 140 million hectares of land-an area the size of South Africa. Rice is thus the main source of income for farmers in Asia. Worldwide, rice is the agricultural commodity with the third-highest worldwide production, with about 761.5 million tons produced in 2018. Rice is produced in about 120 countries worldwide, but China (about 214 million tonnes) and India (about 173 million tonnes) together account for more than 50% of both rice production globally. Rice provides 21% of global human per capita energy and 15% of per capita protein (IRRI, 2012). Although rice protein ranks high in nutritional quality among cereals, protein content is modest. Rice is primarily composed of carbohydrate, which makes almost 80% Trusted Source of its total dry weight. Most of the carbohydrate in rice is starch. Rice also provides minerals, vitamins, and fiber, although all constituents except carbohydrates are reduced by milling. Rice (*Oryza sativa* L.) is considered as the staple food for more than half of the world’s population (Kosolsaksakul *et al.*, 2014). There are three types of rice cultivars are grown in worldwide which are indica, japonica, and javanica classified on the basis of morphological characters of rice (Purseglove, 1985). Indica rice cultivars are generally adapted to the areas of tropical and sub-tropical monsoon climate. The rice cultivar which grown in Bangladesh is belongs to the sub-spices indica (Alim, 1982). Rice is the fundamental source of food for more than one third of the world’s population. It is the second most important crop in the world after wheat, more than 90 per cent of which is grown in Asia.

## 2.2 Abiotic Stress

In agriculture, abiotic stress is one of the critical issues impacting the crop productivity and yield. World agriculture is facing a lot of challenges like producing 70% more food for an additional 9.7 billion people in world by 2050 while at the same time fighting with poverty and hunger, consuming scarce natural resources more efficiently and adapting to climate change (Wilmoth, 2015). However, the productivity of different crops is not increasing in collateral with the food requirement. The lower productivity in most of the cases is assigned to different abiotic stresses. A major area of concern to cope with the increasing food requirements is reducing crop losses due to various environmental stresses (Shanker and Venkateswarlu, 2011). Plants can feel various abiotic stress caused by higher concentrated toxic substances. Sometimes it caused by much water (flood), shortage of water (drought) also by using too much fertilizer. Abiotic stresses change the plant metabolisms which are affect plant growth, development and productivity. Due to higher stress condition intolerable metabolic activities occur in plant cells and reducing plant growth, at extreme cases plants may die (Hasanuzzaman *et al.*, 2012).

Plants encounter various abiotic stresses due to their sessile nature which include heavy metals, salt, drought, nutrient deficiency, light intensity, pesticide contamination, as well as extreme temperatures. These stresses impose major constraints limiting crop production and food security worldwide. Abiotic stresses primarily reduce the photosynthetic efficiency of plants, due to their negative consequences on chlorophyll biosynthesis, performance of the photosystems, electron transport mechanisms, gas exchange parameters, and many others. The extreme environmental conditions trigger excessive production of ROS. ROS damage molecular and cellular components due to the oxidation of biomolecules (lipid, carbohydrates, proteins, enzymes, DNA) and cause plant death (Bhuyan *et al.*, 2020). To avert the damages, plants tightly regulate ROS production via the recruitment of enzymatic and non-enzymatic antioxidants. The enzymatic antioxidant system comprising superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), peroxidase (POX), etc and non-enzymatic antioxidants such as vitamins, flavonoids, stilbenes, and carotenoids quench the excess ROS, thereby providing a shield against oxidative stress (Hasanuzzaman *et al.*, 2020).

Over the year metalloids and heavy metals have received substantial consideration in multidisciplinary areas of environmental and geosciences due to their bio-magnification, bioaccumulation along with negative ecological impacts (Shahid *et al.,* 2020). Plants have the capability to uptake and translocate toxic metals in different parts of their body as reported in several studies (Sofy *et al.,* 2020). Uptake of HMs in plants not only inhibits the growth attributes of plants, but also induces toxic effects on consumer health (Peters *et al.,* 2018). Various studies have revealed the harmful impacts of HMs on human health and plant growth and development (Farid *et al.,* 2018). High concentrations of harmful metals particularly in soil significantly affects physiological and morphological traits of the plants (Shanying *et al.,* 2017). Under HM stress, plants showed evident symptoms of structural transformation and inhibition of the photosynthesis process (Khan *et al.,*2015). Different HMs such as zinc (Zn), cadmium (Cd), aluminum (Al), chromium (Cr), nickel (Ni) and metalloids like arsenic (As) diminish plant development and growth by producing a number of metabolic alterations in plants (Anjum *et al.*, 2017). Most likely, heavy metal ions stay in the cytoplasm and cause oxidative stress through the excessive formation of reactive oxygen species (ROS), which ultimately restrict cell metabolism (Pongrac *et al.*, 2009). All environmental stresses (biotic and abiotic) generate oxidative stress which can easily harm cell components and cause their dysfunction pursued by the uptake and over production of ROS at high rates. Enzyme complexes of nicotinamide adenine dinucleotide phosphate (NADPH) oxidases generally involved in the generation of ROS, which then usually accumulate in different organelles of cell especially cytoplasm, mitochondria and nucleus (Zaid and Wani, 2019). Uncontrolled production of ROS results in protein denaturation, carbohydrates oxidation, oxidation of RNA and DNA, lipid peroxidation in cellular compartments, and it severely affects enzymatic activity in plants (Noctor and Foyer, 1998). Heavy metals produce oxidative stress by disturbing the ROS stability in cells, and then induce the antioxidant mechanism of plants. For examples, excessive accumulation of Cr leads towards the generation of ROS and without doubt oxidative stress as well (Xu *et al.,* 2018). Heavy metals at toxic levels obstructs normal metabolic processes in different ways including displacement and disturbance of protein building blocks (Hall, 2002), adversely altering and affecting the authenticity of the cytoplasmic membrane, repressing critical events in plants such as respiration, photosynthesis and enzymatic activities (Hossain *et al.,* 2012). In plants, heavy metal stress starts diverse signaling paths including signaling of mitogen activated protein kinase (MAPKs) are group of protein kinases that perform a vital role during signal transduction through modulating gene transcription in the nucleus as an appropriate response to changes occurs in the cellular environment, calcium dependent signaling, hormone signaling and signaling of ROS (Kumar and Trivedi, 2016). These signaling pathways increase the expression of responsive stress genes in plants (Tiwari and Lata, 2018).

### 2.3 Arsenic Stress

Arsenic (As), a potentially toxic metalloid released in the soil environment as a result of natural as well as anthropogenic processes, is subsequently taken up by crop plants. In rice grains, As has been reported in Asia, North America and Europe, suggesting a future threat to food security and crop production. Specific transporters mediate the transport of different species of As from roots to the aboveground parts of the plant body. Accumulation of As leads to toxic reactions in plants, affecting its growth and productivity. Increase in As uptake leads to oxidative stress and production of antioxidants to counteract this stress. Arsenic (As), a potentially toxic metalloid, is a naturally occurring element ubiquitous to all soils (Williams *et al*., 2005). It is the 20th most common element in the earth's crust (Mandal and Suzuki, 2002). Soil contains 1.5–3.0 mg $kg^{-1}$ As. As is present in inorganic forms in various minerals in soil, the important ones being realgar, arsenopyrite, etc. From the minerals, As in both inorganic and organic forms, gets mobilized due to natural and human activities, and becomes more readily available to living organisms. Among the natural sources of mobilization of As, weathering of As-containing minerals (Smedley, 2006) and the activities of microorganisms (Turpeinen *et al.,* 2002) are prominent. On methylation by microbes, As is released as monomethyl arsenate (MMA) or dimethyl arsenate (DMA) (Bentley and Chasteen, 2002). Some microbes utilize As in their metabolism, and release the toxic trimethyl arsine oxide (TMAsO) gas which is subsequently released to the atmosphere (Mandal and Suzuki, 2002). Human activities that release more As in bio-available forms include application of As pesticides, use of As in paints to be subsequently released by molds and bacteria, mining of metals and heavy extraction of groundwater. Insecticides [calcium arsenate ($Ca_3As_2O_8$) and lead arsenate ($PbHAsO_4$)], herbicide [sodium arsenite ($NaAsO_2$)], rodenticides arsenious oxide ($As_2O_3$) and sodium arsenite ($NaAsO_2$) contain As (ICAR, 2009). Their residues remain in the soil, get dissolved in the groundwater and become easily available to living organisms. As-containing wallpaper paints, fed upon by molds and bacteria, led to release of the 'Gosio' gas, which was identified to be trimethyl arsine (Woolson, 1977). Metal mining, processing of ores and related activities, has contributed to the release of As in the soil and groundwater (Smedley, 2006) in several countries including Thailand, Ghana, Turkey, England, Serbia, Bosnia, Poland, USA and Canada (Barringer and Reilly, 2013).

A very significant means of mobilization of As is the extraction of groundwater from shallow aquifers in various countries, where surface water is contaminated by disease causing microorganisms. Groundwater is generally more vulnerable to As contamination than surface water because of the interaction of groundwater with aquifer minerals and the increased potential in aquifers for the generation of the physiochemical conditions favorable for As release (Smedley, 2006). As contaminated groundwater is found in many countries like Bangladesh, India, China, Argentina, Chile, Vietnam, Hungary and Mexico (Smedley, 2006; Chakraborti *et al.*, 2008). Deep aquifers containing high As levels have also been reported in the Mekong Valley, Vietnam, where high amount of groundwater extraction has resulted in subsidence of the land level by almost 3cm per year as measured by satellite based radar images from 2007 to 2010 (Erban *et al.*, 2013), leading to speculations that similar deep aquifers of groundwater may not remain As free over the years to come.

In soil, As exhibits a number of oxidation states, the common ones being $As^{5+,}$ $As^{3+}$ and $As^{3-}$. In aerobic soils, As is mainly present in the oxidized form as arsenate ($As^{5+}$). While in anaerobic environments like paddy soil, it mainly exists in the reduced form as arsenite ($As^{3+}$) (Takahashi *et al.*, 2004). $As^{3+}$ by dint of its availability, mobility and phytotoxicity, is the most harmful species of As for the rice crop. With the use of As-contaminated water in irrigation and due to the various processes of its mobilization, the levels of As in soils have escalated, affecting the agricultural system and resulting in the uptake of the element by crop plants. The translocation factor (TF) for As is higher in rice (0.8) compared to the other crops like wheat (0.1) and barley (0.2) (Xu *et al.*, 2008). The amount of As in paddy soil and soil solution stand elevated (Brammer and Ravenscroft, 2009). The scenario is the worst in rice fields in affected areas such as Bangladesh, India, China and Thailand, where rice is the staple food for most of the people (Garnier *et al.*, 2010). Humans also have been exposed to As through drinking of As-contaminated water and consumption of As-contaminated crops. The contaminated groundwater used to cultivate vegetables and rice for human consumption may be an important pathway of As ingestion and exposure to chronic As (Chakraborti *et al.,* 2004). In July 2014, the WHO set worldwide guidelines for what it considers to be safe levels of As in rice, suggesting the maximum of 200 µgkg$^{-1}$ for white rice and 400 µgkg$^{-1}$ for brown rice (Sohn, 2014). According to the US Environmental Protection Agency (EPA) and the International Agency for Research on Cancer (IARC), As and its compounds have been ranked as a Group 1 human carcinogen. As present in drinking water or contaminated crops can have severe impact on the health of human beings as well as other animals (Ng *et al.,* 2003). As being taken up from soils, plant roots are the initial tissues to be exposed. The metalloid inhibits the extension and proliferation of roots. Upon its translocation to the shoot, As can inhibit plant growth severely by hindering the expansion and biomass accumulation as well as compromising the plant reproductive capacity through losses in fertility and yield (Rahman *et al.,* 2008). At sufficiently high concentrations, As interferes with critical physiological and metabolic processes such as cellular membrane damage, and increase in antioxidant mechanisms, which can even lead to the death of the plant (Garg and Singla, 2011).

The rice fields of Bangladesh are heavily irrigated with As contaminated water obtained from millions of shallow tube wells installed by the country's farmers. As a result, in several areas of the country, As content in rice grain has been found to rise to alarming levels which bears threat to food safety and also crop production in future (Rahman and Hasegawa, 2011). The highest mean As content in rice grains is found in Faridpur (0.95 µgg$^{-1}$), followed by Rajbari (0.76 µgg$^{-1}$), Golapganj (0.57 µgg$^{-1}$), Dinajpur (0.54 µgg$^{-1}$), Srinagar (0.48–0.53 µgg$^{-1}$) and Sonargaon (0.46 µgg$^{-1}$) districts of the country (Williams *et al.,* 2006). Of the overall mean of 0.13 µg/g As in Bangladesh rice grains, about 80% is found to be inorganic, which is more toxic than the organic forms (Williams *et al.,* 2006; Zavala *et al.,* 2008). Organic As species are more easily transported to the shoots in rice plants than the inorganic species (Carey *et al.,* 2010). The amount of As accumulated in different parts of rice plants follows the order: straw > husk > grain (Abedin *et al.,* 2002; Bhattacharya *et al.,* 2010). Abedin *et al.* (2002) also showed that the concentrations of As in root and straw do not vary too much, suggesting that translocation from roots to straw takes place readily. However, its concentration decreases considerably in husk and yet further in grains. Accumulation of As by rice plants results in various toxic reactions and affects the growth, morphological and physiological processes of the plant (Abbas *et al.*, 2018). Increasing As content of growing medium or increased accumulation reduces chlorophyll content of the rice plants, the extent of reduction varying slightly among different varieties. This results in reduction in the rate of photosynthesis, leading to reduction in root and shoot growth and grain yield (Halim *et al.*, 2014). Yield reduction has also been observed with the increase in As content in soils in subsequent cropping years due to retention of As from previous years' contaminated irrigation water in the soils (Panaullah *et al.*, 2009). A physiological disease called straight head is also attributed to As toxicity (Rahman *et al.*, 2008). The symptoms of this disease include reduction in the sterility of spikelets, decreased grain yield, and in severe cases, non- formation of the panicles or heads. The level of sensitivity to As toxicity varies in different rice genotypes (Chaturvedi, 2013). Moreover, all the As species cause dissimilar levels of toxicity in rice plants. It is well established that As accumulation leads to various toxic reactions in plants. However, some plants are tolerant to elevated levels of As and accumulate high levels of the metalloid in their bodies.

## 2.4 Abiotic Stress Induced Oxidative Stress

Plants are sessile organisms that normally grow under field conditions. Therefore, in most regions of the world, they face excess light (sunny hours) during the hot season. Besides, different environmental/abiotic stresses generated due to anthropogenic activities and harsh climate changes are contributing in inducing oxidative stress through over generation of ROS. It is well established that chloroplasts, mitochondria, peroxisomes, apoplast, and plasma membranes are the primary sites of cellular ROS generation but chloroplasts are the leading sites for ROS production (Singh *et al.*, 2019). Most of the abiotic stresses reduce the availability of $CO_2$ and hinder carbon fixation and contribute to successive reduction of molecular oxygen, which yields excess ROS and impairs the performance of chloroplasts, thus disturbing photosynthetic processes (Gill and Tuteja, 2010). However, ROS generation greatly varies with plant species, genotypes, stress tolerance level, and duration of stress exposure. Redox reactions (transfer of electrons between a donor and an acceptor) are very common in living organisms, which is responsible for the production of ROS (Decros *et al.*, 2019). In plant cells, redox homeostasis is developed in consequence of the equilibrium between the generation of ROS and the functioning of the antioxidant enzymes where efficient defense system in plants keeps the proper balance between

ROS generation and elimination (Paciolla *et al.,* 2016). A basal level of ROS, which is maintained above cytostatic or below cytotoxic concentration is, therefore, indispensable for proper ROS or redox signaling in cells, and this level is maintained by the balance between ROS production and ROS scavenging (Hasanuzzaman *et al.,* 2019). Therefore, scientists used the term “redox biology” to refer to ROS as signaling molecules to control and uphold the usual physiological activities of plants (Schieber and Chandel, 2014). Redox signaling has been discerned as the equilibrium between low levels of ROS functioning as signals to activate signaling cascades that adjust usual plant functions and high levels of ROS causing oxidative cellular damage (Decros *et al.,* 2019). Therefore, a steady balance between ROS generation and ROS scavenging systems is strongly synchronized over time and space, working together with the cellular redox-sensitive components to shape and finely adapt downstream signaling procedures in a cell-specific and context-specific approach (Panieri and Santoro, 2011). However, any disturbance in the equilibrium of ROS generation and ROS scavenging by antioxidants leads to ROS over accumulation resulting in oxidative stress under various abiotic stress conditions (Hasanuzzaman *et al.,* 2012). Oxidative stress causes lipid peroxidation, damages nucleic acids and proteins, and alters carbohydrate metabolism, resulting in cell dysfunction and death (Hasanuzzaman *et al.,* 2019).

## 2.5 Effect of Arsenic on Rice

Rice (*Oryza sativa* L.) grown under flooded conditions favor greater soil arsenic solubility and uptake into the plant. Movement of arsenic into rice is mediated by silicon transporters (Ma *et al.*, 2008) that inadvertently transport arsenite due to its similarity to silicic acid. This makes rice a major dietary source of arsenic, especially for populations with relatively low drinking water concentrations of arsenic (Davis *et al.,* 2017). Thus, awareness of the human health risk posed by arsenic contaminated rice consumption has become a more widely recognized threat to food safety (Davis *et al.,* 2017).

The most common rice cultivation practice is flooded irrigation, and in absence of rains, groundwater is used. The repeated use of As-laden groundwater has resulted in As build-up in soil through the years (Upadhyay *et al.,* 2019). Rice can accumulate As in several-fold higher levels than other cereal crops such as wheat and maize (Upadhyay *et al.,* 2019). In reducing conditions of flooded paddy fields, arsenite [As(III)] is present in higher concentrations than arsenate [As(V)] (Meharg and Jardine, 2003). Other As forms also exist, which include organic methylated forms such as methylarsenate [MAs(V)], methylarsenite [MAs(III)], dimethylarsenate [DMAs(V)], dimethylarsenite [DMAs(III)], trimethylarsine [TMAs(III)] trimethylarsineoxide [TMAs(V)O] (Upadhyay *et al.,* 2019b), and thio-arsenates (Kerl *et al.,* 2019). Various factors such as pH, redox potential, dissolved organic carbon, organic matter, and biotic factors play a significant role in determining the bioavailability of various As species in the soil system (Majumdar and Bose, 2018). Rice plants release oxygen through their roots, and this leads to iron plaque formation on the root surface (Majumdar *et al.,* 2020). Iron plaque can act as a major sink or source of As to rice plants (Tripathi *et al.,* 2014). The adsorption of As by iron plaque increases the rhizospheric concentration of As around rice roots (Hu *et al.,* 2019). Further, As itself affects iron plaque formation, and there are also varietal influences on iron plaque formation due to differences in root oxidation abilities of different varieties (Lee *et al.,* 2013). The transporters involved in the uptake of As and translocation from root to shoot and grains play a crucial role in As build-up in plants. Significant progress has been made in understanding holistic biochemical, proteomic, and transcriptomic changes in response to As stress in plants (Srivastava *et al.,* 2015). However, the development of low-As accumulating rice varieties through the use of genes/proteins by employing molecular techniques is not yet feasible. The As accumulation in rice grains varies significantly in different rice varieties (Norton *et al.,* 2009).

Rice is a semiaquatic plant, and sufficient water availability plays a key role in achieving proper rice growth and productivity (Islam *et al.,* 2019). The As cycle is a complex phenomenon and is influenced by various factors. Owing to continued water stagnancy in rice fields, anaerobic conditions are generated, and As release from the dissolution of Fe oxy-hydroxides is promoted (Majumdar and Bose, 2018). As(III) tends to become attached to Fe oxides more frequently than As (V) and its concomitant release by microbial reduction, alteration in pH and redox coupling, and changes in organic matter results in increased bioavailability of more soluble As(III) (Majumdar and Banik, 2019). Water management must be practiced in such a way so as to reduce As loading in rice grains without affecting rice yields.

Rahman *et al.* (2007) conducted a pot experiment by used sodium arsenate ($Na_2HAsO_4.7\ H_2O$) as the source of arsenic with five high yielding, popular and widely cultivated Boro rice varieties of Bangladesh, namely BRRI dhan28, BRRI dhan29, BRRI dhan35, BRRI dhan36 and BRRI hybrid dhan1 and the result was increased soil arsenic concentrations drastically reduced rice yield in all varieties. The number of panicle was found to be decreased significantly ($p < 0.05$) with the increase of soil arsenic concentrations as well. True (filled) grain production was also found to be decreased significantly ($p < 0.05$).

## 2.6 Organic Amendments and Crop Productivity

Application of organic matter (OM) to soils can improve soil physical (soil structure and aeration), chemical (cation exchange capacity, nutrients, and pH buffer), and biological (microbial activity, nutrient mineralization and immobilization) properties, and is therefore a common agricultural practice. However, many studies have shown that the addition of OM to soils may affect the bioavailability and mobility of As through redox reactions, anions (phosphate, silicate, and DOC), competitive adsorption, and As–OM complexation (Wang and Mulligan, 2006). Soil organic matter affects soil physical, chemical and biological properties and is thus agronomically important because these factors affect crop yields. Organic amendments such as manures, composts and plant residues are frequently used in crop production systems as alternatives to inorganic fertilizers, to restore degraded soils and ameliorate physicochemical constraints. Soil organic matter is a critical component of productive soils. It influences a wide range of physical, chemical and biological attributes and processes, including the formation and stabilization of soil aggregates, nutrient cycling, water retention, disease suppression, pH buffering and cation exchange capacity (Murphy, 2015). Consequently, organic matter is important from an agronomic perspective because it has the potential to influence crop yields via any of these processes (Oelofse *et al.,* 2015). Soil organic matter is a complex mixture of organic compounds such as plant residues, microbial products and rhizosphere inputs in various stages of decomposition (Kögel-Knabner and Rumpel, 2018). Soil organic matter contains around 50% carbon (C) (Pribyl, 2010), it also contains high levels of nitrogen (N), phosphorous (P) and sulfur (S) in fixed ratios (Kirkby *et al.*, 2011) that are released to the plant as they mineralize. This CNPS stoichiometry has implications for building soil organic matter: every unit increase in C requires a fixed input of N, P and S (Kirkby *et al.,* 2013). Increasing soil organic matter content can be achieved by the application of organic amendments, which contain C and other nutrients, or by the addition of inorganic fertilizers and a source of C such as in crop residues (Alvarez, 2005). Organic amendments have been applied to soils in order to restore or maintain soil fertility, structure and productive capacity since the beginning of agriculture (Churchman and Landa, 2014).

Additionally, organic amendments may be used to improve plant nutrient uptake, maximize nutrient-use efficiencies or reduce environmental impacts compared to inorganic fertilizers (Edmeades, 2003). Organic amendments can increase soil carbon and, by a series of interdependent processes, improve biological activity, soil structure, cation exchange, water holding capacity and so on (Lal, 2006). These changes can ultimately lead to an increase in crop yields (Diacono and Montemurro, 2010). However, organic amendments can also provide significant nutrients to the plant in mineral form, which directly improve crop yields via fertilization. Organic matter has a greater affinity for As adsorption due to the formation of an organo-As complex, thereby reducing As availability to plants (Mitra *et al.,* 2017).

### 2.7 Effect of organic amendments on rice under arsenic stressed condition

Arsenic (As) is a toxic and carcinogenic metalloid that has received significant attention due to its geogenic release as a major source of groundwater As contamination globally, especially in South and Southeast Asian countries (Hussain *et al.,* 2021). In paddy soils, irrigation to rice (*Oryza sativa* L.) crop with As-rich groundwater may increase As accumulation in rice grain causing potential risk for human food chain contamination (Chen *et al.,* 2016). This is because approximately half of the world's population consume rice grain as staple food (Islam *et al.,* 2019). Rice plants possess high As uptake due to cultivation under reduced (paddy) soil conditions that favor As(V) transformation to As(III), and ultimately translocation to rice grain. Arsenic transfer to rice grain also depends on the presence of microbial species, redox potential, pH and organic or inorganic amendments (Majumder and Banik, 2019). For example, some researchers have reported that As concentration in rice tissue may increase up to 1 mg $kg^{-1}$ DW irrigated with As contaminated water (0.13 mg $L^{-1}$) (Carrijo *et al.,* 2019). Similarly, Irem *et al.* (2019) conducted a pot experiment using two different rice genotypes. The authors reported that application of different amendments (farmyard manure, phosphate and iron sulfate) reduced grain As concentration by 24% in Basmati-385 and 14% in BR-1 genotype grown in As-contaminated soil from Punjab, Pakistan. In another study, Kang *et al.* (2018) reported that addition of the poultry manure, agri-lime, steel slag and gypsum had significant effect on reducing soil As mobilization. The treatments having agri-lime increased As concentration by 32%, while steel slag and gypsum decreased As by 65% and 63%, respectively. In a recent study, Alam et *al.* (2020) observed 66–76% decrease in grain As content under the influence of biochar, arbuscular mycorrhizal fungi, silica gel, selenium and sulfur (S) amendments.

Chlorophyll content is an important stress index as a significant reduction in leaf chlorophyll content is a common phenomenon under stressful conditions (Dehshiri and Paknyat, 2014). Similarly, proline and catalase (CAT) both are sensitive indicators of salt and other environmental stresses in different food crops (Gharsallah *et al.*, 2016). These biochemical parameters change during salt and drought as well as arsenic (As) stress conditions in food crops (Swarnakar, 2014). Reactive oxygen species (ROS) are the by-products of aerobic metabolism, and these components are often confined to certain cellular compartments (Gill and Tuteja, 2010). Stress conditions trigger ROS production, leading to the occurrence of oxidative stress in plant cells. The antioxidant enzymes are a group of important defense compounds that actively participate in the detoxification of ROS such as superoxide and $H_2O_2$, especially under stress conditions (Esfandiari *et al.*, 2007). For instance, CAT is involved in scavenging $H_2O_2$ (Horemans *et al.*, 2000) and thus contributes to the plant defense mechanism against oxidative stress (Gill and Tuteja, 2010).

Arsenic contamination is one of the main abiotic stresses that limit plant growth and leads to the deterioration of food quality by its entry into the food chain. Over the last several decades, the severity of As stress has increased to become a global problem for the cultivation of various food crops (Bailey-Serres *et al.*, 2012). This metal has severe effects on seedling growth, root anatomy, lipid peroxidation, electrolyte leakage, $H_2O_2$ accumulation, root oxidizability, and the activities of antioxidant enzymes in rice. CAT activity decreases in response to As exposure and corresponds to the decrease of $H_2O_2$ content as well. Arsenic causes the reduction of root elongation in food crops due to the increase of lipid peroxidation (Singh *et al.*, 2007). Arsenic-stressed plants show reduced growth and pigment content in food crops. Total chlorophyll, CAT, and ascorbic acid content are drastically reduced in food crops due to the imposition of excess metals or metalloids such as As, cadmium, and lead ( Srivastava and Sharma, 2013).

Organic amendments influence plant growth and As accumulation in rice plants (Norton *et al.*, 2013). Arsenic mobility is strongly dependent on redox potential ($E_h$). The $E_h$ of soils can be decreased by amending with OMs, which leads to an increase in As dissolution from iron oxides and hydroxides and the transformation of the As species in soils (methylation and volatilization) (Jia *et al.,* 2013). Some reports have shown that dissolved OM is able to mobilize As from the sorption sites of soil minerals by competition (Sharma *et al.*, 2011). Bauer and Blodau (2006) also found that competition adsorption is more important than redox reactions for As from soil solid phases released by dissolved OM. Addition of amendments, possibly triggered antioxidants defense mechanism in rice plants producing high glutathione. Glutathione in rice plants can decrease As stress by conversion of As(III) to As(V) and it can improve photosynthetic activity (Irem *et al.*, 2019).

Application of organic amendments such as CD, FYM, and SCB to rice plants under As stress can provide essential macro- and micro-nutrients (e.g., N, P, K, Zn). Banik *et al.* (2006) reported that under inorganic fertilizer and CD application, CD improved annual grain yield by 3.47 t $ha^{-1}$ compared to other amendments. Also, CD application enhanced soil organic carbon and total N contents (0.72% and 6.21 g $kg^{-1}$). Hence, CD application under As-contaminated irrigation water application could possibly enhance soil organic carbon that may help in immobilizing As in paddy soil and improving rice plant growth (Khaleda *et al.*, 2018). In another study, Khaleda *et al.* (2018) indicated that application of Zn and CD separately to two rice genotypes increased grain yield significantly in a field experiment in Bangladesh. Arsenic accumulation by rice in paddy soil is controlled by many factors, including pH, $E_h$, organic matter content and nutrients (Hussain *et al.,* 2021). The paddy soil under flooded conditions has dynamic redox conditions that may alter As speciation and mobility. Iron concentration in soil can lead to formation of Fe plaque on rice plant roots, and the presence of Fe-hydro(oxide)s at root-soil interface bind As in paddy soils (Qiao *et al.,* 2018). Microbes can play an important role in As speciation under reducing conditions. The CD contains methanogenic bacteria and As methylating bacteria (Islam *et al.*, 2005). For instance, Rahman *et al.* (2014) reported that As(V) was transformed to As (III) form before As methylation in the presence of CD as an amendment that acts as the prime source for As(V) reduction. The authors reported that CD have the anaerobic bacteria that remained in soil solution after the depletion of aerobic bacteria and as such converted As(V) to As(III) ending ultimately with methylated As species.

Vermicompost characterized as an excellent nutrient-rich biological fertilizer that has good physical structure, is associated with high microbial activity, and has large amounts of humic substances (Doan *et al.,* 2014). VC contains a mass of humic acids that have the capacity to alter the fraction distribution of heavy metals and possesses carboxylic acids on the molecular structure that responses to protons exchange processes between weak organic acids and heavy metal cations. Thus, VC may be identified as an environment friendly soil amendments (Durán et al., 2006). Shohel *et al.* (2020) conducted a pot experiment at which, arsenic toxicity adversely affects all the yield related attributes of BRRI dhan47 and the application of vermicompost with inorganic fertilizers decreased the adverse effects of high arsenic toxicity on rice plant and improved plant growth parameters. The use of organic soil amendment such as vermicompost could be an approach to alleviate the accumulation of As of rice plants.

Biochar as a black carbon or a charcoal is produced from the pyrolysis of biomass process in the absence of oxygen or the presence of partial oxygen (Woolf *et al.*, 2010). Biochar application to soils has multiple benefits on agriculture. The high cation exchange capacity (Thomas *et al.,* 2013), specific surface area (Forján *et al.,* 2018), and porosity (Nie *et al.,* 2018) of biochar have increase the soil hydraulic conductivity (Chaganti and Crohn, 2015), water holding capacity (Cornelissen *et al.,* 2013), organic matter content (Tang *et al.,* 2020), bulk density (Huang *et al.,* 2019). Moreover, adding the biochar to soils led to an increase in the soil cation exchange capacity (CEC) (Liang *et al*., 2006). Lehmann *et al*. (2003) also indicated that the application of charcoal to the soil improved the soil available potassium. Application of biochar also can improve nutrient cycles. In wheat culture, biochar addition at 10% in combination with urea improved agronomic efficiency of N by 63% (Abbas *et al.*, 2017). Many studies have reported that biochar has the potential to mitigate plant stresses (Paneque *et al.,* 2016; Kumar *et al.,* 2018). Paneque *et al*. (2016) found that biochar dosed at 15 t ha$^{-1}$ significantly increased water-use efficiency of sunflower plants grown under water-deficient conditions. Likewise, biochar can alleviate Zn uptake in Zn-contaminated soil and improve the growth of *Ficus benjamina* under metal stress conditions (Kumar *et al.,* 2018).

## CHAPTER III

# MATERIALS AND METHODS

This chapter shows a short description about experimental period, site description, climatic condition, crop or planting materials, treatments, experimental design and layout, crop growing procedure, fertilizer application, uprooting of seedlings, intercultural operations, data collection and statistical analysis.

### 3.1 Location

The experiment was conducted at the Experimental shed of the Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka during the period from January-May, 2021. The location of the experimental site has been shown in Appendix III.

### 3.2 Soil

The soil of the experimental area belonged to the Modhupur tract (AEZ No. 28). It was a medium high land with non-calcarious dark grey soil. The pH value of the soil was 5.7.

### 3.3 Climate

The experimental area was present under the subtropical climate and characterized by high temperature, high humidity and heavy precipitation with occasional a blast of winds during the period from March-April.

### 3.4 Materials

### 3.4.1 Plant materials

BRRI dhan89 was used in the experiment. Features of this variety are given below:

**BRRI dhan89:**

BRRI dhan89 variety is grown in boro season. It released by Bangladesh Rice Research Institute (BRRI) in 2018. It completes its life cycle 154-158 DAS. It attains a plant height 106 cm. 1000-seed weight is 24.4g. Protein content is 28.5% and yield is 8-9.7ton ha$^{-1}$.

### 3.4.2 Earthen pot

Empty earthen pots with 18inch depth were used for the experiment. Twelve kilogram sun-dried soils were put in each pot. After that, pots were prepared for seedling transplanting.

## 3.5 Arsenic treatment

Sodium Arsenate (0.5Nm, $Na_2HAsO_4$) as $As_1$ and Sodium (meta) arsenite (0.5Mm, $NaAsO_2$) considered as $As_2$. The number of total treatment application doses was 4 which is applied at 21DAT, 32DAT, 42DAT and 52DAT.

## 3.6 Protectant treatment

Cow-dung, Vermi-compost and Biochar used as protectant. 1.5kg cow-dung, 600g vermi-compost and 500g biochar mixed respectively to prepare the pot soil before transplanting of seedling.

## 3.7 Treatments

The experiment consisted of single factor as mentioned below:

a. Total number of treatments: 12

i. Control(C) +$As_0$ (No arsenic)
ii. Cowdung(CD)+ $As_0$
iii. Vermicompost (VC)+$As_0$
iv. Biochar (B)+$As_0$
v. $As_1$ (0.5 mM; Sodium arsenate, $Na_2HAsO_4$)
vi. $As_1$+CD
vii. $As_1$+VC
viii. $As_1$+B
ix. $As_2$ (0.5 mM; Sodium meta arsenite, $NaAsO_2$)
x. $As_2$ +CD
xi. $As_2$ +VC
xii. $As_2$+B

## 3.8 Design and layout

The experiment was laid out in Randomized Completely Block Design (RCBD) with three replications. There were all together 36 pots in the experiment.

The layout is given below:

| $R_1$ | $R_2$ | $R_3$ |
|---|---|---|
| Control (C) | Control (C) | Control (C) |
| Cowdung (CD), 1.5kg | Cowdung (CD), 1.5kg | Cowdung (CD), 1.5kg |
| Vermicompost (VC), 600g | Vermicompost (VC), 600g | Vermicompost (VC), 600g |
| Biochar (B), 500 g | Biochar (B), 500 g | Biochar (B), 500 g |
| $As_1$ | $As_1$ | $As_1$ |
| $As_1$+CD | $As_1$+CD | $As_1$+CD |
| $As_1$+VC | $As_1$+VC | $As_1$+VC |
| $As_1$+B | $As_1$+B | $As_1$+B |
| $As_2$ | $As_2$ | $As_2$ |
| $As_2$+CD | $As_2$+CD | $As_2$+CD |
| $As_2$+VC | $As_2$+VC | $As_2$+VC |
| $As_2$+B | $As_2$+B | $As_2$+B |

## 3.9 Seed collection

Seeds of BRRI dhan89 were collected from Bangladesh Rice Research Institute, Joydebpur, Gazipur.

## 3.10 Pot Preparation

The soil was collected from the field of Sher-e-Bangla Agricultural University was sun dried, crushed sand sieved. The soil, cowdung, vermicompost, biochar and other fertilizers were mixed well before placing the soils in the pots. Each pot was filled up with 12 kg soil. Pots were placed at the net house of Sher-e-Bangla Agricultural University. The pots were pre-label for each treatment. Finally, water was added to bring soil water level to field capacity.

### 3.11 Fertilizer Application

The recommended dose of nitrogenous, phosphatic, potassic, sulphur fertilizer for rice is @ 250 kg/ha, 110 kgha$^{-1}$, 140 kgha$^{-1}$, 50 kgha$^{-1}$in the form of urea, triple super phosphate, muriate of potash, gypsum respectively and cowdung @5tonha$^{-1}$. For pot experiment per pot requires 350 g of urea, 180 g of triple super phosphate, 175 g of muriate of potash and 80 g of gypsum and 30 g of cowdung. One-third of urea and the whole amount of cowdung and other fertilizers were incorporated with soil at final pot preparation before transplanting. Rest of the urea were applied in two equal splits one at 30 days after transplanting (DAT) and second at 45 days after transplanting (DAT).

### 3.12 Sowing of seeds in seedbed

Previously collected seeds were soaked for 48 hours and then washed thoroughly in fresh water and incubated for sprouting. The sprouted seeds were sown in the wet seedbed.

### 3.13 Uprooting and transplanting of seedlings

Seedlings of 30 days old were uprooted carefully from the seedbed and transplanted in the respective pots at the rate of two seedlings hill-1 and one hill pot$^{-1}$ on January 23, 2021.

### 3.14 Intercultural operations

#### 3.14.1 Weeding and irrigation

Sometimes there were some small aquatic weeds observed in pots that were uprooted by hand pulling. About 3-4 cm depth of water was maintained in the pot until the crop attained maturity.

#### 3.14.2 Plant protection measures

Rice bug attacked at milking stage of grain and it was controlled by the application of Cypermathrin at 5 ml 10 L$^{-1}$ water. From heading emergence, the pots were netted to protect the rice grain from the attack of birds.

### 3.15 General observation of the experimental pots

Observations were made frequently and the plants looked normal green. No lodging was observed at any stage. The maximum tillering, panicle initiation, and flowering stages were not uniform.

### 3.16 Detecting maximum tillering and panicle initiation stages

Maximum tillering and panicle initiation stages were detected through field observations When the number of tillers hill$^{-1}$ attained the highest number and thereafter had tendency to decrease the number, was indicated at maximum tillering stage. When a small growth at the top of upper most nodes of main stem was noted like a dome indicated the beginning of panicle initiation stage. These stages were not uniform. These were varied with arsenic and organic amendments treatments.

### 3.17 Collection of data

Data were recorded on the following parameters:

- Plant height (cm)
- Leaf number
- Leaf area (cm$^2$)
- Shoot fresh weight (g)
- Root fresh weight (g)
- Shoot dry weight(g)
- Root dry weight(g)
- Tiller no. hill$^{-1}$
- SPAD value of leaf
- Relative water content (RWC)
- Panicle length
- Number of rachis panicle$^{-1}$

### 3.18 Procedure of sampling for growth study during the crop growth period

#### 3.18.1 Plant height (cm)

The height of the rice plants was recorded from 35 days after transplanting (DAT) at 7days interval up to 49DAT, beginning from the ground level up to tip of the leaf was counted as height of the plant.

### 3.18.2 Leaf number

The number of leaf was recorded manually at 35DAT and 49DAT at 15days interval.

### 3.18.3 Leaf area ($cm^2$)

Leaf area determined from K (L × W) equation at 42DAT and 49DAT, where L is the leaf length, W is the maximum width of the leaf, and K is 0.75 for wet-season crops. This method is unbiased; estimates should have no more than 5% error.

### 3.18.4 Shoot fresh weight (g)

In order to measure fresh weight, at 42DAT, 3plants with root as sample are randomly collected from each pot. To get precise data excess water and whole root was removed from the plant and weight immediately and then averaged them. This process was done quickly to avoid the plant water losing.

### 3.18.5 Root fresh weight (g)

At the same date, at 42DAT, 3roots are separated from rice plant during measured shoot fresh weight and weight immediately and then averaged them.

### 3.18.6 Shoot and root dry weight (g)

After weighting the fresh weight, shoot and root of rice plants were kept in an electric oven maintaining 60°c for 24 hours. Then it was weighted in balance to take dry weight and then averaged them.

### 3.18.7 Tiller no. $hill^{-1}$

Total tiller number was taken from 35 DAT, 42DAT and 49DAT at 7days interval.

### 3.18.8 SPAD value

Five leaves were randomly selected per pot. The top, middle and base of each leaf were measured with atLEAF as atLEAF value. Then it was averaged and total chlorophyll content was measured by the conversion of atLEAF value into SPAD units and then totals chlorophyll content.

### 3.18.9 Relative water content (%)

Three leaves were randomly selected per pot and cut with scissors. Relative water content (RWC) of leaf was measured according to Barrs and Weatherley (1962). Fresh leaf laminas were weighed (fresh weight, FW), then placed immediately between two layers of filter paper, and immersed in distilled water in a petri dish for 24 h in a dark place. Turgid weight (TW) was measured after gently removing excess water with a paper towel. Dry weight (DW) of leaf laminas was measured after 48 h oven-drying at 80°C. Finally, RWC was determined using the following formula:

RWC (%) = $\frac{FW - DW}{TW - DW} \times 100.$

### 3.18.10 Panicle length (cm)

Panicle length was recorded from the basal nodes of the rachis to apex of each panicle.

### 3.18.11 Number of rachis panicle$^{-1}$

After harvesting, number of rachis/panicle was counted from 5 selected sample.

### 3.19

### Statistical analysis

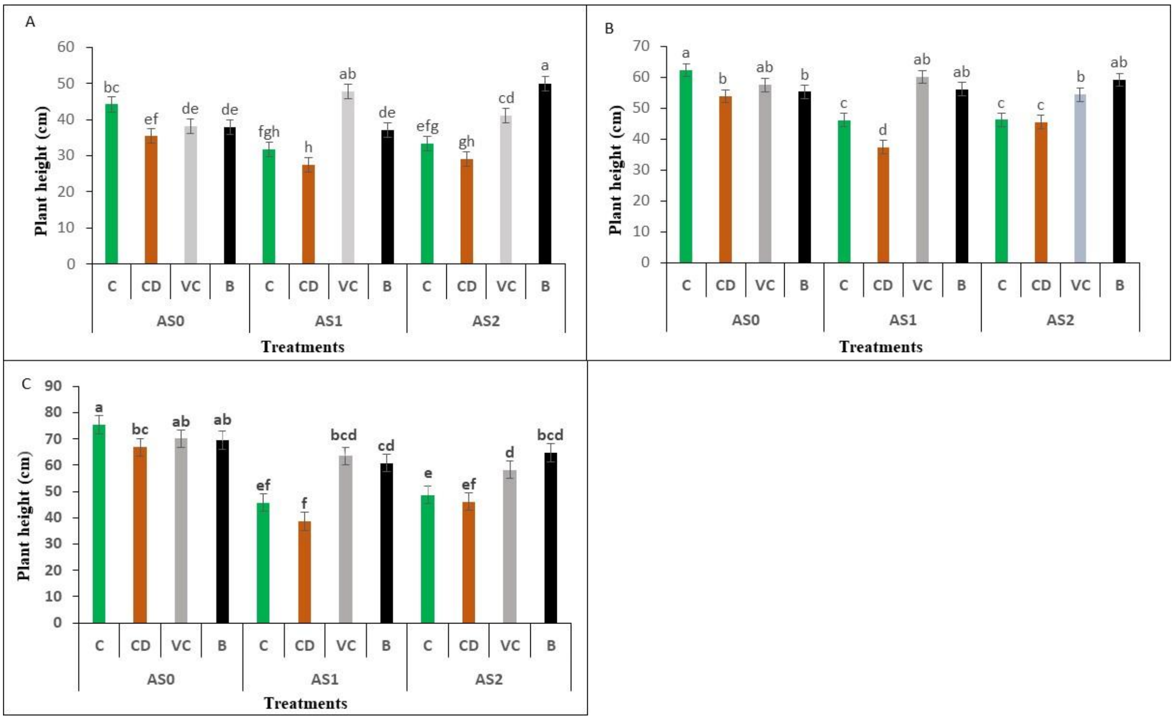
The data obtained for different parameters were statistically analyzed following computer based software Statistics 10.0 and mean separation was done by LSD at 5% level of significance.

## CHAPTER IV

# RESULTS AND DISCUSSION

### 4.1. Plant height

At 35DAT, there is no significant difference in control condition. In case of $As_1$ condition cow-dung didn't show any significant improvement in plant height. On the other hand, when vermicompost and biochar was applied plant height improve by 51% and 17% compared to As1condition. Similarly, when exposed to $As_2$ condition cowdung had no significant improvement and when vermicompost and biochar was applied plant height increased by 23% and 50% respectively (Figure A). At 42 DAT, Control condition shows the highest plant height (62.28cm). In case of $As_1$ and $As_2$ treated plants vermicompost and biochar shows significant improvement of 30% and 22% compared to control conditions. For both treatment cow-dung shows the lowest plant height (37.45cm and 45.525cm) (Figure B). Similar result is found at 49DAT as well. Control condition shows the highest plant height where cow-dung shows the lowest plant height (Figure C). Lange *et al.* (2020) reported that As plays no biological functions in plants and exerts a negative impact on the growth, development, and yield of rice plants. Enhanced uptake of arsenic will influence plant growth negatively. Rekaby *et al.* (2020) reported, the application of organic amendments increased the plant height significantly. Accumulation of As in rice reduced plant growth and productivity, affects macro- and micro-nutrition and results in the generation of reactive oxygen species (ROS) (Panaullah *et al.,* 2009). Ghorbani *et al.* (2019) indicated that As stress decreased the growth of rice by reducing stomatal conductance. Arsenic-induced oxidative stress hinders the seed germination and plant growth (Seneviratne *et al.,* 2019). Similar observations were reported by Chathurika *et al.* (2014) with some amendments, growth and yield parameters of barley plant were significantly improved with the organic amendments treatment. As stress not only reduces plant growth, and yield but also can result in the death of the rice plant by excessive generation of oxidative stress (Hasanuzzaman *et al*., 2012).

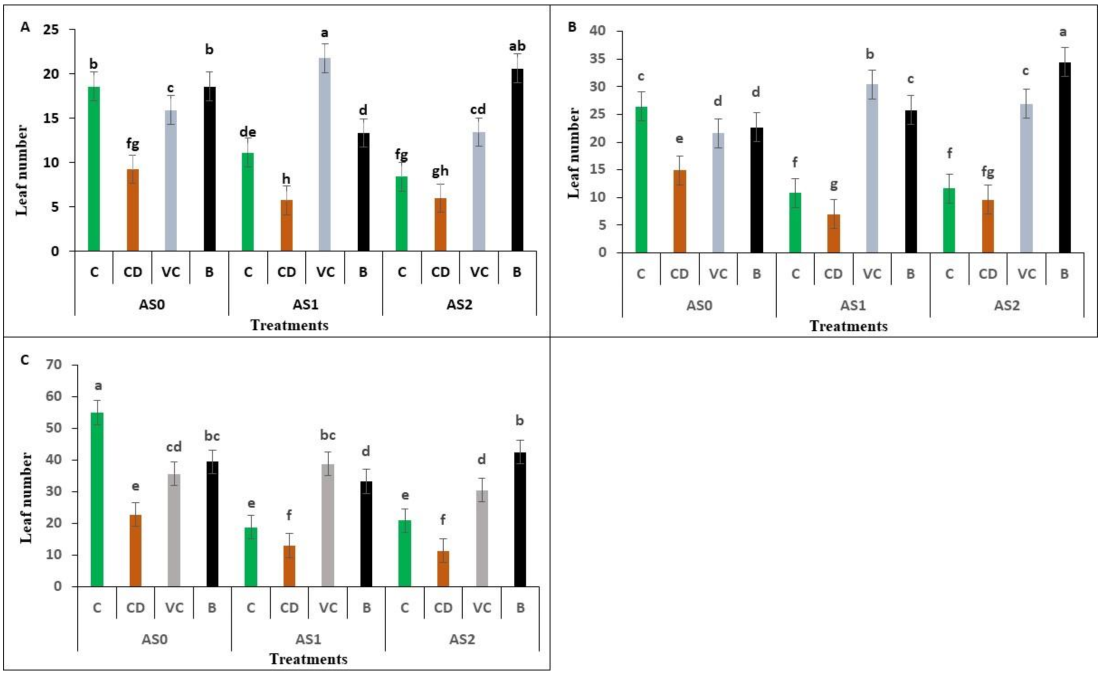


Here, C= Control, CD (cowdung), VC (vermicompost), B (biochar); As0 (Without arsenic), $As_1$ (Sodium arsenate), $As_2$ (Sodium meta arsenite); DAT (days after transplanting).

Figure 1. Plant height at different stress conditions (A) 35DAT, (B) 42DAT, (C) 49DAT. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

## 4.2. Leaf numbers

At 35DAT, control and biochar conditions shows the highest results. There is no significant different between them and they are statistically similar. Cow-dung shows a decrease result of 50% compared to control condition. In case of $As_1$ conditions, Vermicompost shows the highest result of 96% compared its control condition. There is no significant difference between control and biochar condition. For $As_2$ condition, biochar shows the highest result of 146% compared its control conditions. Vermicompost also shows an increasing result of 60% as well. For both $As_1$ and $As_2$ conditions Cow-dung shows the lowest and decrease result of 48% and 29% respectively (Figure A). At 42DAT, we observe the similar result as 35DAT. In case of $As_0$ control condition shows the highest result and cow-dung shows the lowest result. The result of vermicompost and biochar are statistically similar. For $As_1$, after adding vermicompost and biochar it increases leaf number by 182% and 139% respectively compared to $As_1$ condition. Similarly, when exposed to $As_2$ condition vermicompost and biochar shows a significant improvement by 131% and 196% respectively compared to control ($As_2$) conditions (Figure B). At 49DAT, control shows the highest result as well. In case of $As_1$, after adding vermicompost and biochar it improves leaf number by 106% and 76% compared to $As_1$ condition. Similarly, when plant exposed to $As_2$ condition vermicompost and biochar increase leaf number by 46% and 105% compared to control ($As_2$) conditions (Figure C). At all the conditions 35DAT, 42DAT, 49DAT for all treatment $As_0$, $As_1$ and $As_2$, we observe that cow-dung shows a decreasing and lowest result. Arsenic exposure affects different morpho-physiological processes in plants leading to decrease in plant height, leaf number, root length and biomass (Farooq *et al.*, 2016).

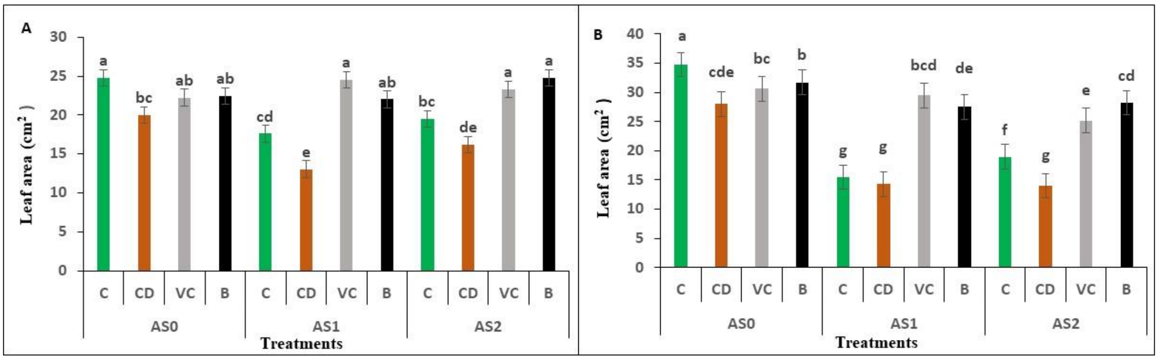


Here, C= Control, CD (cowdung), VC (vermicompost), B (biochar); $As_0$ (Without arsenic), $As_1$ (Sodium arsenate), $As_2$ (Sodium meta arsenite); DAT (days after transplanting).

Figure 2. Leaf number at different stress conditions (A) 35DAT, (B) 42DAT, (C) 49DAT. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

## 4.3 Leaf area

At 42DAT, in case of As0, control condition shows the highest result and cow-dung shows the lowest result. The result of vermicompost and biochar are statistically similar. For $As_1$ condition, after adding vermicompost and biochar it increases leaf number by 39% and 25% respectively compared to control ($As_1$) condition. Similarly, when exposed to $As_2$ condition vermicompost and biochar shows a significant improvement by 20% and 28% respectively compared to control ($As_2$) conditions (Figure A). At 49DAT, control conditions showed the highest result as well. In case of $As_1$, after adding vermicompost and biochar it improves leaf number by 90% and 78% compared to $As_1$ condition. Similarly, when plant exposed to $As_2$ condition vermicompost and biochar increase leaf number by 33% and 49% compared to control ($As_2$) conditions (Figure B). At all the conditions 42DAT, 49DAT for all treatment $As_0$, $As_1$ and $As_2$, we observe that cow-dung shows a decreasing and lowest result by 19%, 8%, 26% and 20%, 26%, 17% respectively. The number and size of the rice leaves vary with the different growth periods.



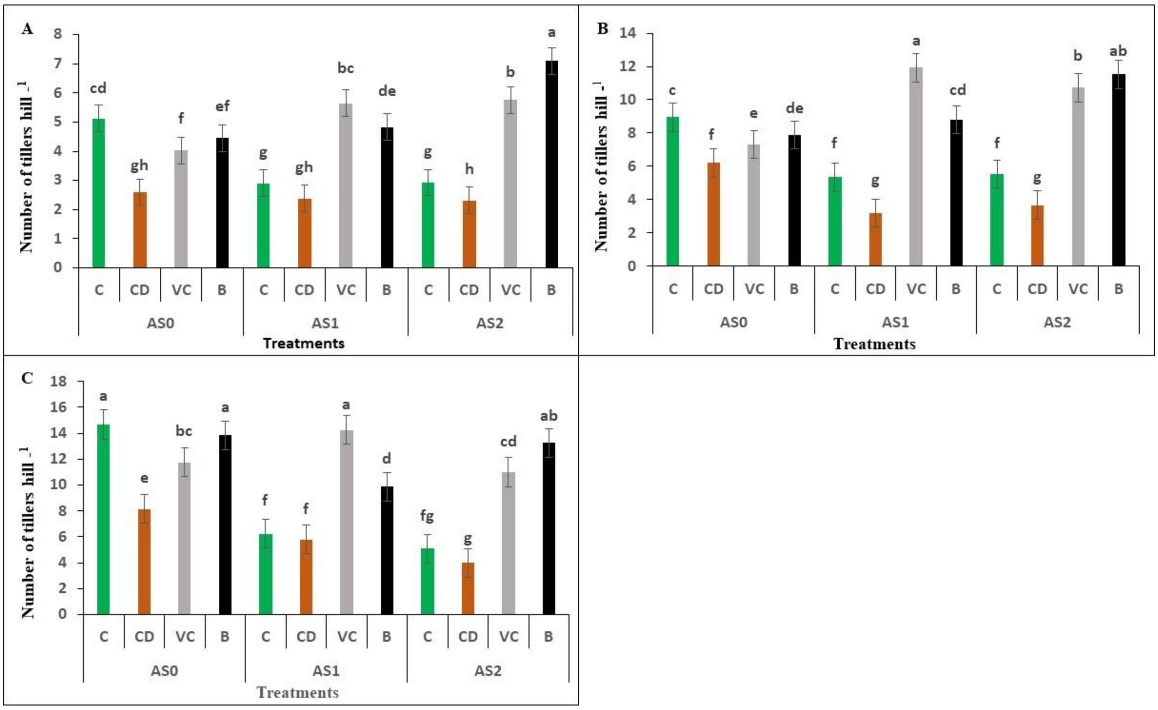
Here, C= Control, CD (cowdung), VC (vermicompost), B (biochar); $As_0$ (Without arsenic), $As_1$ (Sodium arsenate), $As_2$ (Sodium meta arsenite); DAT (days after transplanting).

Figure 3. Leaf area at different stress conditions (A) 42DAT, (B) 49DAT. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Das *et al*., (2013) found that arsenic accumulation and translocation factors are same for rice grown in pot and on field. Arsenic gets accumulated and translocated first in the root, reaches the stem and finally spreads to the leaf and grains (Roychowdhury, 2008a). An index of depression, calculated on the basis of morphological parameters (e.g. leaf area), showed a stimulation of plant growth in case of 15 mg $kg^{-1}$ As in soil and depression of plant growth in case of 50 and 100 mg $kg^{-1}$ As in soil (Miteva, 2002).

### 4.4 Tillers hill$^{-1}$

At 35DAT, in case of As0; control conditions showed the highest results and cow-dung shows the lowest results. There is no significant different between vermicompost and biochar, they are statistically similar. In case of $As_1$ conditions, after adding vermicompost and biochar it increases tiller number by 95% and 66% respectively and vermicompost shows the highest result compared to control ($As_1$) condition. Similarly, when exposed to $As_2$ condition vermicompost and biochar shows a significant improvement by 97% and 142% respectively and biochar shows the highest results compared its control ($As_2$) conditions (Figure A). At 42DAT, we observe the similar result as 35DAT. In case of $As_0$, control condition shows the highest result and cow-dung shows the lowest result. For $As_1$, after adding vermicompost and biochar it increases tiller number by 122% and 65% respectively compared to $As_1$ condition. Similarly, when plant exposed to $As_2$ condition vermicompost and biochar shows a significant improvement by 93% and 107% respectively compared to control ($As_2$) conditions (Figure B). At 49DAT, in case of $As_0$; control and biochar shows the highest result as well. In case of $As_1$, after adding vermicompost and biochar it improves tiller number by 128% and 58% compared to $As_1$ condition. Similarly, when plant exposed to $As_2$ condition vermicompost and biochar increase tiller number by 116% and 161% compared to control ($As_2$) conditions (Figure C). At all the conditions 35DAT, 42DAT, 49DAT for all treatment As0, As1 and $As_2$, we observe that cow-dung shows a decreasing and lowest result compared with control conditions. The reduction of rice plant growth, in terms of tillering was the ultimate result of arsenic phytotoxicity at high soil arsenic concentrations (Rahman *et al.*, 2004).

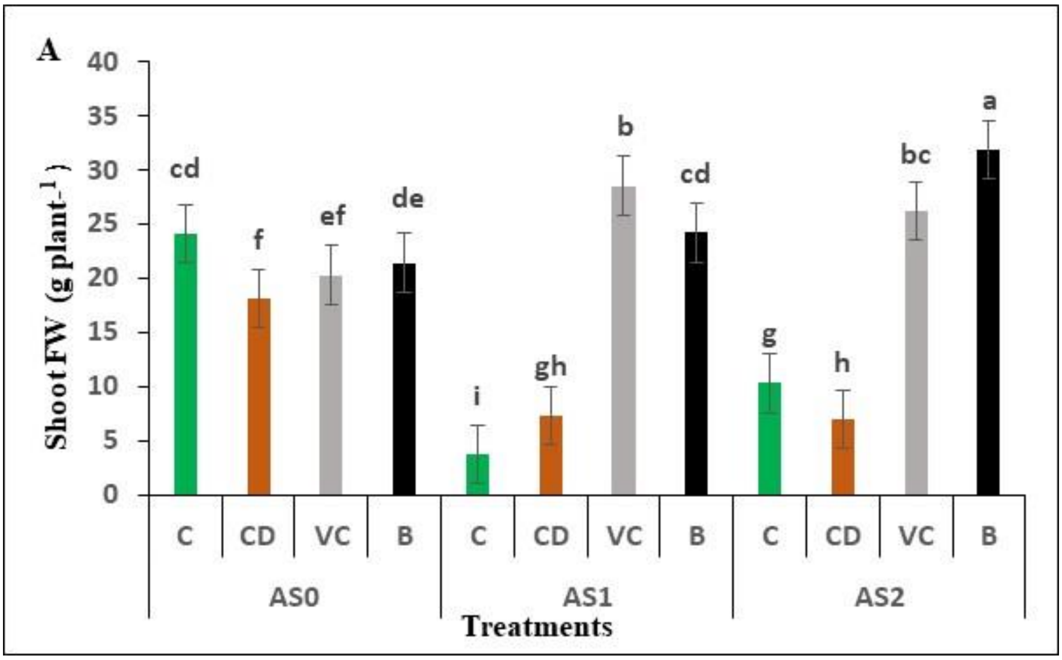


Here, C= Control, CD (cowdung), VC (vermicompost), B (biochar); $As_0$ (Without arsenic), $As_1$ (Sodium arsenate), $As_2$ (Sodium meta arsenite); DAT (days after transplanting).

Figure 4. Number of tiller hill$^{-1}$ at different stress conditions (A) 35DAT, (B) 42DAT, (C) 49DAT. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

## 4.5 Shoot fresh weight

A noticeable change had occurred in case of shoot fresh weight compared to control. In case of As0; control conditions showed the highest results. There is no significant different between vermicompost and biochar, they are statistically similar. In case of $As_1$ conditions, after adding vermicompost and biochar it increases fresh weight by 655% and 541% respectively and vermicompost (28.55g) shows the highest results compared to control ($As_1$) condition. Similarly, when plants exposed to $As_2$ condition vermicompost and biochar shows a significant improvement by 153% and 208% respectively and biochar (31.88g) shows the highest results compared its control ($As_2$) conditions. At $As_0$ and $As_2$ cow-dung shows a decreased result by 25% ,32% respectively but in case of As1 conditions cow-dung shows an increasing result by 94% compared with control conditions (Figure A). A decrease in plants biomass with increasing As concentration in irrigation water has been reported by Pigna *et al.* (2008).

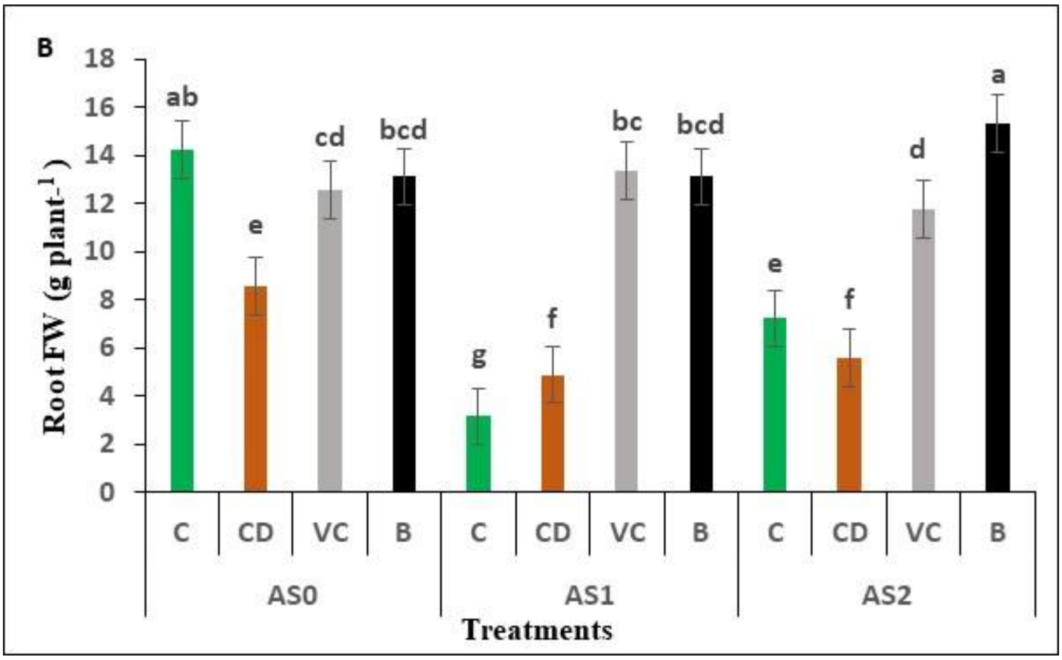


Here, C= Control, CD (cowdung), VC (vermicompost), B (biochar); $As_0$ (Without arsenic), $As_1$ (Sodium arsenate), $As_2$ (Sodium meta arsenite); DAT (days after transplanting).

Figure 5. Shoot fresh weight at different stress conditions (A) 42DAT. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

## 4.6 Root Fresh Weight

A noticeable change had occurred in case of root dry weight compared to control. In case of As0; control conditions showed the highest results. There is no significant different between vermicompost and biochar, they are statistically similar. In case of $As_1$ conditions, after adding vermicompost and biochar it increases fresh weight by 323% and 315% respectively compared to control ($As_1$) condition. Similarly, when plants exposed to $As_2$ condition vermicompost and biochar shows a significant improvement by 63% and 112% respectively and biochar (15.33g) shows the highest results compared its control ($As_2$) conditions. At $As_0$ and $As_2$ cowdung shows a decreased result by 39% ,23 % respectively but in case of As1 conditions cowdung shows an increasing result by 55% compared to control conditions (Figure A). There are several reports regarding the loss of fresh and dry biomass of roots as well as shoots, loss of yield and fruit production, morphological changes; when the plants are grown in As-treated soils (Shaibur *et al*., 2008; Srivastava *et al*., 2009). Liu *et al.* (2005) reported a significant decline in root biomass production in wheat seedlings with the increase in As(III) and As(V) concentrations for all six varieties of *Triticum aestivum* studied.

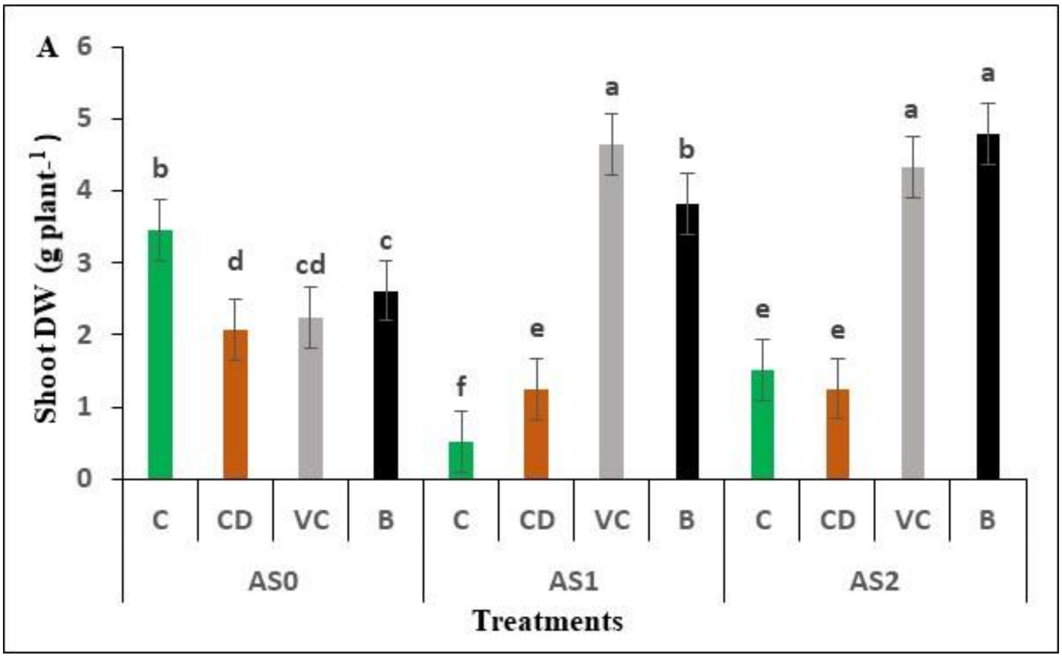


Here, C= Control, CD (cowdung), VC (vermicompost), B (biochar); $As_0$ (Without arsenic), $As_1$ (Sodium arsenate), $As_2$ (Sodium meta arsenite); DAT (days after transplanting).

Figure 6. Root fresh weight at different stress conditions (A) 42DAT. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

## 4.7 Shoot dry weight

A noticeable change had occurred in case of shoot dry weight compared to control. In case of $As_0$; control conditions showed the highest results. Shoot dry weight has been decreased by 40%, 35%, 24% in case of plants treated with cow-dung, vermicompost and biochar respectively compared to control conditions. There is no significant difference between vermicompost and biochar, they are statistically similar. In case of $As_1$ conditions, after adding vermicompost and biochar it increases fresh weight by 808% and 645% respectively and vermicompost (28.55g) shows the highest results compared to control ($As_1$) condition. Similarly, when plants exposed to $As_2$ condition vermicompost and biochar shows a significant improvement by 187% and 218% respectively and biochar (4.79g) shows the highest results compared its control ($As_2$) conditions. When plants exposed to $As_2$, cowdung shows a decreased result by 17%, but in case of As1 conditions cowdung shows an increasing result by 145% compared with control conditions (Figure A). Arsenic in the growth medium negatively changed physiological conditions, including DW, RWC, Pro accumulation, chl content, and the glyoxalase system (Rahman *et al.*, 2015). The reduction of shoot biomass production, was the ultimate result of arsenic phytotoxicity at high soil arsenic concentrations (Rahman *et al.*, 2004).



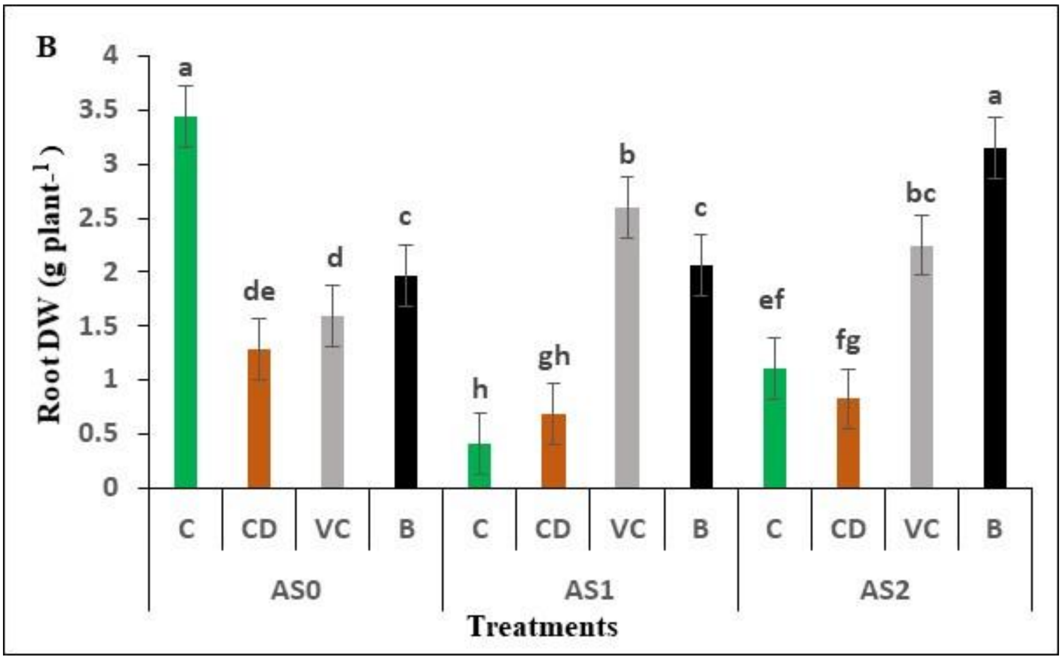
Here, C= Control, CD (cowdung), VC (vermicompost), B (biochar); $As_0$ (Without arsenic), $As_1$ (Sodium arsenate), $As_2$ (Sodium meta arsenite); DAT (days after transplanting).

Figure 7. Shoot dry weight at different stress conditions (A) 42DAT. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

### 4.8 Root dry weight

A noticeable change had occurred in case of root dry weight compared to control. In case of $As_0$; control conditions showed the highest results. Root dry weight has been decreased by 63%, 54%, 43% in case of plants treated with cow-dung, vermicompost and biochar respectively compared to control conditions. In case of $As_1$ conditions, after adding vermicompost and biochar it increases dry weight by 533% and 403% respectively and vermicompost (2.59g), shows the highest results compared to control ($As_1$) condition. Similarly, when plants exposed to $As_2$ condition vermicompost and biochar shows a significant improvement by 101% and 183% respectively and biochar

(3.15g) shows the highest results compared its control ($As_2$) conditions. When plants exposed to $As_2$, cowdung shows a decreased result by 26%, but in case of $As_1$ conditions cowdung shows an increasing result by 65% compared with control conditions (Figure B).



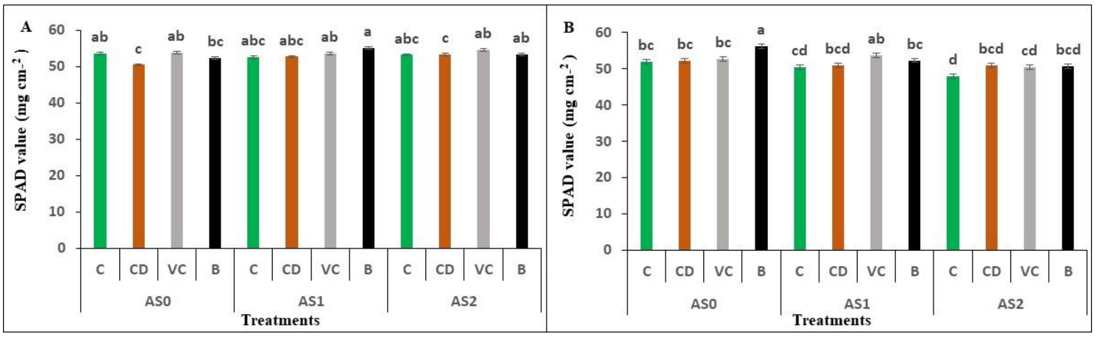
Here, C= Control, CD (cowdung), VC (vermicompost), B (biochar); $As_0$ (Without arsenic), $As_1$ (Sodium arsenate), $As_2$ (Sodium meta arsenite); DAT (days after transplanting).

Figure 8. Root dry weight at different stress conditions (A) 42DAT. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Root fresh and dry weight increased with the application of organic amendments compared to inorganic amendments. With the application of organic amendments improvement in rice growth could possibly be attributed to supply of additional nutrients from these organic materials (Irem *et al.*, 2019). The chlorophyll content of seedling leaves decreased with both arsenate(V) and arsenite(III). This observation explains the lower biomass and growth. Roots show decreased biomass and lower root vigor (Zhao *et al*., 2009).

## 4.9 SPAD value

At 35DAT, in case of $As_0$; Cow-dung shows the lowest result and decreased leaf chlorophyll content by 6% compared to control condition. There is no significant difference between control, vermicompost and biochar, they are statistically similar. In case of $As_1$ conditions, after adding vermicompost and biochar it increases chlorophyll content by 95% and 66% respectively and biochar shows the highest result compared to control ($As_1$) condition. Similarly, when exposed to $As_2$ condition vermicompost shows a significant improvement by 97% compared to control ($As_2$) Figure A). At 49DAT; in case of $As_0$, biochar shows the highest result by 8 % compared to control conditions. In case of $As_1$, after adding vermicompost and biochar it improves chlorophyll content of leaf by 6% and 4% compared to control ($As_1$) condition. Similarly, when plant exposed to $As_2$ condition cow-dung, vermicompost and biochar increases chlorophyll content by 6%, 5% and 6% respectively compared to control ($As_2$) conditions (Figure B).



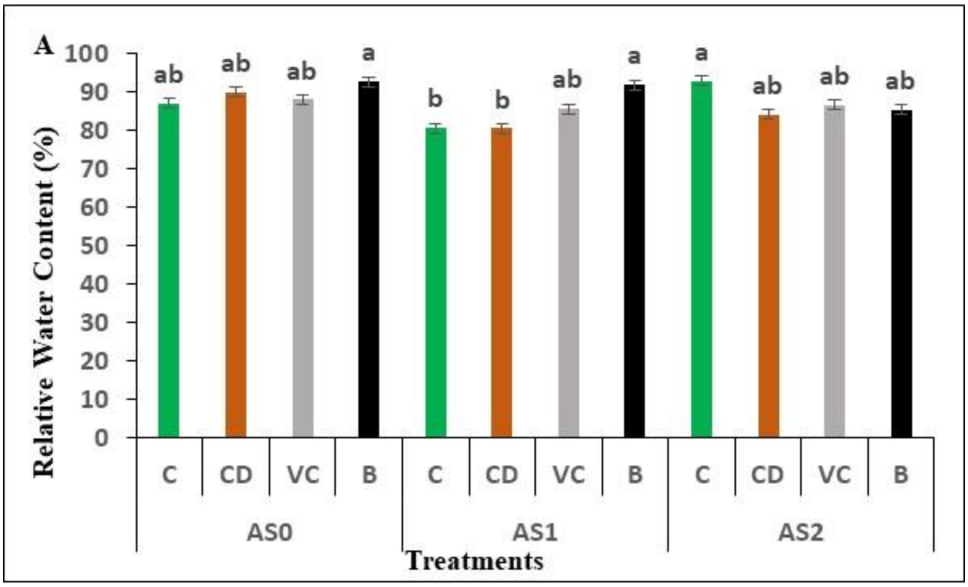
Here, C= Control, CD (cowdung), VC (vermicompost), B (biochar); As0 (Without arsenic), As1 (Sodium arsenate), As2 (Sodium meta arsenite); DAT (days after transplanting).

Figure 9. SPAD value at different stress conditions (A) 35DAT, (B) 49DAT. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Rahman *et al*. (2007) found that the chlorophyll a and b contents in rice leaves (*Oryza sativa* L.) decrease significantly ($p < 0.05$) with increasing soil arsenic content in a glass house experiment. Murugaiyan *et al*. (2019) reported that a concentration of As above 9 mg kg$^{-1}$ leads to damage of photosynthetic pigment. Recently, Asgher *et al.* (2021) reported that As stress down regulated the photosynthesis process of the rice plant by inhibition of PS II activity. Arsenic has been reported to retard biosynthesis of chlorophyll in plants because As toxicity causes chlorophyll degradation, growth retardation, nutrient deficiency, poor photosynthesis and membrane degradation in plants (Khalid *et al.*, 2017).

### 4.10 Relative Water Content

In case of control ($As_0$) condition, cow-dung, vermicompost and biochar showing an increasing result by 4%, 2% and 7% respectively and biochar showing the highest percentage of relative water content (92%) compare to control conditions. Similarly, when plants exposed to $As_1$ conditions cow-dung had no significance improvement and when vermicompost and biochar was applied it increases relative water content by 6% and 14% respectively. Biochar showed the highest percentage of relative water content (92%) as well as $As_0$ conditions. Different result was observed in case of $As_2$ conditions. Control condition showed the highest result and cow-dung, vermicompost and biochar showed a decreased result by 9%, 7% and 8% respectively compared to control conditions. Stoeva *et al*. (2004) reported a result of leaf water potential (w) and transpiration rate in the As-treated plants while the relative water content decreased slightly.

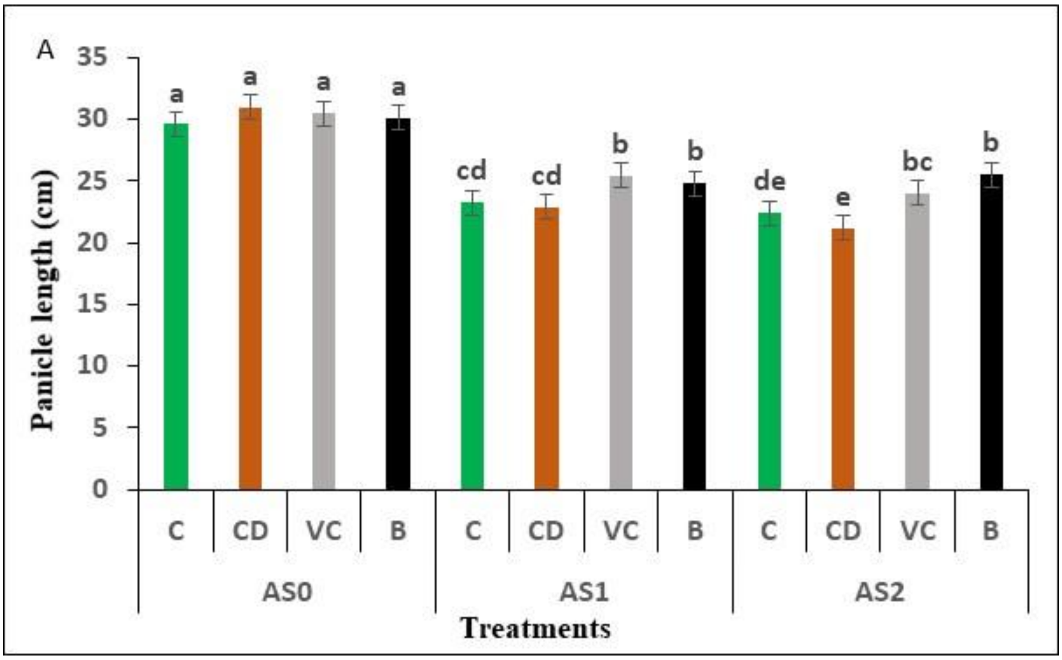


Here, C= Control, CD (cowdung), VC (vermicompost), B (biochar); $As_0$ (Without arsenic), $As_1$ (Sodium arsenate), $As_2$ (Sodium meta arsenite); DAT (days after transplanting).

Figure 10. Relative water content at 42DAT. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

## 4.11 Panicle length

Arsenic caused a significant reduction of panicle length compared to control and control combined with cow-dung, vermicompost and biochar. Number of panicle length has been increasing by 5%, 3% and 2% in case of cow-dung, vermicompost and biochar respectively compared to control $As_0$ conditions. Under $As_1$ conditions, panicle length has been significantly decreased compared to $As_0$ conditions. Plant treated with vermicompost and biochar has been shown an increasing length of panicle by 10% and 7% respectively compared to control $As_1$ conditions. Similarly, when plant exposed to $As_2$ conditions, cow-dung has been shown a decreasing result of panicle length by 5% and vermicompost and biochar has been shown an increasing result of panicle length by 8% and 14% respectively compared to control $As_2$ conditions.

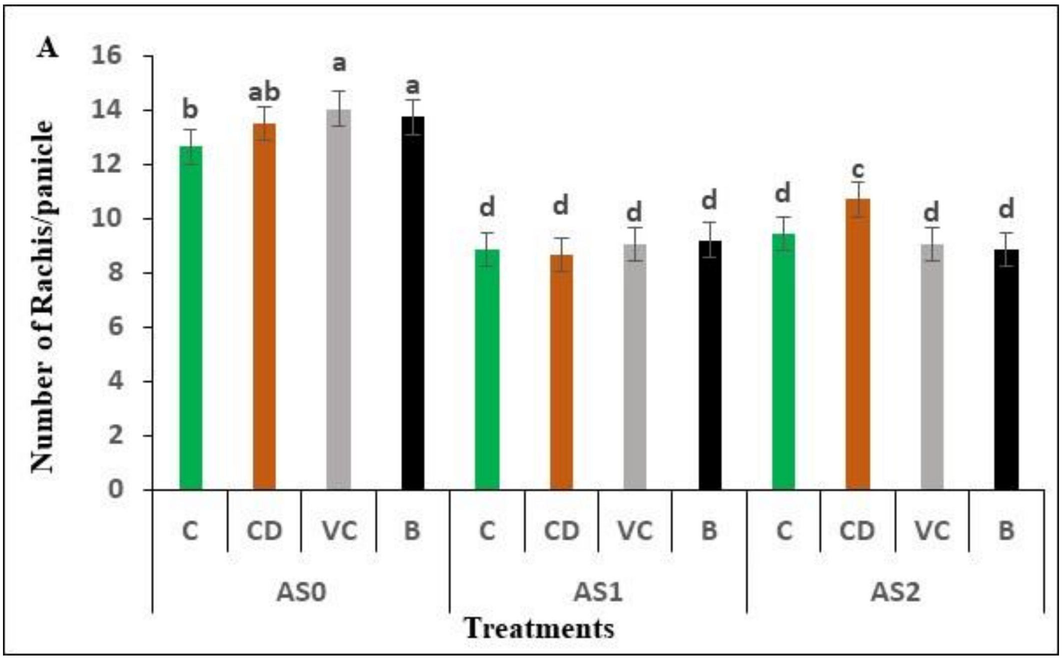


Here, C= Control, CD (cow-dung), VC (vermicompost), B (biochar); $As_0$ (Without arsenic), $As_1$ (Sodium arsenate), $As_2$ (Sodium meta arsenite); DAT (days after transplanting).

Figure 11. Panicle length (at harvest) different stress conditions (. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

## 4.12 Number of rachis panicle$^{-1}$

Arsenic caused a significant reduction of rachis number per panicle compared to control and control combined with cow-dung, vermicompost and biochar. Number of rachis per panicle has been increasing by 7%, 11% and 9% respectively in case of cow-dung, vermicompost and biochar compared to control $As_0$ conditions. Under $As_1$ conditions, panicle length has been significantly decreased compared to $As_0$ conditions. There is no significant difference between all the treatment combined with $As_1$. Plant treated with vermicompost and biochar has been increased rachis number per panicle by 3% and 4% respectively comrpare to control $As_1$ conditions. Similarly, when plant exposed to $As_2$ conditions, cow-dung has been shown an increasing result of rachis number per panicle by 10% and vermicompost and biochar has been shown a decreasing result of rachis number per panicle 4% and 6% respectively compared to control $As_2$ conditions. Rice yield was measured on the basis of panicle number, filled grain production and weight of total grain. Results imply that increasing soil arsenic concentrations drastically reduces rice yield in all varieties. The number of panicle was found to be decreased significantly ($p < 0.05$) with the increase of soil arsenic concentrations (Rahman *et al.*, 2007).



Here, C= Control, CD (cowdung), VC (vermicompost), B (biochar); $As_0$ (Without arsenic), $As_1$ (Sodium arsenate), $As_2$ (Sodium meta arsenite); DAT (days after transplanting).

Figure 12. Number of rachis panicle$^{-1}$ at harvest at different stress conditions. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

## CHAPTER V

# SUMMARY AND CONCLUSION

This study was conducted to mitigate arsenic stress in boro rice by exogenous application of organic amendments (cowdung, vermicompost, biochar). BRRI dhan89 was used for this experiment which was conducted at the Experimental shed of the Department of Agronomy, Sher-e-Bangla Agricultural University, in Dhaka during the period of January to May,2021. BRRI dhan89 was collected from Bangladesh Rice Research Institute (BRRI), Gazipur. The experiment was placed out in a Randomized Completely Block Design (RCBD) with three replications. There were 36 pots all together replication with the given factors. Empty earthen pots with 18inch depth were used for the experiment. There were 12 treatment combinations. The treatments were control (C), control+cowdung (C+CD), control+vermicompost (C+VC), control+biochar (C+BC); $As_1$ (0.5 mM, Sodium arsenate), $As_1$+CD, $As_1$+VC, $As_1$+BC; $As_2$ (0.5 mM, Sodium meta arsenite), $As_2$+CD, $As_2$+VC, $As_2$+BC.

Different arsenic with or without organic amendment treatments had significant effect on crop growth parameters e.g. plant height and tillers hill$^{-1}$ at different DAT. The highest plant height was found at control condition at 35DAT, 42DAT and 49DAT (44.185cm, 64.52cm, 75.4 cm respectively). In case of $As_1$ at all the DAT, vermicompost shows the highest result of plant height (47.85cm, 60.08cm, 63.525cm respectively) and in $As_2$ condition biochar shows the highest plant height (49.87cm, 59.17cm, 64.733 cm respectively). For both leaf number at 35DAT, 42DAT and 49DAT and leaf area at 42DAT and 49DAT vermicompost (21.8, 30.45, 38.86; 24.48, 29.48) shows the highest result when plant exposed to $As_1$ and biochar (20.65, 34.45, 42.58; 24.77, 28.2) shows the highest result in case of $As_2$ respectively.

Arsenic treatments had significant effect on the physiological parameters viz. relative water content. For both control (92.802%) and As1 condition biochar (91.94%) shows the highest percentage of relative water content and in terms of $As_2$ condition, $As_2$ (92.94%) shows the highest percentage of relative water content.

Arsenic treatments had significant effect on the yield and yield contributing characters viz. plant height, effective and non-effective tillers hill$^{-1}$, length of panicle, rachis panicle$^{-1}$. For both $As_1$ and $As_2$ treated plants combined with vermicompost and biochar shows an increasing percentage at all the data parameters compare to $As_1$ and $As_2$ treated plant only.

Based on result of the present experiment, together with results found in the available literature, we therefore concluded that exogenous organic amendments (cowdung, vermicompost, biochar) application is an effective way to overcome the adverse effects of arsenic stress on growth, physiology and yield components of rice effectively. Also these amendments increased the fresh and dry weight of shoot and root of rice plants. All parameters decreased significantly at 0.5 mM of arsenic stress. Exceptions were panicle length, non-effective tiller hill$^{-1}$ which increased in response to arsenic stress.

Considering these responses, we can conclude that vermicompost shows the better percentage of increasing result when plant exposed to $As_1$ (0.5 mM; Sodium arsenate, $Na_2HAsO_4$**)** and in case of $As_2$ (0.5 mM; Sodium meta arsenite, $NaAsO_2$) biochar shows an increasing result compare to all the treatments.

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# APPENDICES

Appendix I. Phenotypic pictures of rice plant under arsenic stress treated with cowdung, vermicompost and biochar.

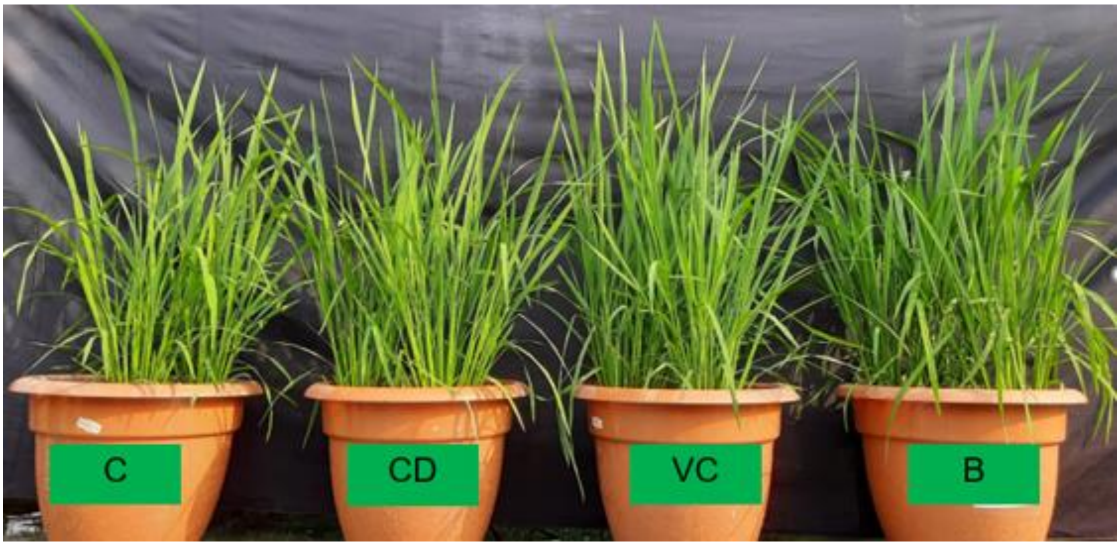

Plate 1: Control condition of rice plant at 42 DAT.

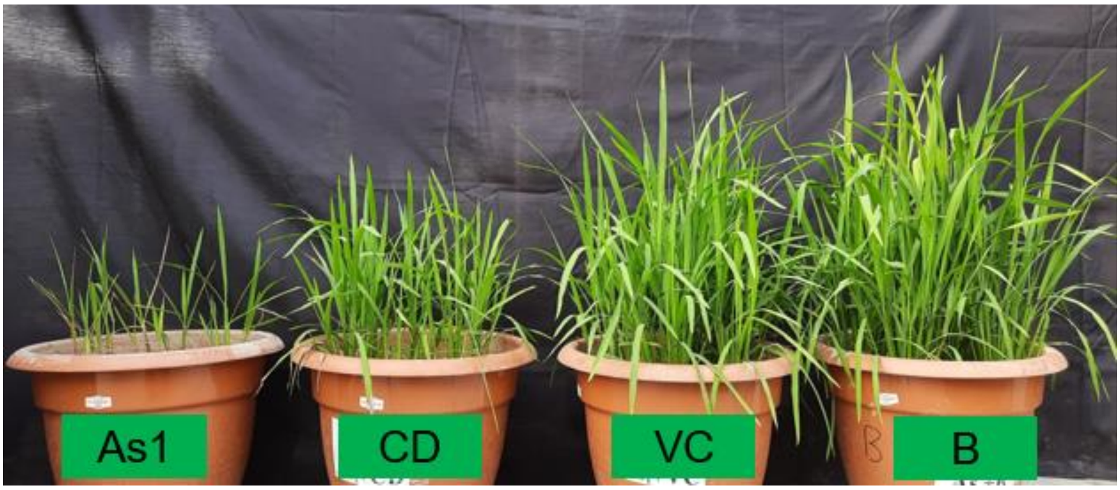

Plate 2: Rice plant under $As_1$ stress treated with cowdung, vermicompost and biochar at 42 DAT.

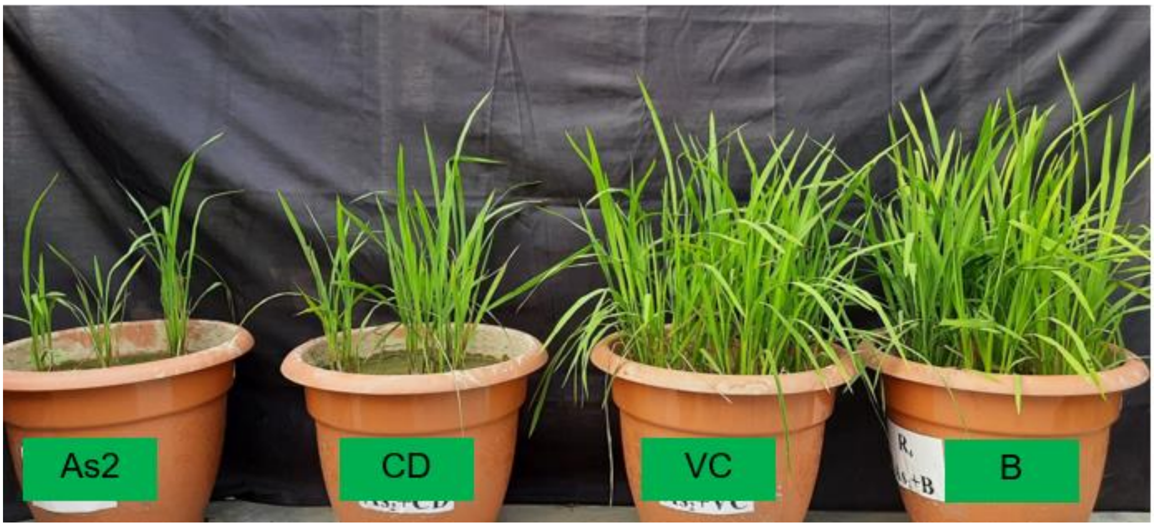

Plate 3: Rice plant under $As_2$ stress treated with cowdung, vermicompost and biochar at 42 DAT.

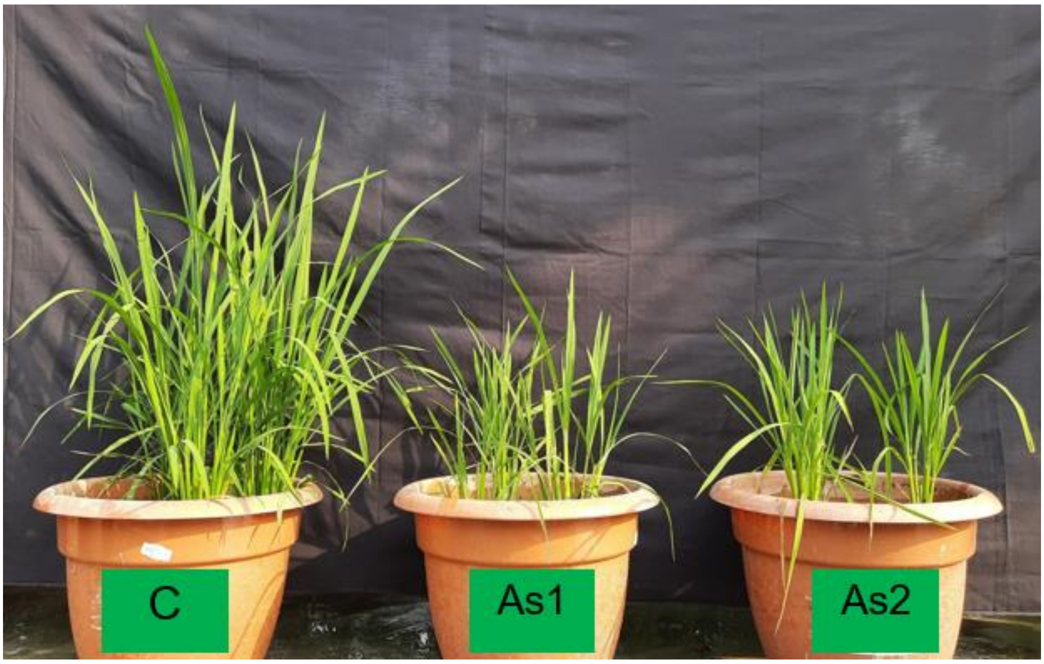

Plate 4: $As_1$ and $As_2$ treated rice plant compare with control ($As_0$) plant at 42 DAT.

Appendix II. Supervision of my experiment.

Plate 5: Supervision of my experiment plot by SAURES at 8march, 2021

Appendix III. Map showing the location of the experiment