MITIGATION OF CADMIUM STRESS IN RICE BY EXOGENOUS APPLICATION OF SODIUM NITROPRUSSIDE (NITRIC OXIDE DONOR)

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CERTIFICATE

*This is to certify that thesis entitled, "***MITIGATION OF CADMIUM STRESS IN RICE BY EXOGENOUS APPLICATION OF SODIUM NITROPRUSSIDE (NITRIC OXIDE DONOR)***" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE (MS) IN AGRONOMY, embodies the result of a piece of bona-fide research work carried out by MD. ZAHIDUL ISLAM HAWLADER TUHIN, Registration no. 10-03973 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

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ABSTRACT

A pot experiment with BRRI dhan48 was conducted at the Experimental shed of the Department of Agronomy, Sher-e Bangla Agricultural University, Bangladesh during the summer season (April-August) to investigate the role of exogenous sodium nitroprusside (SNP) in growth and yield of rice under different cadmium stress condition. The experiment was carried out ten cadmium stress treatments viz. control (without cadmium), control+SNP (without cadmium with 0.2 mM SNP), Cd0.5 (0.5 mM $CdCl_2$), Cd0.5+SNP (0.5 mM $CdCl_2$ with 0.2 mM SNP), Cd1 (1 mM $CdCl_2$), Cd1+SNP (1 mM $CdCl_2$ with 0.2 mM SNP), Cd1.5 (1.5 mM $CdCl_2$) and Cd1.5+SNP (1.5 mM $CdCl_2$ with 0.2 mM SNP), Cd2 (2 mM $CdCl_2$) and Cd2+SNP (2 mM $CdCl_2$ with 0.2 mM SNP). Cadmium stresses significantly reduced the plant height and tillers $hill^{-1}$ at all growth duration. Leaf relative water content (RWC) and chlorophyll (chl) content also reduced due to cadmium stresses. At harvest, cadmium stresses reduced the effective tillers $hill^{-1}$, number of filled grains $panicle^{-1}$, 1000-grain weight, grain yield, straw yield and harvest index. On the other hand, exogenous application of sodium nitroprusside (SNP) improved the plant height, effective tillers $hill^{-1}$, panicle length, filled grains $panicle^{-1}$, 1000-grain weight, straw yield and grain yield upto 1 mM cadmium stress. But SNP application could not improve crop growth parameters, physiological parameters and yield at higher level of cadmium stress (1.5 and 2 mM $CdCl_2$) significantly.

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LIST OF ABBREVIATIONS

ABA	Abscisic acid
APX	Ascorbate peroxidase
AsA	Ascorbic acid (ascorbate)
ASC	Ascorbic acid
BRRI	Bangladesh Rice Research Institute
Car	Carotinoids
CAT	Catalase
Cd	Cadmium
Chl	Chlorophyll
cGMP	Cyclic guanosine monophosphate
DHAR	Dehydroascorbate reductase
DNA	Deoxyribonucleic acid
EL	Electrolyte leakage
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Corporate
GPX	Glutathione peroxidase
GR	Glutathione reductase
GS	Glutamine synthetase
GSH	Reduced glutathione
GSSG	Oxidized glutathione
GST	Glutathione *S*-transferase
HM	Heavy metal
HR	Hypersensitive Response
IAA	Indole acetic acid
IRRI	International Rice Research Institute
MAP	Mitogen-activated protein
MDA	Malondialdehyde
MDHAR	Monodehydroascorbate reductase
MST	Membrane thermostability
NO	Nitric oxide
NADPH	Nicotinamide adenine dinucleotide phosphate
PAL	Phenylalanine ammonia-lyase

LIST OF ABBREVIATIONS (Cont'd)

PCs	phytochelatins
POD	Peroxidase
POX	Phosphylated oximes
RAN	Ribonucleic acid
QTLs	Quantitative trait loci
REL	Relative electrolyte leakage
RFO	Raffinose oligosaccharide
ROS	Reactive oxygen species
RuBP	Ribulose-1, 5-bisphosphate
SA	Salicylic acid
RWC	Relative water content
SAR	Systemic acquired resistance
SIPK	Salicylic acid induced protein kinase
SOD	Superoxide dismutase
SNP	Sodium nitroprusside
SRDI	Soil Resource Development Institute
TBARS	Thiobarbituric acid reactive substance
TTC	2, 3, 5-triphenyl tetrazolium chloride
USDA	United States Department of Agriculture
WHO	World Health Organization
WUE	Water use efficiency

Chapter 1
INTRODUCTION

Rice (*Oryza sativa* L.) belongs to the Poaceae family and a crop grown in all seasons. According to economic and social aspect of Bangladesh rice outruns all other crops. It is also chief cereal crop in Bangladesh. In worldwide, 483.3 million metric tons of rice was produced from 159.64 million hectares of land during the year of 2015-16 which is 4.5 million metric tons higher than last season (USDA, 2016). FAO estimates Bangladesh to produce around 52.3 million tons of rice in 2015-16, in an area about 120 million hectare. During the year 2015-16, 3.4 million metric tons of aus rice was produced from 1.45 million hectares of land with an average yield of 2.34 Mt/ha in Bangladesh (BBS, 2016). Rice is the main crop in Bangladesh and its production need to be increased to fulfill the food necessity of an over populated country where the size of the population is increasing gradually.

Cadmium (Cd) is one of the main pollutants in crop fields which make environmental stress on crop. All of the environmental stresses contamination of soil with cadmium will be a widespread environmental problem that has been found to affect huge area, around 30,000 tones cadmium (Cd) are released into the environment annually, of which 13,000 tones resulted from human activity (Gallego *et al.*, 2012). Cadmium (Cd) is a highly water-soluble toxic heavy metal that can easily accumulate in water and soil from industrial activities and phosphate fertilizers (Kuriakose and Prasad, 2008). It is well known that food is the main origin of Cd intake for human beings and animals through food chain (Yu *et al.*, 2006; Uraguchi *et al.*, 2009). Rice (*Oryza sativa* L.) is the major food for more than half of the world's population (Meharg *et al.*, 2013; Kosolsaksakul *et al.*, 2014). It was indicated that Cd can be easily taken up by rice from soil solution and translocated to shoot and then gathered to grains (Wang *et al.*, 2014; Song *et al.*, 2015). Rapid climate change throughout the world has exposed plants to various environmental adversities that prevent them from reaching their full genetic potential and limit their productivity (Hasanuzzaman *et al.*, 2012b).

Cadmium can metamorphose the uptake of minerals by plants through its effects on the availability of minerals from the soil or through a reduction in the population of soil microbs (Moreno *et al.*, 1999). Stomatal opening, transpiration and

photosynthesis have been reported to be affected by cadmium in nutrient solutions but the metal is taken up into plants more rapidly from nutrient solutions than from soil (Sanita di Toppi and Gabrielli, 1999). Chlorosis and stunting are the most common visible symptoms of cadmium toxicity in plants. Chlorosis may appear due to deficiency of Fe (Haghiri, 1973). Usually, Cd has been shown to interfere with the uptake, transport and use of several elements such as Ca, Mg, P, K and water through plants (Das *et al.*, 1997). Plants can survive in Cd contaminated condition through physiological and biological change (Tanou *et al.*, 2009a; El-Shabrawi *et al.*, 2010). The production of reactive oxygen species (ROS), such as superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}HO$), and singlet oxygen (O_2), as a result of oxidative stress in the target organs, is one of the mechanisms by which Cd induces its toxicity (Casalino *et al.*, 2002).

Cadmium is recognized as a non essential element and is the fifth most toxic metal to vertebrates and the fourth most toxic metal to vascular plants.

Nitric oxide (NO) is a diffusible gaseous free radical. Its emission from plants has been reported several years ago in soybean plants (Klepper, 1997). Two mechanisms by which NO might abate stress has been postulated. Firstly, NO behaves as an antioxidant, which directly remove the reactive oxygen species (ROS), such as superoxide radicals to form peroxynitrite (Radi *et al.*, 1991), which is notably less toxic than peroxides and thus lower cellular damage. Secondly, NO might function as signaling molecule which works as a source of changing of gene expression (Lamattina *et al.*, 2003). Recently NO has emerged as an important signaling molecule and it was indicated that the application of exogenous NO donors increases tolerance mechanism of plants to various abiotic stresses (Hasanuzzaman *et al.*, 2010; Hasanuzzaman *et al.*, 2013a). Nitric oxide (NO) is a widespread gaseous signaling molecule in plants. NO regulates numerous key physiological and biochemical processes, including plant growth, germination and organogenesis (Wilson *et al.*, 2008). Recently, the number of studies that have examined the exogenous NO effects on reducing heavy metal toxicity in plants has increased. Application of sodium nitroprusside (SNP) under Cd toxic conditions may protect rice seedlings from Cd stress (Panda *et al.*, 2011).

NO is considered as a stress inducing agent (Leshem, 1996) and also as a protective element (Hsu and Kao, 2004) depending on its concentration, the plant tissue or age, and the type of stress. Nitric oxide (NO) is a highly reactive, membrane-permeable free radical that has recently emerged as an important signaling molecule and antioxidant. NO triggers many kinds of redox-regulated (defense-related) gene expressions, directly or indirectly, to establish plant stress tolerance (Polverari *et al.*, 2003; Sung and Hong, 2010). The application of an NO donor, sodium nitroprusside (SNP), confers tolerance to various abiotic stresses in plants by enhancing their antioxidant defense system (Neill *et al.*, 2002; Tian and Lei, 2006; Sheokand *et al.*, 2008; Zheng *et al.*, 2009; Singh *et al.*, 2009; Xu *et al.*, 2010). Several lines of study have shown that the protective effect of NO against abiotic stress is closely related to the NO-mediated reduction of ROS in plants (Hasanuzzaman *et al.*, 2010).

Nitric oxide is itself an effective molecule and along with other chemical compound such as abscisic acid and jasmonates, it may work to mitigate or trigger torment in several plant species (Leshem and Kuiper, 1996). Laxalt *et al.* (1997) have indicated that NO was able to partially prevent the chlorophyll damage through producing *Phytophtora infestans* in potato leaves. Beligni and Lamattina (1999) and Hung *et al.* (2002) have indicated that NO is able to repel the toxicity of diquat and paraquat on rice leaves.

NO donors [N-tert-butyl-phenylnitrone, 3-morpholinosydonimine, sodium nitroprusside (SNP) etc.] were effectively reduced $CdCl_2$-induced toxicity and $CdCl_2$ increased MDA content. SNP checked $CdCl_2$-induced increase in the contents of H_2O_2 and MDA and increased the specific activities of antioxidant enzymes. SNP also prevented $CdCl_2$-induced accumulation of NH_4^+, decrease in the activity of glutamine synthetase (GS), and increase in the specific activity of phenylalanine ammonia-lyase (PAL) (Hsu and Kao, 2004). However, under field condition the effect of NO donor has rarely been tested under Cd stress.

Considering the above entitled prospect, the present study was commenced with the following objectives:

i. To investigate the effect of cadmium on growth and yield of rice
ii. To investigate the role of exogenous SNP in mitigating cadmium stress-induced damages in rice.

Chapter 2

REVIEW OF LITERATURE

2.1 Rice

Rice (*Oryza sativa* L.) is the staple food of more than half of the world's population is more than 3.5 billion people depend on rice for more than 20% of their daily calories. Rice provided 19% of global human per capita energy and 13% of per capita protein in 2009. Asia accounts for 90% of global rice consumption, and total rice demand there continues to rise. In 2015/16 growing season total milled rice production in Asia is 118.2 million tons where 47.0 million hectares of land which approximately 45 percent or 21.0 million hectares are irrigated were harvested in Southeast Asia alone (approximately 87% of total world rice production) (USDA, 2016). 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 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 (Meharg *et al.*, 2013; Kosolsaksakul *et al.*, 2014). Rice is grown in many countries of the world; most of them are grown in Asia. There are three types of rice cultivars are grown in world-wide 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. In 2015-16, the production of rice is about 483.3 million tons (FAOSTAT, 2016). Rice is one of the most widely grown crops in irrigated area also in coastal and flood areas. According to USDA rice (*Oryza sativa* L.) is rated as one of the major food crops in the world, but it has been informed that Cd stress caused a reduction in rice growth and biomass (Ahsan *et al.*, 2007; Li *et al.*, 2012a; Li *et al.*, 2012b; Wang *et al.*, 2014).

2.2 Abiotic stress

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). Gradual changes of global climatic conditions adversely affect our natural environment condition which is producing different abiotic stress for crop production (Mittler and Blumwald, 2010). 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.*, 2012a, b).

Besides curtailing crop productivity abiotic stress influence the distribution of different plant species in different types of area and environment in worldwide (Araus *et al.*, 2002). In the period of climate change, plants have continuously endured from environmental adversity that inhibits them from reaching and completing their full genetic potential and limits crop productivity worldwide (Hasanuzzaman *et al.*, 2009, 2010a, b; Hasanuzzaman and Fujita, 2011; Hasanuzzaman *et al.*, 2011a, b; 2012a–c; 2013a–d). Abiotic stress resposible for changes soil-plant atmosphere causes reducing productivity of different major crop in various parts of the world (Ahmad and Prasad, 2012). Industrial waste materials cause water and soil pollution with deposition of heavy metals which creates abiotic stress. This heavy metal widely present in rivers, estuaries, near shore waters, and marine sediments because of the discharge in industrial activities (Mangal *et al.*, 2016). These stress produce harmful chemical compound in plants called reactive oxygen species (ROS), which include hydrogen peroxide (H_2O_2), superoxide radical (O^{2-}), hydroxyl radical (OH^-), etc. (Choudhury *et al.*, 2013).

Abiotic stresses are a major determining factor in crop and forage productivity (Boyer, 1982; Rao *et al*., 2013), and also influences the differential ordination of the plant species across various types of environments (Chaves *et al.*, 2003). Now a-days climate change is a major problem which enhances abiotic stress on a global scale, with increased irregularity and unpredictability, so adaptation strategies need to be developed for crops to specific environments (Beebe *et al*., 2011). Higher temperature also can make abiotic stress through accelerate mineralization of soil organic matter, making soil confines more intense and these severally can limit root penetration into soil and plant development, further intensifying the upshots unfavorable climate (Beebe *et al*., 2013). Different stress factors interact with each other will probably increase damage to crop yields (Beebe, 2012; Yang *et al*., 2013).

Primary processes of plant such as photosynthesis, cell growth are affected by drought stress. Abiotic stress such as water deficit on carbon metabolism results in changes in the pool of sugars used for signaling cellular processes or substrates for biopolymers like cellulose, starch and proteins (Chaves *et al*., 2009; Liu *et al*., 2013). Liu *et al*. (2004) reported that decrease of carbohydrate flux from leaves to pods, together with decreased hexose to sucrose ratio in drought-stressed in pods of soybean are suggested as potential factors contributing to pod abortion. Mishra *et al.* (2011) reported that plants those are growing under environmental stresses condition increases lipid per-oxidation (degradation) and protein oxidation. Flexas and Medrano (2002) reported that under severe water deficit condition production of Ribulose-1, 5-bisphosphate (RuBP) and Rubisco carboxylation efficiency were both decreased.

Atkinson and Urwin (2012) reported that the activation of multiple responses involving complex gene interactions and "cross-talk," with many pathways at the whole-plant, physiological, biochemical, cellular and molecular levels can adapt plant to abiotic stresses.

Environmental stress such as energetic short wavelength ultraviolet (UV) photon which comes from sunlight is detrimental for amino acids of essential proteins, nucleic acids bases or membrane lipids because of destruction interaction with them. Accumulation of phytotoxic metals such as Cd, Zn, Cu are mass pollutants causing in stunted growth, chlorosis and necrosis (Oncel *et al.*, 2000). Heavy metals such as

cadmium treatments in mung bean seedlings decrease the levels of germinating and the chlorophyll by bringing of lipoxgenase with the synchronous inhibition of the antioxidative enzyme SOD and CAT (Somashekaraiah *et al.*, 1992).

Salinity stress is one of the major abiotic stress which reduces the relative water content (RWC), at the rate of 100mM NaCl treatment in plants reduce RWC at 20% and also 10% chlorophyll content (Sheokand *et al.*, 2008). Weggler *et al.* (2000) found that Cd uptake was increased when plants were grown in higher NaCl content soil. Muhling and Lauchli (2003) described that Cd and NaCl stress in combination results higher plasma membrane permeability and raise the production of oxygen radicals and H_2O_2 in plants.

Many researchers estimated the crop losses due to abiotic stress, according to Bray *et al.* (2000), abiotic stress is the primary reason of crop loss in worldwide on an average yield loss of major crops is more than 50%. According to report of Thakur *et al.* (2010) yield loss and reduce of biomass production of staple food crops up to 70%. It is difficult to understand the abiotic stress response in plants because of complexity, interrelationship, and variability of mechanisms (Patakas, 2012).

2.3 Cadmium stress

The heavy metal contamination in soil and water is a worldwide problem due to its harmful impacts on plants and its potential transfer through the food chain (Nagajyoti *et al.*, 2012; Monteiro *et al.*, 2012). Cadmium is one of the most dangerous carcinogenic elements and is mainly produced by anthropogenic activity which moves easily to the food chain through soil to plant root absorption and accumulates an appreciable amount in the living body without showing stress. In terms of toxicity to plants and human, Cd is one of the most toxic heavy metals (Dong *et al.*, 2006; Li *et al.*, 2014) due to its high water solubility, relative mobility, and long half- life in living organisms (Juang *et al.*, 2012; Wu *et al.*, 2014).A major source of Cd input in agricultural soils is atmospheric deposition. Cadmium (Cd) is a toxic heavy metal widely present in rivers, estuaries, near shore waters, and marine sediments through the discharge of Cd compounds in industrial activities (Yuan *et al.*, 2004; Mangal *et al.*, 2016). The water-born cadmium level increases seriously may reach 1 mg/L (9 µM) and polluted water areas (Ma *et al.*, 2008). It is well known that important heavy

metals posing threats to soil quality and human health include Cd is used for a wide variety of industrial, urban, and agricultural applications. Higher concentration of heavy metal in may adversely affect crop growth also interfere physiological and biochemical activities of crop (Atafar *et al.*, 2010). Heavy metal may also change the soil microbial community. Around 1000 meter area from brick kiln considers higher accumulation of cadmium which is harmful for agro-environment (Sikder *et al.*, 2015). Cadmium poisoning is a common phenomenon in the irrigated areas of rice field through wastewater release from industry and mines. In Bangladesh many rivers and land areas are contaminated by heavy metals those are present beside different industries (soap and detergent), garments, pharmaceuticals, dyeing, aluminum, carbide, match and ink manufacturing, textile pain, paper pulp and bar factories, steel workshop etc. (Rahman *et al.*, 2012). These types of industries are mainly present beside some main rivers such Turag, Buriganga, Sitalakkha, Dhaleshwari in Bangladesh.

Ordinarily, the allocation different heavy metals in soil is influenced by the nature of parent materials, climatic conditions and their relative mobility depending on soil parameters, such as mineralogy, texture and classification of soil (Mohiuddin *et al.*, 2011). Some important properties of soil such as pH and organic carbon that governance the accumulation and the availability of heavy metals in soil. Cadmium (Cd) is a highly toxic heavy metal pollutant classified as human carcinogen (Henkel and Krebs, 2004). Excessive exposure to Cd^{2+} can lead to 'itai-itai' disease, affecting cardiovascular system and intestine (Shah and Nahakpam, 2012). The continuous release of cadmium from different industries such as paint, batteries and jewelry and the low permissible limit (0.01 mg/L) assert significant threat to the environment and human health (Nawrot *et al.*, 2010; Eichler *et al.*, 2014).

Various experiments were conducted on availability of cadmium different area in Bangladesh was found 0.8 µg -7 µg per gram of soil. Gradually, cadmium concentration is increasing in our crop field. Whereas Bangladesh is a floodplain area for this cadmium spread out mostly cultivated area through flood water in rainy season. Heavy metal pollution of aquatic system is growing at an alarming rate worldwide due to anthropogenic activity (Malik *et al.*, 2010). Metals like Cr, Pb, Cd, As *etc.* exhibit high toxicity even at trace levels. In Bangladesh all most all the

industries are present at the bank of the river, in this case those industries are drainage their waste material in the river water. In 9th January 2017, daily newspaper Prothom Alo reported that industries at the side of the river mainly leather industry deposits different 11 items of heavy metal in river. Also increasing water pollution and reducing dissolved oxygen in water which reducing aquatic plants and fishes. Rivers are a main pathway for metals transport in cultivable land (Miller *et al.*, 2003) and become significant pollutants of many riverine systems. Outflow of cadmium in soil varied depending on the source (Hasanuzzaman and Fujita, 2012a).

Cadmium is a toxic pollutant that can easily be taken up by plant roots and gathered into the xylem of the leaves, causing inhibition of plant growth, changing in the photosynthesis rate, the uptake of micro and macronutrients and water use efficiency conducting to a reduction in crop production (Benavides *et al*., 2005). The main problem of Cd is that it can be shifted to the food chain and assert a threat to human health (Clemens, 2006). The FAO/WHO recommended maximum tolerable intake of Cd is 400–500 μg $week^{-1}$ or 70 μg d^{-1}. Cadmium is a non essential element for plants and is the fifth most toxic metal to vertebrates and the fourth most toxic metal to vascular plants. It is suspected that the agricultural land with fertilizers and agrochemicals for long periods of time is the main reason for accumulation of Cd in crop field. Premarathna *et al.* (2011) reported that the triple super phosphate (TSP) used by the Sri Lankan farmers carried 23.50 to 71.4 mg/Cd/kg of P_2O_5. He also proved that commonly used weedicides in rice cultivation in Sri Lanka known as bispyribac sodium contains 0.5mg/l of Cd. Cultivation of crop in cadmium polluted soil may also minimize water and nutrient uptake (Li *et al.*, 2008), as well as causes chlorosis and necrosis of the leaves. One of the most target sites of cadmium is photosynthetic apparatus of crop as well as inhibits biosynthesis of photosynthetic pigments, can decrease electron transport efficiency, inhibits the enzymes involved in photosynthesis, reduces photosynthetic carbon assimilation, and causes oxidative damage to sub-organelles (Maksymiec *et al.*, 2007). Cadmium induced abnormal seed germination, reduced and irregular growth, disorganized development of reproductive organs and yield components, and reduced yield (Gill and Tuteja, 2011). Also cadmium hampered the seed germination rate, root elongation, shoot elongation, and seedling growth of wheat (*Triticum aestivum*) (Chen *et al.*, 2010). Shekar *et al.* (2011) reported on germination, according to his report the seedling survival percentage of

beans was gradually reduced from the control as Cd stress levels increased, the survival percentage of seedlings in the control and in Cd 50, 100, 200, 300, and 400 mg kg^{-1} soil samples was 89.0 and 83.0, 76.0, 70.0, 62.0, and 54.0% respectively. Growth of tomato plants were inhibited when nutrient media contained 10 μM of cadmium solution. Therefore, chlorosis of leaves, reduced length, and the browning of shoots were the main toxicity symptoms (Cherif *et al.*, 2011). Asgher *et al.* (2014) also reported that reduced plant growth was correlated to Cd-mediated reduction in the maximum photochemical efficiency of photosystem II (PS II), enhanced impairments in the net CO_2 assimilation rate and decreased ribulose 1,5-bisphosphate carboxylase (Rubisco) activity.

Gill and Tuteja (2010); Anjum *et al.* (2012) reported that metals/metalloids can be prompted the formation of ROS and a powerful inducers of lipid per-oxidation in plants. Markedly, redox active metals (such as Cu, Cr, and Fe) can cause lipid per-oxidation through producing ROS in redox cycling. However, redox inactive metals (such as Cd, As, Co, Hg, Al, Ni, Pb, Se, Zn etc.) fetch significant damage in antioxidant defense components such as thiol-containing antioxidants and enzymes, and at last cause lipid per-oxidation. Many studies showed that by inducing lipid per-oxidation Cd strongly alterations in the functionality of membranes and troubling in chloroplast metabolism through inhibiting chlorophyll biosynthesis and reducing the activity of enzyme which is related in CO_2 fixation. (Cuypers *et al.*, 2011; Gallego *et al.*, 2012; Gill *et al.*, 2013). Different levels of lipid per-oxidation occur in different organs of the same plant under Cd exposure. For example, Talukdar (2012) showed that MDA (a lipid per-oxidation product) accumulation was more pronounced in shoots than in roots of the Cd exposed lentil (*Lens culinaris*) seedlings. Stohs and Bagchi (1995) seen that some metals such as Cd, Pb, and Hg depleted the protein-bound thiol groups.

Bansal *et al.* (2002) documented that Cd also inhibit mitochondrial enzymes, such as α-ketoglutarate, isocitrate dehydrogenase, succinate dehydrogenase and malate dehydrogenase. Cd also intervene with the uptake, transport and use of some essential mineral elements, such as calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K) and iron (Fe) by inducing deficiency in plants (Nedjimi and Daoud, 2009). Dias *et*

al. (2013) described that Cd-mediated disruption in the coordination between carbon (C), nitrogen (N) and sulfur (S) metabolism in plant cells.

2.4 Abiotic stress-induced oxidative stress

Minimal levels of ROS may act as significant signal transduction molecules to varied stresses but a chaos in the ROS in any cell compartment conducts a situation called oxidative stress. Anjum *et al.* (2012) ; Krasensky and Jonak (2012) showed that oxidative stress (residual ROS and their reaction products within cells) can cause significant physiological damages including cell death, and the arrest of plant growth and development, mainly by stimulant oxidative modification of vital bio-molecules including membrane lipids, cellular amino acids, proteins and DNA.

Abiotic and biotic stresses both are affecting on the production of active oxygen species in plants and making oxidative stress. Foyer and Noctor (2003) reported that oxidative stress is caused through serious imbalance in any compartment between the production of reactive oxygen species (ROS) and antioxidant defense. It is necessary for cells to control the concentration of ROS tightly, but not to expel them entirely (Schutzendubel and Polle, 2002). ROS can be seen as cellular indicators of stress and secondary messengers involved in all aspects of plant biology from gene expression and rendering to enzyme chemistry (Foyer and Noctor, 2003). Many reports have showed that the negative effect of environmental stresses may be partially due to the generation of reactive oxygen species and interdict of the system which protects against them.

At time of the reduction of O_2 to H_2O, a transfer of one, two or three electrons to O_2 can occur to superoxide ($O_2^{\cdot-}$), hydroxyl radicals ($\cdot OH$)**,** hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) are most active, toxic and destructive. At the membrane level in most plant cell organelles the superoxide radical is produced and hydrogen peroxide is the product of superoxide dismutase and of various oxidases of the peroxisomes. These reactive molecules, particularly $\cdot OH$, are highly exterminatory to lipids, nucleic acids, and proteins. Albeit, ROS such as $O_2^{\cdot-}$ and H_2O_2 are required for lignification and function as signals in the defense repercussion to pathogen infection (Gratao *et al.*, 2005).

Asada (1997) reported that the photosynthetic electron transport system is the principal source of active oxygen species in plant tissues, have the potential to originate singlet oxygen $^1O_2$ and superoxide O_2^-. Olga *et al.* (2003) resolved that generation of reactive oxygen species (ROS) is characteristic for hypoxia and particularly for re-oxygenation. A number of cellular reactions are produced by both hydrogen peroxide (H_2O_2) and superoxide (O_2^-), including the iron-catalysed Fenton reaction, and by several enzymes such as lipoxygenases, peroxidases, NADPH oxidase and xanthine oxidase. The main cellular components are lipids (peroxidation of unsaturated fatty acids in membranes), proteins (denaturation), carbohydrates and nucleic acids are susceptible to damage by free radicals.

Noctor (2005) informed that plants use reactive oxygen species (ROS) as secondary messengers in signal transduction cascades in processes as assorted as mitosis, tropisms and cell death, so their accumulation is crucial factor to plant development and defense. . If ROS escape from destruction by antioxidants or consumption in a ROS cascade only then direct ROS signal transduction will proceed.

Proteins sheltering RNA chaperone activity, RNA- binding proteins (RBPs), have been recently described to prevent RNA miss-folding or support resolving non-functional structures, thereby playing a significant role in post-transcriptional gene regulation under stress conditions (Cabello and Chan, 2012).

Even though the plant growth is controlled by a multitude of physiological, biochemical, and molecular processes, photosynthesis is a key phenomenon, which contributes substantially to the plant growth and development. Stress-induced stomatal or non-stomatal limitations reduce the photosynthetic rate (Saibo *et al.*, 2009; Rahnama *et al.*, 2010). For instance, drought stress, especially at its mild intensity, can inhibit leaf photosynthesis and stomatal conductance in most green parts of plants (Medrano *et al.*, 2002). Different reports showed that stomata generally close during the initial stages of drought stress resulting in increased WUE (net CO_2 assimilation rate/transpiration). Transpiration of water is inhibited by closure of Stomata than that on CO_2 diffusion into the leaf tissues (Chaves *et al.*, 2009; Sikuku *et al.*, 2010). Though, in opposite, under severe drought stress, dehydration of mesophyll cells takes place resulting noticeable inhibition of basic metabolic

processes of photosynthesis as well as a reduction of plant WUE (Damayanthi *et al.*, 2010; Anjum *et al*., 2011). The efficiency of mesophyll cells to utilize the available CO_2 is reduced by drought stress (Karaba *et al*., 2007; Dias and Bruggemann, 2010a, b).

Different stressful conditions have been showed to decrease photosynthetic pigment. For example, Li *et al*. (2010) and Yang *et al*. (2011) reported that salt stress can break down chlorophyll (Chl), the effect imposed to increased level of the toxic cation, Na^+. Under abiotic or biotic stress carotenoids (Car) are necessary for photo-protection of photosynthesis and they play an essential role as a vanguard in signaling during the plant development. Carotenoids (Car) have an effective potential to promote nutritional quality and plant yield. Gomathi and Rakkiyapan (2011) found that under abiotic stress such as salt stress (7–8 dS m^{-1}) at various plant-growth stages caused a remarkable reduction of Chl and Car contents. Abiotic stress (salinity stress, drought stress etc.) causes not only an effective damage to photosynthetic pigments, but it also leads to deterioration of thylakoid membranes (Huseynova *et al*., 2009; Anjum *et al*., 2011; Kannan and Kulandaivelu, 2011).

Signorelli *et al*. (2013) showed that as a consequence of the water stress makes differential spatial distribution of oxidative and nitrosative stress. The oxidative and the nitrosative stress component were higher in leaves and roots, respectively. Fallen *et al.* (2013) reported that reliable Quantitative Trait Loci (QTLs) for drought resistant improved soybean tolerance to drought stress. Vandecasteele *et al*. (2011) investigated that the composition or amount of soluble sugars (sucrose) and raffinose oligosaccharide family (RFO) was part of the genetic determinants of seed vigor in *M. truncatula.* But the correlation and co-location of sucrose/RFO ratio with germination and radicle growth QTLs suggested that an increased sucrose/RFO ratio in seeds might negatively affect seed vigor.

2.5 Antioxidant defense system

Gout *et al.* (2001) concluded that the formation of ROS is checked by an antioxidant system, low molecular mass antioxidants (such as ascorbic acid, glutathione, and tocopherols), enzymes regenerating the reduced forms of antioxidants and ROS-interacting enzymes such as SOD, peroxidases and catalases. Tanoua *et al.* (2009)

reported that sodium nitroprusside (SNP) increased the activities of leaf SOD, CAT, APX and GR antioxidant enzyme along with the inductionof related-isoform(s).

Gill and Tuteja (2010); Anjum *et al.* (2012) reported that generally aerobic metabolism in plants produce reactive oxygen species (ROS; such as $O_2^{\bullet -}$, H_2O_2, and $^{\bullet}OH$), where antioxidant defense mechanism (consisting enzymes such as superoxide dismutase, SOD; catalase, CAT; guaiacol peroxidase, GPX; glutathione sulfo-transferase, GST; ascorbate peroxidase, APX; monodehydroascorbate reductase, MDHAR; dehydroascorbate reductase, DHAR; glutathione reductase, GR; and non-enzymes such as ascorbate, AsA; glutathione, GSH; carotenoids; tocopherols; phenolics) efficiently scavenge ROS and also keep up their levels at non-damaging levels.

Photosynthetic cells are affected by oxidative stress causes they contain photo sensitive pigment; also produce and consume oxygen. According to Asada (1997) key source of active oxygen species in plant tissues is photosynthetic electron transport system which produces singlet oxygen $^1O_2$ and superoxide O^-_2. Olga *et al.* (2003) showed that various phenolic compounds such as flavonoids, tannins and lignin in plant tissues work as antioxidant and ROS scavenging compounds. Under oxygen deprivation condition antioxidants are not work on this situation compartmentalization of ROS formation and antioxidant localization, synthesis and transport of antioxidants are effective for their defense (Olga *et al.*, 2003).

According to Noctor and Foyer (1998) reduced glutathione (GSH), a disulphide reductant that protects thiols of enzymes and react with reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($^{\bullet}OH$) together with ascorbate and protect the plants. Ederli *et al.* (2004) showed that to compete with toxic metals plants make sulfur pathway to supply huge amount of glutathione for the biosynthesis of phytochelatins (PCs) which sequestrate the metals.

Fatima and Ahmad (2005) reported that SOD is a major enzyme in protecting cells from oxidative stress, which constitutes initial line of defense as to dismutate superoxide radicals to H_2O_2. Mittler (2002) documented that deterioration of H_2O_2 to

water and oxygen is carried out by CAT in peroxisomes or by POD in vacuoles, cell wall and cytosol.Most of the ROS-scavenging pathways in plants involve superoxide dismutase (SOD) that found in almost all cellular compartments, the water–water cycle in chloroplasts, the ascorbic acid (AsA)–glutathione (GSH) cycle in chloroplasts, cytosol, mitochondria, apoplast and peroxisomes, glutathione peroxidases, and catalase (CAT) in peroxisomes (Mittler, 2002). Aravind and Prasad (2005) showed that the SOD catalyzes the O_2^-radicals to molecular oxygen and hydrogen peroxide (H_2O_2) which is then reduced to H_2O and O_2 by CAT, guaiacol peroxidase (GPX), and ascorbate peroxidase (APX).

Maier *et al.* (2003) described that GSH (γ-glutamylcysteinyl glycine) activity well as an antioxidant because of its supreme structural properties, broad redox potential, abundance and wide distribution in plants. Maier *et al.* (2003) found that increased GSH levels were found to be related with exogenous Cd stress tolerance and May *et al.* (1998) showed that increased GSH biosynthesis has been reported to be an intrinsic response of plants against Cd stress. Noctor *et al.* (2002) also described that GSH is a substrate for GPX and GST, which is involved in the removal of ROS. GHS also helps to formation of phytochelatins (PCs), which have a relation to heavy metal (HM) and are transported as complexes into the vacuole, thus allowing plants to have some level of resistance to HM (Sharma and Dietz, 2006). Other function of GHS is detoxification of xenobiotics and as a storage and transport form of reduced sulfur (Srivalli and Khanna-Chopra, 2008). Hasanuzzaman *et al.* (2011a, b) suggested that increased GHS content in plant increases protection to various abiotic stresses.

Ascorbate is synthesized in the cytosol of higher plants primarily from the conversion of D-glucose to AsA which act as an important antioxidant in plant tissues. According to Smirnoff (2005) it reacts with a range of reactive oxygen species (ROS such as H_2O_2, $O_2^{\cdot -}$, $^1O_2$ and $OH^{\cdot}$) at diffusion-controlled rates. De Tullio (2004) reported that AsA is responsible for keeping prosthetic metal ions in a reduced form and also maintaining the activity of various antioxidant enzymes. Hasanuzzaman *et al.* (2011a) showed that AsA plays an important role in plant stress tolerance. Shalata and Neumann (2001) also found that exogenous application of AsA increasing the activity of different enzymes which helps to minimize the damage caused by oxidative processes through synergic function with other antioxidants.

Different antioxidant enzyme situated in different sites of plant cells and work together for detoxification of ROS. Hasanuzzaman *et al.* (2012a) documented that the AsA-GSH cycle includes 4 enzymes (APX, MDHAR, DHAR and GR) as well as AsA, GSH and NADPH which work together to detoxify H_2O_2 in a series of cyclic reactions and again regenerate AsA and GSH.

Catalases (CATs) are present in peroxisomes, glyoxysomes, and related organelles (Mittler, 2002) where H_2O_2-generating enzymes are located (Agarwal *et al.*, 2009). CAT is one of the most important enzymes whose one molecule can convert around six million molecules of H_2O_2 to H_2O and O_2 per minute. Gill and Tuteja (2010) showed that CAT is an important enzyme in removing of H_2O_2 generated in peroxisomes by oxidases involved in β-oxidation of fatty acids, photorespiration, and purine catabolism. It has also been reported that seperate from reaction with H_2O_2, CAT also react with some hydroperoxides such as methyl hydrogen peroxide (MeOOH) (Ali and Alqurainy, 2006). Many response of CAT activity has been observed under metal stress, its activity increased in *O. sativa* (Hsu and Kao, 2004), *Brassica juncea* (Mobin and Khan, 2007), *Triticum aestivum* (Khan *et al.*, 2007), *Cicer arietinum* (Hasan *et al.*, 2008) and *Vigna mungo* roots (Singh *et al.*, 2008) under Cd stress. Hsu and Kao (2007) also documented that pretreatment of rice seedlings with H_2O_2 under non-heat shock conditions increasing the CAT activity and protected rice seedlings from subsequent Cd stress.

Noctor and Foyer (1998) found that APX family consist with five different isoforms including mitochondrial (mAPX), thylakoid (tAPX) and glyoxisome membrane forms (gmAPX), as well as chloroplast stromal soluble form (sAPX), cytosolic form (cAPX). Hasanuzzzaman and Fujita (2011a, b) reported that APX activity in increased in plants during different abiotic stress conditions.

Glutathione reductase (GR) is a dynamic enzyme of the AsA-GSH cycle which catalyzes the reduction of GSH. Pang and Wang (2010) described that GR maintains a high ratio of GSH/GSSG in plant cells that is necessary for enhancing the H_2O_2 scavenging pathway mainly under stress conditions.

Dixon *et al.* (2010) described that GST is a multifunctional enzymes of super-family which catalyse the conjugation of electrophilic xenobiotic substrates with GSH. Gullner and Komives (2001) also showed that besides catalyzing the conjugation of electrophilic compounds GST isoenzymes also exhibit the activity of POX.

2.6 Effect of cadmium on rice

Cadmium (Cd) is one of the major pollutants in paddy fields, and its accumulation in rice (*Oryza sativa* L.) and next is a global environmental issue as transfer to food. Ahsan *et al.* (2007) found that excessive Cd (between 0.2 and 1.0mM) in the growth medium reduced rice seed germination. When growing media of seeds are contaminated by heavy metal such as cadmium prevents water uptake and water movement in the embryo axis, this is one of the main reasons for low germination (Vijayaragavan *et al.*, 2011), slow germination and imperfect seedling development (Rahoui *et al.*, 2010). Cadmium toxicity reduced rice growth as calculated in terms of root and shoot length, leaf and root area, and number of leaves and roots per plant (Yu *et al.*, 2006; Song *et al.*, 2015). Photosynthesis and gas exchange characteristics (chlorophyll a, chlorophyll b, carotenoids, net photosynthetic rate, stomata conductance, transpiration rate, and water use efficiency) is decreased in rice under cadmium stress (Rascio *et al.*, 2008; Wang *et al.*, 2014). In general, Cd stress restricted rice growth and biomass (Zhou *et al.*, 2014; Mostofa *et al.*, 2015; Rahman *et al.*, 2015).

Cadmium stress also inhibits the uptake and translocation important nutrients by rice (Li *et al.*, 2012a). Several studies informed that in rice seedlings, Cd toxicity increased oxidative stress by release of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), malondialdehyde (MDA) contents, and electrolyte leakage (EL) (Yu *et al.*, 2013; Srivastava *et al.*, 2014). Smeets *et al.* (2008) reported that the bindings of Cd to sulfhydryl and carbonyl groups or the replacement of essential cofactors which can finally lead to oxidative stress. Cadmium toxicity deflected leaf and root ultra-structure and caused structural damages to photosynthetic apparatus of rice. DNA polymorphism is induced by cadmium in preferential mutation sites in rice roots is a cadmium dose-dependent manner (Aina *et al.*, 2007). It has been reported that Cd treatment for 24 h compared to control is regulated 36 proteins were up or down in rice under 100 μM (Lee *et al.*, 2010). Lee *et al.* (2013) also expressed that

under Cd stress condition rice may over-expression of glutamine synthetase (GS) gene which modulated the oxidative response in rice that may result in plant death.

Liu *et al.* (2003a, b) reported that the Cd interactions with iron (Fe), zinc (Zn), and copper (Cu) are synergistic in uptake and translocation from rice root to shoot. Rodda *et al.* (2011) found that Cd could transfer to rice grains and decreased grain yield, quality, and nutrient uptake. Response of rice to Cd stress varies with rice genotypes, the dose and duration of Cd stress applied (Yu *et al.*, 2006; Song *et al.*, 2015).

Fe and Cd are closely linked together in plants (Rizwan *et al.*, 2016) because they have similar chemical properties and share the same transporters (Ogo *et al.*, 2014). It was documented that Cd could be absorbed and transported by the transporters which under to essential metals. These transporters show broad substrate specificity towards divalent metals including Fe^{2+}, Zn^{2+}, Mn^{2+}, and Cd^{2+} (Vert *et al.*, 2002). Fe deficiency symptom induces if Cd exists in plants, where Cd inhibits not only Fe absorption (Sharma *et al.*, 2004), but also Fe transportation from underground parts to aboveground parts (Yoshihara *et al.*, 2006; Solti *et al.*, 2011; Xu *et al.*, 2015). At the rate of increasing iron (Fe) deficiency increasing of cadmium (Cd) absorption through the Fe uptake systems (Lombi *et al.*, 2002). Increasing the supplementary application of Fe reduces the Cd concentration in rice and decreases Cd toxicity (Sebastian and Prasad, 2015). Dynamic increase in $CdCl_2$ concentration increased the carbonylated derivative formation in two rice (*Oryza sativa*) varieties (IR-29 and Nonabokra) (Roychoudhury *et al.*, 2012), which can increase the local ROS in the peroxisomes.

Chang *et al.* (2012a, b) showed that Fe concentration decreased in both shoots and roots of rice seedlings when treated with $CdCl_2$ and chlorosis was visually observed in the leaves of Fe deficient and Cd-treated rice seedlings. Chang *et al.* (2012a) also showed that Fe deficiency is caused by inducing the expression of *OsIRT1* gene in rice roots and Cd induces the expression of *OsIRT1* gene in rice roots (Chang *et al.*, 2012b).

Major physiological functions of potassium (K) include osmoregulation, cell elongation, regulating stomatal aperture, enzyme activation, phloem solute transport,

chloroplast structure and functioning, cation/anion balancing, protein and starch synthesis, and energy conservation across membranes (Hafsi *et al.*, 2014; Shabala and Pottosin, 2014). Liu *et al.* (2012) reported that on treatment of $CdCl_2$ in Cd sensitive rice cultivar with increasing Cd concentration decreasing K concentration in root and shoot of seedlings and reduces activity of the physiological function. Hsu and Kao (2007a) shown that Cd toxicity in the leaves of rice seedlings is due to accumulation of H_2O_2 and Cd-dependent H_2O_2 production originates from plasma membrane NADPH oxidase.

Lin *et al.* (2011) documented that in rice seedlings, N deficiency increases subsequent Cd toxicity and decreased the activities of ascorbate peroxidase, glutathione reductase, and catalase and the content of ascorbate in rice leaves.

Poschenrieder *et al.* (1989) showed that heavy metals such as Cd increases the abscisic acid (ABA) in plants derived from xanthophylls. Fediuc *et al.* (2005) demonstrated that Cd-induced ABA accumulated in roots, but not in shoots, of *Typha* and *Phragmites* plants. Hsu *et al.* (2006) shown that Cd-induced ABA content increased in the rice seedling leaves at high temperature (35/30 °C, day/night).

Cd damages the photosynthetic apparatus, lowers chlorophyll and alters proline and polyamine contents (Sharma and Dietz, 2006). Asada (1999) shown that oxygen is important for the subsistence of aerobic life, but toxic reactive oxygen species (ROS), which include the superoxide anion O_2^-, hydroxyl radical (OH•) and hydrogen peroxide (H_2O_2), are generated in all aerobic cells during metabolic processes and ROS only considered as damaging to cells. $CdCl_2$ increased the content of H_2O_2 and MDA, decrease the GSH and ASC contents and protein loss and lipid per-oxidation in rice leaves. Cobbett and Goldsbrough (2002) reported that GSH is the vanguard of phytochelatins, cysteine-rich peptides, synthesized via phytochelatin synthase. An acute depletion of GSH is a general response to Cd caused by an enhanced consumption of GSH for phytochelatin production (Schutzendubel and Polle, 2002).

In plants, Cd affects physiological and morphological system causes stunted growth, chlorosis and decreased reproducibility. Hernandez and Cooke (1997) demonstrated that leaf chlorosis, leaf and root necrosis, primarily decrease in growth are the main

symptoms of Cd toxicity in plants. Khan *et al.* (2007) reported that cadmium (Cd) interacts with proteins and nucleic acids, as a result affects enzyme activities and causes alteration in membrane permeability and leading to the loss of membrane function.

Uraguchi and Fujiwara (2012) revealed in their study that the xylem- arbitrate Cd translocation from roots to shoots as the main decisive factor for shoot Cd accumulation has been ensured in a number of plants including rice. Sasaki *et al.* (2014) reported that OsHMA2 and OsHMA3 were to take effect in this process. OsHMA3 plays a critical role in Cd compartmentalize into vacuoles in root cells; while OsHMA2 is involved in the distribution of Cd to developing tissues (Takahashi *et al.*, 2012). Uraguchi *et al.* (2011) observed that after transfer from xylem to phloem at nodes, Cd is favorably transported to the upper nodes and at last into the panicle instead of into leaves. (Wu *et al.*, 2015b) found in several studies that further accumulation of Cd into grains is mediated by phloem. Fujimaki *et al.* (2010) observed that nodes are the central organ for xylem to phloem transfer, which play a vital role in Cd translocation from soil to grains at the grain-filling stage. Uraguchi *et al.* (2014) have identified OsLCT1 as a Cd transporter gene that revealed at the nodes for transporting of Cd into grains.

Redjala *et al.* (2011) described that cadmium ions move into the root via the rhizodermis cell walls, from soil solution towards vascular cylinder. Sasaki *et al.* (2012) reported that as a member of the Natural Resistance-Associated Macrophage Protein (NRAMP), OsNRAMP5 is responsible for the transport of Fe, Mn and Cd from the external solution to root cells in rice. Takahashi *et al.* (2011b) indicated that another NRAMP gene, OsNRAMP1 is localized to the plasma membrane in plants which was demonstrated to participate in cellular Cd uptake. Takahashi *et al.* (2011a) also discovered that OsNRAMP1 development in roots was increased in the presence of 1 mM Cd under Fe deficiency, causing in increased uptake of Cd in rice. Ye *et al.* (2012) investigated on rice genotype to Cd uptake and they found that Cd accumulation in indica polished grain were higher than that in hybrid and japonica grain. Liu *et al*. (2003) reported that the average Cd accumulation in rice roots were much higher than in stems and leaves and rice grains. Liu *et al*. (2003) also found that the average Cd concentrations in roots were much higher than in stems and leaves at

the two periods in rice plants. The ratios of average Cd concentration in roots to stems to leaves were 139:5:1 at heading stage and 53:4.5:1 at ripening stage.

Herath *et al*. (2014) found that with increasing soil cadmium concentration the plant height, number of tillers, flag leaf chlorophyll content, leaf area of the flag leaf, and root dry weight was decreased significantly new improved varieties such as Bg 300 and Bg 358 as well as traditional varieties such as kaluheenati and kuruluthudu in Sri Lanka.

2.7 Nitric oxide and crop productivity

Nitric oxide (NO) is known as a biological messenger in various tissues of plants to regulate diverse range of physiological process along with growth, development and response to abiotic and biotic stresses. Beligni and Lamattina (2002) mentioned that NO is a gaseous multifunctional messenger molecule. Exogenously applied NO has multiple effects on different physiological and biochemical processes of plants. Planchet *et al*. (2005) described that in the cell free system NO produce by nitrate reductase (NR) enzyme, it reduces the nitrate to nitrite in the expense of NAD(P)H, further catalyzes a 1-electron transfer from NAD(P)H to nitrite resulting in formation of NO. At present heavy metal toxicity is one of the major abiotic stresses in plants, accumulate excessive amount of reactive oxygen species (ROS) which leads to peroxidation of lipids, oxidation of protein, inactivation of enzymes, DNA damage and interact with different component of plant cells. Different articles reported that exogenous application of NO mitigating heavy metal toxicity in plants.

NO was considered as toxic compound released from industrial waste, vehicle exhaust, cigarette smoke etc before discovering as a signaling molecule to free radical. Beligni and Lamattina (2001) discovered that nitric oxide (NO) is a bioactive molecule acts a key role in diverse physiological process in plants. Arasimovic and Floryszak-Wieczorek (2007) reported that NO can be produced in plants by enzymatic and non-enzymatic systems. According to Misra *et al.* (2011) NO can decrease stress response through two processes one by a signaling molecule in various cellular events leading to changes in the gene expression and another by an antioxidant by directly scavenging ROS, such as O_2^-, to form peroxynitrite ($ONOO^-$) which is considered less toxic than peroxides. NO can interact with ROS due to

existence of unpaired e^- within NO molecule. Wink *et al.* (1993) reported that lower amount of NO acts as a signal for activation of defense response in plants but at higher concentration it causes acute injury due to fluent ROS generation.

Under heavy metal stress condition plant produces NO can play a vital role against damage due to stress (Hung and Kao, 2005). Hung and Kao also found that exogenous NO similarly worked against in the destructive action of ethylene, herbicides and other abiotic stress. Polverari *et al.* (2003) described that NO helps to produce various redox-regulated defense related gene expression which directly or indirectly helps to establish plant stress tolerance. Rao and Davis (2001) found that pretreatment of wheat seeds with NO could significantly improve germination and reduce oxidative stress against Cu toxicity. Soybean plants grown $CdCl_2$ contaminated soil at the level of 200µM, the exogenous application of NO protected soybean plants against oxidative damage caused by this metal stress, increasing the level of heme oxygenase-1 expression, as it occur with other gene involved in the antioxidant defense system (Orozco-Cardenas Ryan, 2002).

Uchida *et al.* (2002) discovered that pretreatment of seedlings with 1µM Sodium nitroprusside (SNP) protected rice leaves against Cd-induced oxidative stress. According to Singh *et al.* (2004) information in *Lupinus* roots grown with 50µM Cd, protection of NO could consist of stimulation of SOD activity to counteract over production of O_2^-, thus supersede formulation of $ONOO^-$ from NO to O_2^-. Zhang *et al.* (2008) in their another experiment with *Cassia tora* plant observed that pretreated for 12h with 0.4 mM SNP and subsequently exposed to24h to10µM Al shown a significantly greater root elongation and a decrease in Al accumulation in root apexes as compared with plants without SNP treatment. Hsu and Kao (2004) reported that exogenous NO prevent Cd induced, increase in the contents of H_2O_2 and malondialdehyde (MDA), decrease the contents of GSH and ascorbate and increase the specific activities of antioxidant enzymes in rice. Xu *et al.* (2010) also reported that NO prevents Cd induced accumulation of $NH4^+$, decrease the activity of GS and increase the specific activity of Glutamine synthetase. NO provides resistance to rice against As induced toxicity. They again found that NO also improve Cd tolerance in *M. truncatula* roots by enhance in the production of proline and total GSH content. Cui *et al.* (2009) discovered that exogenous application of SNP developed ROS scavenging enzymes reduced accumulation of H_2O_2 and induced the activity of

H^+ATPase and H^+PPase in plasma membrane or tonoplast, also significantly reduced the growth inhibition included with $CuCl_2$ in tomato plants.

Singh *et al.* (2008) documented that 24h treatment of wheat seeds and seedlings with $CdCl_2$ increasing MDA contents 11-21%, REL (relative electrolyte leakage) 33-55%, SOD, CAT and GR 32-67% and GPX 8-31% with increasing of Cd concentration from 50µM to 250µM. they also found that application on NO donor SNP with Cd significantly reduces the MDA, REL, SOD, CAT and GR percentage in wheat roots.

Bethke *et al.* (2004) reported that lower amount of SNP (25µM) reduced dormancy and stimulated germination, but SNP at 250 µM or more impaired seedling development, including root growth, and inhibited germination of *Arabidopsis* seeds. He also noted that dormancy was reduced when *Arabidopsis* seeds were exposed to gasses which are generated by solutions of SNP. An *et al.* (2005) showed that exo and endogenous NO promote shoot growth of maize, NO stimulate activation of cell division and embryogenic cell formation (Otvos *et al.*, 2005), phloem and xylem differentiation (Gabaldon *et al.*, 2005), root development and lateral root formation in tomato and plant–rhizobacterium interaction (Creus, 2005), auxin-induced NO and cGMP (cyclic guanosine monophosphate) mediate gravitropic flexure in soybean roots (Hu *et al.*, 2005) is also regulated by NO. NO donor SNP treatment maize seedlings increase the accumulation of K^+ in leaves and sheath which stimulates chlorophyll biosynthesis and chloroplast differentiation (Zhang *et al.*, 2006). NO also regulated the stoma closure in leaf which is normally done by ABA.

An Arabidopsis mutant protein AtNOS1 was identified that involved NO production, AtNOS1-deficient *Arabidopsis* plants were initiated to flower earlier than wild-type plants when treated with NO (Guo *et al.*, 2003).

NO plays role in root elongation like as auxin. Lamattina *et al.* (2001) revealed that NO treatment in explants of wood species were responsive to induce adventitious root formation. Pagnussat *et al.* (2002) also expressed that IAA treatment in cucumber explants increase in the level of endogenous NO in the basal region of the hypocotyls. Arnaud *et al.* (2006) found that NO appears to be a primary element in the signal

transduction pathway leading to the increase of the iron-storage protein ferritin at both protein and mRNA levels.

Treatment of plants with NaCl reduces the fresh weight of shoot and root. Boldizsar *et al.* (2013) revealed that this decrease is lower when combined application of NaCl and an NO-donor ((Z)-1-[N-(2- aminoethyl)-N-(2-ammonioethyl) amino] diazen-1-ium-1,2-diolate, DETA/NO) in the shoots, while it was greater after simultaneous treatment with NaCl and nitro-$_L$-arginine ($_L$-NNA, inhibitor of NO synthesis) in the root. Also found that Pro. $_L$-NNA (a NO donor) treatment resulted in a significant increase in the Ala, Val, Gly and Tyr amino acid contents.

Singh *et al.* (2013) reported that 5 and 50 µM arsenic (As) declined 15 and 26% growth of *Luffa acutangula* seedlings through significant accumulation of As respectively. SNP (100 µM) protected the *Luffa* seedlings by reducing the arsenic accumulation. Also expressed that SNP developed photosynthetic pigments and chlorophyll fluorescence parameters such as Fv/Fm, Fv/F0, Fm/F0 and q^P (decreased at 10 and 22%, 27 and 49%, 19 and 35%, and 13 and 24% at 5 and 50 µM arsenic respectively) which were decreased by NPQ was raised from As. (Where F0, minimal fluorescence; Fv (Fm-F0), variable fluorescence in dark adapted leaves; Fv/Fm, maximum photochemical efficiency of PS II; Fv/F0, the activity of PS II; Fm/F0, electron transport rate through PS II; qP, photochemical quenching; NPQ, non-photochemical quenching).

Singh *et al.* (2009) documented that treatment with 50µM SNP (a NO donor) developed the root and coleoptile length of rice that was decreased by arsenic toxicity. They also expressed that As-induced malondialdehyde (MDA), superoxide ion (O_2^-), root oxidizability and H_2O_2 content in rice significantly alleviated by supplementary application of NO.

Kong *et al.* (2012) reported that heat stress condition induced increased thiobarbituric acid reactive substance (TBARS) content in mycelia of edible fungi (*Pleurotus eryngii* var. *tuoliensis*) which was dramatically reduced by exogenous application of NO donor SNP under high temperature (37ºC). Gould *et al.* (2003) also found that a quick and significant wave of NO level was activated in tobacco cells by heat stress.

Srivastava and Dubey (2011) discovered that exogenous application of $MnCl_2$ (manganese chloride) to excised rice leaves for 24 and 48 h resulted in increased production of H_2O_2 and lipid peroxides, decline in the levels of antioxidants, glutathione and ascorbic acid, and increased activities of antioxidative enzymes, superoxide dismutase, guaiacol peroxidase, catalase, ascorbate peroxidase, dehydroascorbate reductase, and glutathione reductase. Under treatment of rice leaves with 100 µM sodium nitroprusside (SNP), a NO donor, was effectively reduced Mn-induced increased levels of Hydrogen peroxide (H_2O_2), lipid peroxides and also increased the activity of antioxidative enzymes.

High temperature stress is a complex process. In wheat variety which are heat susceptible a significant decrease in membrane thermostability (MTS) and 2, 3, 5-triphenyl tetrazolium chloride (TTC) cell viability whereas content of lipid peroxide increased in high temperature (33±1º C). Susceptible wheat variety treatment with SNP increased the activities of all antioxidant enzymes with an increase in MTS and TTC (Bavita *et al.*, 2012).

NO also helps to defense against biotic stress by inducing defense gene expression and producing of antimicrobial compounds e.g. phytoalexins. Manifestation of hypersensitive response (HR), lignin deposition and defense-related enzyme phenylalanine ammonia-lyase, accumulation of salicylic acid and activation of pathogenesis related protein (PR-1) and launch of systemic acquired resistance (SAR) were enhanced by NO (Delledonne, 2005, Arasimowicz and Floryszak-Wieczorek, 2007).

Another target of NO is induced MAP kinase work in IAA- induced rooting process. Kumar and Klessing (2000) investigated that MAP kinase (mitogen activated protein kinase) activate in tobacco plant cells in response to SNP (a NO donor). NO and SA also SIPK (SA-induced protein kinase) functions as a downstream of SA (salicylic acid) in NO signaling for defense.

2.8 Effects of nitric oxide on rice under cadmium stressed condition

Wu *et al.* (2015a) mentioned that exogenous application of various signaling molecules such as nitric oxide (NO) reduced the Cd toxicity in rice seedlings. He *et al.* (2014) practiced with the effects of increasing doses of sodium nitroprusside (as exogenous NO donor) on rice seed germination, seedling growth, and activities of antioxidant enzymes under 100 μM Cd stress for 7 days. From this experiment they found that SNP decreased Cd accumulation by plants and decreased Cd-mediated inhibition of seed germination and seedling growth. They also found that SNP reduced oxidative stress, decreased production of H_2O_2 and MDA, and enhanced the activities of antioxidant enzymes, SOD, APX, POD, and CAT, and proline contents in both rice shoots and roots.

Singh and Shah (2014) described that exogenous SNP application decreased Cd uptake and reversed the Cd-induced toxic effects by restoring the membrane integrity on rice seedlings. Panda *et al.* (2011) showed that stress metabolism in rice seedlings in governed by SNP under Cd stress codition. Xiong *et al.* (2009a, b) reported that initiation of crown root primordia and increasing pectin and hemicellulose in the root cell wall enhanced by SNP. Zhao *et al.* (2013a, b) found similar thing that NO significantly reduced Cd uptake and enhanced rice tolerance to Cd stress.

Chapter 3

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 April-August, 2015. The location of the experimental site has been shown in Appendix I.

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. The physical and chemical properties of the experimental soil have been shown in Appendix II.

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 April to July. The detailed meteorological data in respect of air temperature, relative humidity, rainfall and sunshine hour recorded by the meteorology center, Dhaka for the period of experimentation have been presented in Appendix III.

3.4 Materials

3.4.1 Plant materials

BRRI dhan48 was used in the experiment. Features of this variety are given below:

BRRI dhan48: BRRI dhan48 variety is grown in aus season. It released by Bangladesh Rice Research Institute (BRRI) in 2008. It is a moderate leaf and sheath resistant variety. It completes its life cycle 105-110 DAS. It attains a plant height 105-

110 cm. 1000-seed weight is 23 g, grain is medium and white in color. Protein content is 8.5% and yield is 5.5 ton/ha.

3.4.2 Earthen pot

Empty earthen pots with 18 inch 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 Cadmium treatment

The cadmium treatments were applied on 15 DAT to 60 DAT. There were five cadmium levels including control developed by adding respective amount $CdCl_2$ to the soil pot^{-1} as water dissolved solution. The cadmium levels were C (control), $Cd_{0.50}$ (0.50mM), $Cd_{1.00}$ (1.00mM), $Cd_{1.50}$ (1.50mM) and $Cd_{2.00}$ (2.00mM). When no cadmium added it termed as control (C), while 15ml, 30ml, 45ml and 60ml cadmium chloride (1mM) added in 30 liter water was consider as $Cd_{0.50,}$ $Cd_{1.00,}$ $Cd_{1.50}$ and $Cd_{2.00}$ respectively. Solution was applied instead of irrigation @ 4 liter pot^{-1}.

3.6 Protectant treatment

Sodium nitroprusside (SNP) was used as a protectant. The concentration of SNP was 0.2mM and applied as a solution. 0.1192g solid SNP was mixed with 2 liter distilled water to prepare solution.

3.7 Treatments

The experiment consisted of single factor as mentioned below:

a) Total number of treatments: 10

i. Control (No cadmium)
ii. Control+0.2 mM SNP(nitric oxide donor)
iii. 0.50 mM $CdCl_2$
iv. 0.50 mM $CdCl_2$+0.2 mM SNP
v. 1.00 mM $CdCl_2$
vi. 1.00 mM $CdCl_2$+0.2 mM SNP
vii. 1.50 mM $CdCl_2$
viii. 1.50 mM $CdCl_2$+0.2 mM SNP
ix. 2.00 mM $CdCl_2$
x. 2.00 mM $CdCl_2$+0.2 mM SNP

SNP solution was applied as spray @ 1.5 liter for 5 pots.

3.8 Design and layout

The experiment was laid out in Randomized Completely Block Design (RCBD) with three replications. There were all together 30 pots in the experiment. The layout is given below:

R1	R2	R3
C	$Cd_{0.5}$	$Cd_{1.0}$
$Cd_{1.5}$ + SNP	$Cd_{2.0}$+ SNP	$Cd_{1.5}$
$Cd_{0.5}$	$Cd_{1.0}$	$Cd_{1.0}$ + SNP
$Cd_{1.0}$	C	$Cd_{1.5}$ + SNP
$Cd_{0.5}$ + SNP	$Cd_{1.5}$	$Cd_{0.5}$
$Cd_{1.0}$ + SNP	$Cd_{1.5}$ + SNP	$Cd_{2.0}$+ SNP
$Cd_{2.0}$	$Cd_{2.0}$	C
C+SNP	$Cd_{0.5}$ + SNP	C+SNP
$Cd_{1.5}$	$Cd_{1.0}$ + SNP	$Cd_{2.0}$
$Cd_{2.0}$+ SNP	C+SNP	$Cd_{0.5}$ + SNP

Conduction of the experiment

3.9 Seed collection

Seeds of BRRI dhan48 were collected from Bangladesh Rice Research Institute, Joydebpur, Gazipur.

3.10 Pot Preparation

The collected soil was sun dried, crushed sand sieved. The soil, cowdung and fertilizers were mixed well before placing the soils in the pots. Soils of the pots were poured in polythene bag. 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-labeled 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 kg/ha, 140 kg/ha, 50 kg/ha in the form of urea, triple super phosphate, muriate of potash , gypsum respectively and cowdung @5ton/ha. 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 15 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 April 23, 2015.

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

Before panicle initiation leaf roller infestations were observed in the crop and they were successfully controlled by applying Durshban two times on 55 DAT and 62 DAT at 20ml/10L of water. Rice bug also attacked at milking stage of grain and it was controlled by the application of Cypermathrin at 8 ml/10 L 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 cadmium and SNP treatments.

3.17 Germination test

Germination test for harvested seeds of different treatments was conducted in laboratory. For laboratory test, petridishes were used. Filter paper was placed inside petridishes. Firstly seeds were soaked in 10ml of 70% alcohol for 10 minutes. Then half amounts of seeds were soaked in SNP solution for 1 hr. The filter paper soaked with 10 ml water for Control and 10 ml of 0.5 mM, 1.0 mM, 1.5 mM and 2.0 mM $CdCl_2$ solution. Seeds were placed in petridishes randomly. 10 days after germination data was collected.

3.18 Collection of data

Data were recorded on the following parameters:

1. Phenological parameters

- Days to flowering
- Days to grain formation
- Days to maturity

2. Crop growth parameters:

- Plant height (cm) at 15 days interval up to harvest
- Tiller no. hill^{-1} at 15 days interval up to harvest

3. Physiological parameters:

- Chlorophyll (SPAD) value of leaf
- Relative water content (RWC)

4. Yield contributing parameter:

- 1000-grain weight
- Number of effective tillers hill^{-1}
- Number of ineffective tillers hill^{-1}

5. Yields:

- Grain yield pot^{-1}
- Straw yield pot^{-1}
- Harvest index (%)

3.19 Procedure of sampling germination parameter

3.19.1 Germination percentage

Germination percentage was measured by the following formula-

$$\text{Germination percentage} = \frac{\text{Number of germinated seed}}{\text{Number of seed placed}} \times 100$$

3.19.2 Normal and abnormal seedlings percentage

The normal seedlings and abnormal seedlings were classified according to the formal rules given by ISTA (1999)**.**

3.19.3 Shoot and root length of seedling

Shoot and root length was measured from ten seedlings randomly.

3.19.4 Fresh weight of shoot and root seedling^{-1}

Ten sample seedlings were given for taking fresh weight. Then seedlings shoot and root were separated and weighed in balance separately and averaged them to take fresh weight seedling^{-1}.

3.19.5 Dry weight of shoot and root seedling^{-1}

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

3.20 Procedure of sampling for growth study during the crop growth period

3.20.1 Plant height

The height of the rice plants was recorded from 30 days after transplanting (DAT) at 15 days interval up to harvest DAT, beginning from the ground level up to tip of the leaf was counted as height of the plant.

3.20.2 Tiller no. hill^{-1}

Total tiller number was taken from 30 DAT at 15 days interval up to harvest DAT.

3.21 Procedure of sampling phenological parameters

3.21.1 Chlorophyll content

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.

3.21.2 Relative water content (RWC) %

Three leaves were randomly selected per pot and cut with scissors. Leaves were collected from duplicate set of experimental pots. Relative water content (RWC) was measured according to Barrs and Weatherley, (1962). Leaf laminas were weighed (fresh wt., FW) and then immediately floated on distilled water in a petridish for 4 h in the dark. Turgid weights (TW) were obtained after removing excess surface water with paper towels. Dry weights (DW) were measured after drying at 60°C for 48 h. Then calculation was done using the following formula:

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

3.22 Procedure of sampling yield contributing parameter

3.22.1 Plant height

Plant height was measured from the soil level to the apex of the leaf or panicle in randomly 5 plants of each pot.

3.22.2 Effective tillers hill^{-1}

The total number of tillers hill^{-1} was counted from selected samples and were grouped in effective and non-effective tillers hill^{-1}.

3.22.3 Panicle length

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

3.22.4 Number of total grains panicle^{-1}

Grains of 5 randomly selected panicle of each pot were counted and then the average number of grains for each panicle was determined.

3.22.5 Grain yield per pot

The grains were separated by threshing per plant basis and then sun dried and weighed.

3.22.6 Straw yield per pot

The straw were separated by threshing per plant basis and weighed.

3.22.7 1000-grain weight

One thousand clean sun dried grains were counted from the seed stock obtained from the sample plants and weighed by using an electronic balance.

3.22.8 Harvest index

It denotes the ratio of economic yield to biological yield and was calculated following the formula of Gardner *et al*. (1985). It was calculated by using the following formula:

$$\text{Harvest index (HI)} = \frac{\text{Grain yield}}{\text{Biological yield}} \times 100$$

3.23 Statistical analysis

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

Chapter 4

RESULTS AND DISCUSSION

4.1 Crop growth parameters

4.1.1 Plant height

There was a significant variation noticed in plant height as a result of different cadmium treatments. Cadmium treatments reduced the plant height compared to its respective control in all growth duration. However, SNP increased plant height up to 1.0 mM $CdCl_2$ stress for all stage (Table 1). The highest plant height were found in control and only SNP treated plant (28.40 and 29.41cm at 30 DAT, 40.32 and 41.25cm at 45 DAT, 52.17 and 52.96cm at 60 DAT, 89.17 and 89.46cm at 75 DAT, 105.86 and 106.83cm at 90 DAT, 103.89 and 104.87cm at harvest, respectively). Also, the lowest result found in 1.5 and 2 mM cadmium stress condition and SNP could not increase plant in these cadmium level. Song *et al.* (2015) observed that at the vegetative stage cadmium toxicity reduced rice growth in terms of root and shoot length.

Table 1. Effect of cadmium and SNP treatments on plant height of rice at different days after transplanting

Treatments	Plant height (cm)					
	30 DAT	45 DAT	60 DAT	75DAT	90DAT	At Harvest
C	28.40ab	40.32ab	52.17ab	88.17ab	105.86ab	103.89ab
C+SNP	29.41a	41.25a	52.96a	89.46a	106.83a	104.87a
Cd0.5	26.93bcd	38.02abc	48.42bc	82.06bc	98.23bc	99.06bc
Cd0.5+SNP	27.39bc	39.15ab	49.58b	83.51b	99.25abc	100.04b
Cd1	26.29cd	37.57c	47.13c	80.21cd	95.10c	95.14cd
Cd1+SNP	26.91bc	38.09bc	48.03bc	81.12c	96.42bc	95.97c
Cd1.5	26.03d	35.79d	46.22d	77.49d	92.10d	92.61e
Cd1.5+SNP	26.01d	35.58d	46.22d	77.48d	92.14cd	91.98e
Cd2	25.23e	35.07e	45.11e	76.18e	91.03e	90.12f
Cd2+SNP	25.12e	35.11e	45.10e	76.21e	91.01e	90.10f
$LSD_{(0.05)}$	**2.87**	**4.11**	**4.49**	**7.66**	**9.04**	**9.19**
CV (%)	**6.21**	**6.35**	**5.42**	**5.48**	**5.44**	**5.55**

4.1.2 Tillers hill^{-1}

Various cadmium level affected tiller production significantly entire the growing period. Increasing cadmium level reduced tiller number compared to control (Table 2). On the other hand SNP increased tiller number upto 1mM cadmium treatments (9.2%.9.7%, and 7.07% at 30 DAT; 8.5%, 4.4% and 2.35% at 45 DAT; 2.09% and 2.22% at 60 DAT; 2.19%, and 6.1%, at 75DAT; 2.21% and 6.4% at 90 DAT and 1.4% at harvest, compared with control and SNP, 0.5mM cadmium and SNP, 1mM cadmium and SNP, respectively) where SNP application could not response at 1.5 mM and 2 mM of cadmium stress condition at all the growth stage. At times mediocre level of Cd treatment gave similar result with SNP treated Cd treatment. Tiller production serially decreased with increased levels of cadmium.

Table 2. Effect of cadmium and SNP treatments on total tillers hill^{-1} of rice at different days after transplanting

Treatments	Tillers hill^{-1} (no.)					
	30 DAT	45 DAT	60 DAT	75DAT	90DAT	At Harvest
C	25.50ab	33.30ab	39.12ab	35.06ab	33.03ab	28.13ab
C+SNP	27.60a	34.50a	40.56a	36.74a	34.68a	29.10a
Cd0.5	22.80bcd	28.20cd	34.32bc	31.01bc	28.59bc	24.77bc
Cd0.5+SNP	24.90abc	30.60bc	35.01b	31.93b	29.96b	25.63b
Cd1	21.60de	27.00d	31.20d	28.15cd	26.26cd	22.87cd
Cd1+SNP	22.50cde	28.20c	31.89cd	29.87c	27.94bc	23.20bc
Cd1.5	19.80ef	25.50e	30.06e	26.44d	24.58d	21.33c
Cd1.5+SNP	20. 09ef	26.10e	30.06e	26.44d	24.58d	21.33c
Cd2	18.30f	21.60f	29.01f	25.15e	23.90d	21.07c
Cd2+SNP	18.00f	21.60f	29.04f	25.11e	23.57d	21.06c
LSD$_{(0.05)}$	**2.72**	**3.22**	**2.95**	**2.81**	**2.75**	**2.21**
CV (%)	**7.16**	**6.78**	**5.18**	**5.50**	**5.77**	**5.36**

4.2 Physiological parameters

4.2.1 Relative water content

Relative water content was decreased 6.7%, 9.2% 10.85 % and 16.43 at 0.5, 1.0, 1.5 and 2.0 mM of cadmium stressed condition compared to untreated control (Figure 1). Besides, SNP could increase relative water content at control, under, 0.5 and 1.0 mM of cadmium stressed condition. But relative water content under 1.5 mM and 2 mM stressed condition and with SNP application gave statistically similar result. Singh and Tewari (2003) reported that increased Cd concentration decreased leaf water content.

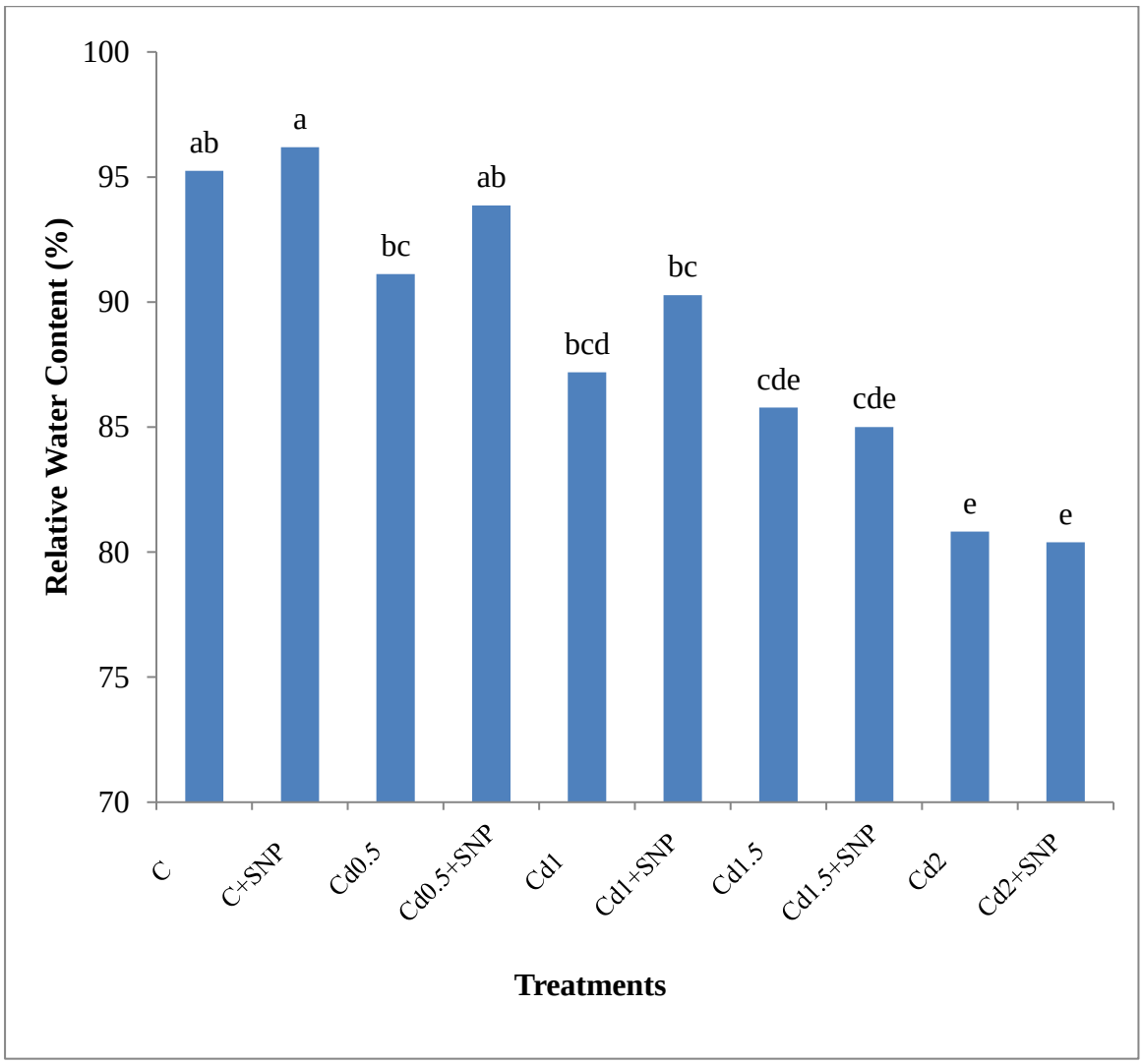

Figure 1. Effect of treatments on relative water content of rice

4.2.2 Chlorophyll content

Various cadmium treatments affected chlorophyll production significantly entire the growing period. Cadmium treatment reduced total chlorophyll content compared to its respective control and control with SNP (Figure 2). Increased cadmium level reduced chlorophyll content 8.49%, 9.74%, 14.26% and 18.85% at 0.5, 1.0, 1.5 and 2.0 mM concentration, respectively. On the other hand, SNP with cadmium treatments increased chlorophyll content (2 and 1% at 0.5 and 1.0 mM, respectively). Cadmium damages the photosynthetic apparatus and lowers the chlorophyll (Sharma and Dietz, 2006). Gill *et al.* (2013) found that cadmium strongly altered the chloroplast metabolism through inhibiting chlorophyll biosynthesis.

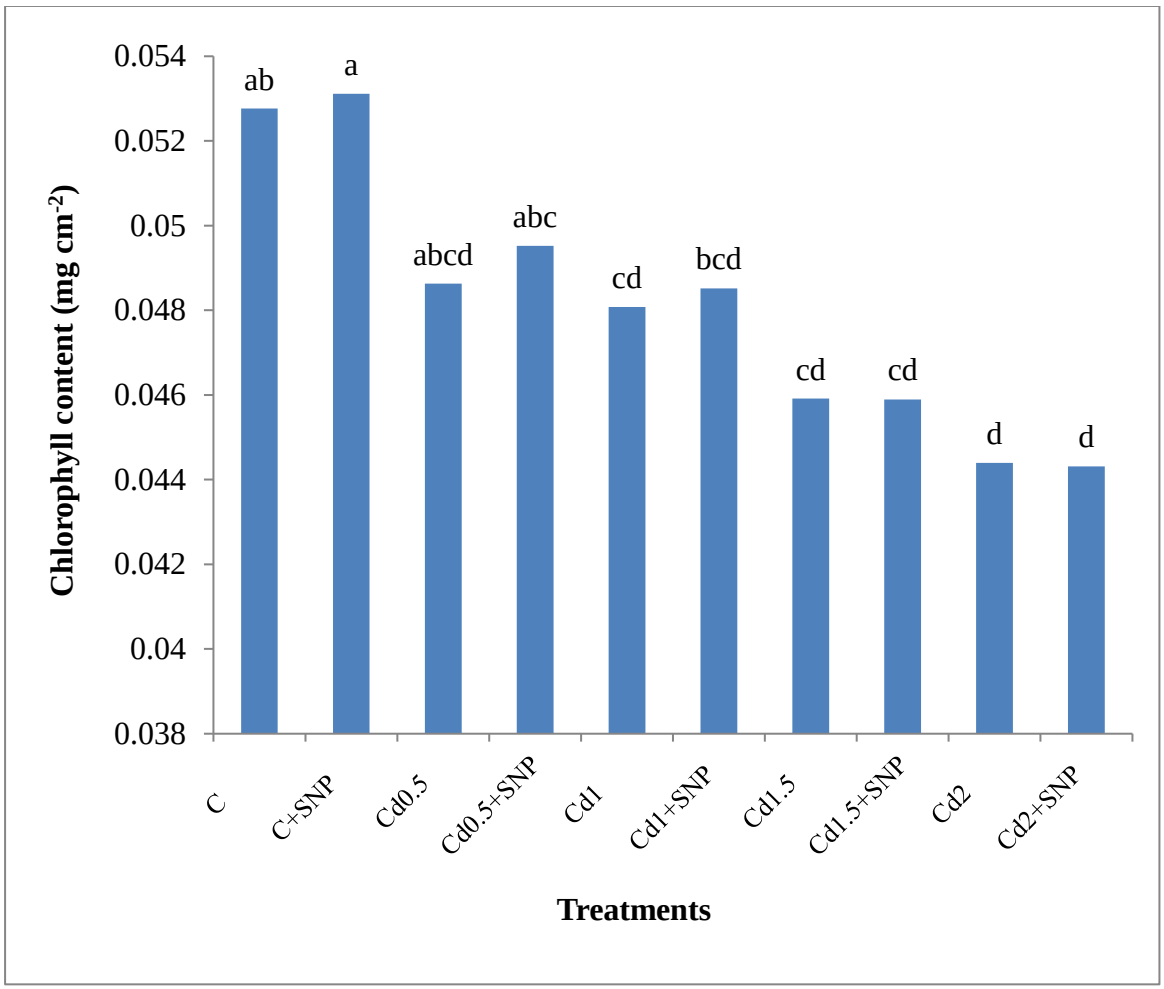

Figure 2. Effect of treatments on chlorophyll content of rice

4.3 Yield contributing characters

4.3.1 Effective tillers hill^{-1}

Cadmium caused a significant reduction of effective tiller compared to control and control with SNP (Figure 3). The highest number of effective tiller was found in control and only SNP treated plant. On the other, SNP increased effective tiller number compared to its respective control but not similar with control and only SNP treated plant (6% at 0.5 and 1.0 mM stressed condition). The 1.5mM and 2.5 mM cadmium stressed condition was not affected by SNP treatment and gave statistically similar result (Figure 3). Increasing soil cadmium concentration number of tillers decreased significantly (Herath *et al*., 2014).

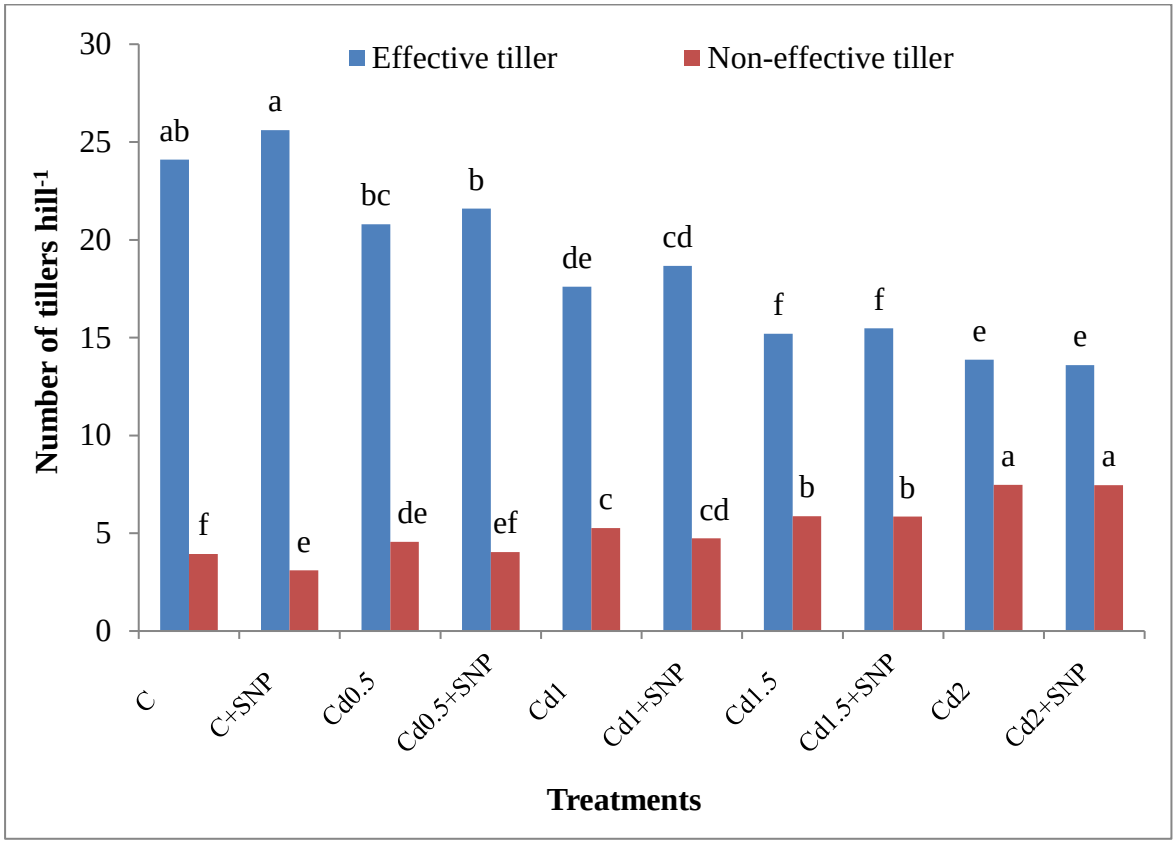

Figure 3. Effect of treatments on tillers hill^{-1} of rice

4.3.2 Non-effective tillers hill^{-1}

Upon exposure to cadmium stress, non-effective tiller increased significantly compared to their controls (Figure 3). The highest non-effective tiller number was found in 2 mM cadmium stressed plant (7.47). In addition, the lowest non-effective tiller was found in only SNP treated plant (3.50). SNP also decreased the non

effective tiller up to 1mM stressed condition. Number of non effective tiller was increased with the increased of cadmium level.

4.3.3 Panicle length (cm)

Length of panicle was also seized by cadmium stress, according to figure 4. Cadmium treatment decreased the length of panicle compared to control and control with SNP treated plant. Increased cadmium level reduced panicle length 9.13%, 12.97%, 18.72% and 24.03% at 0.5, 1.0, 1.5 and 2.0 mM cadmium stress, respectively. Besides, the reduction was less in SNP treated stressed plant compared to its respective control (Figure 4). But effect of SNP could not affect 1.5 mM and 2 mM cadmium stressed condition where it gave lower result according to 1.5 mM and 2 mM stressed condition.

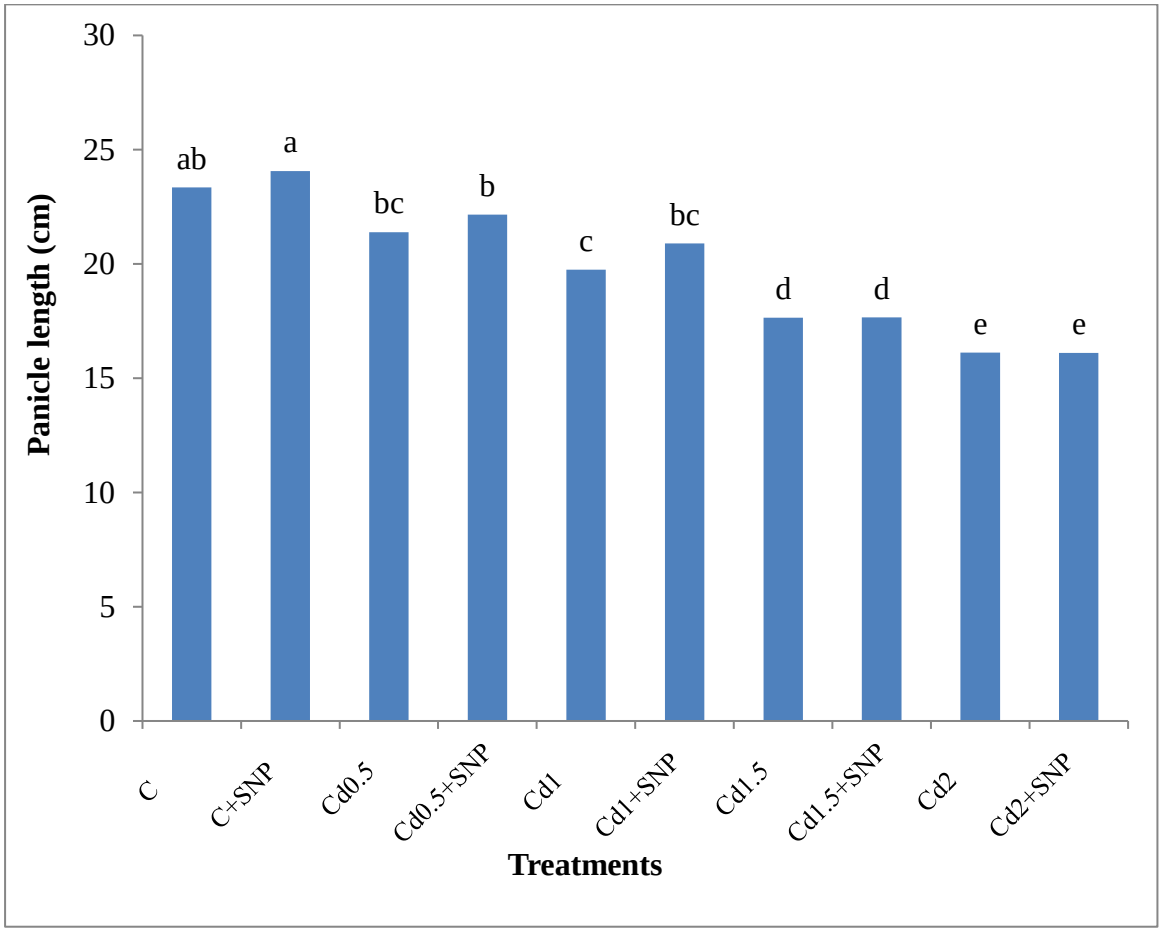

Figure 4. Effect of treatments on panicle length of rice

4.3.4 No. of filled grains panicle^{-1}

Various cadmium treatments affected the number of filled grains panicle^{-1} significantly entire period of grain development. Cadmium treatment reduced total number of filled grains panicle^{-1} compared to its respective control (Figure 5). Cadmium reduced grain number panicle^{-1} as 5.94%, 12.63%, 16.30%, and 32.09% at 0.5, 1.0, 1.5 and 2.0 mM, respectively. On the other hand, SNP with cadmium treatments increased the number of filled grains panicle^{-1} (5.06%, and 3.89% at 0.5, 1.0 mM, respectively). Also SNP increased filled grains at 1.5 mM stressed condition which was statistically similar with 1.5 mM condition. SNP could not effect on 2.0 mM stressed condition. Moreover, filled grains panicle^{-1} decreased significantly with increase in level of cadmium concentration. Liu *et al.* (2007) discovered that treatment with 100mg cadmium per kg soil decreased the number of grains per panicle and filled grain percentage more than 20%.

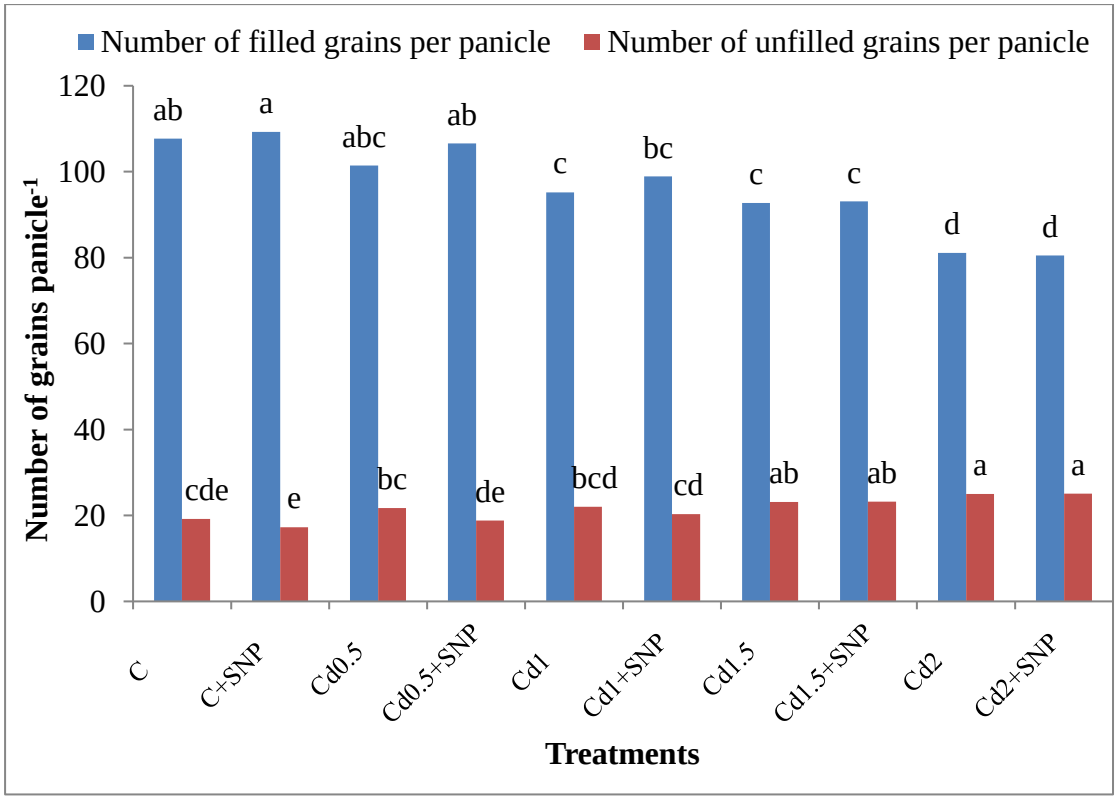

Figure 5. Effect of treatments on grains panicle^{-1} of rice

4.3.5 Number of unfilled grains panicle^{-1}

On exposure to cadmium stress, number of unfilled grains per panicle decreased significantly compared to their controls (Figure 5). The highest number of unfilled

grains panicle^{-1} was found in 2 mM of cadmium stressed plant (25.013). Also, the lowest number of unfilled grains panicle^{-1} was found in only SNP treated plant (17.287).

4.3.6 1000-grain weight

Cadmium reduced 1000-grain weight compared to its control. Cadmium decreased 4.6, 10, 15.7 and 22.1% of grain weight at 0.5, 1.0, 1.5 and 2.0 mM, respectively. SNP treatment increased 1000 grain weight under cadmium stressed condition (4.06% and 3.98% at 0.5 and 1.0 mM, respectively). But in 1.5 and 2 mM stressed condition application of SNP did not affect on the 1000-grain weight (Figure 6). Cadmium could transfer to rice grains and decreased grain yield, quality, and nutrient uptake by inhibiting accumulation of carbohydrates and other food materials (Rodda *et al.*, 2011).

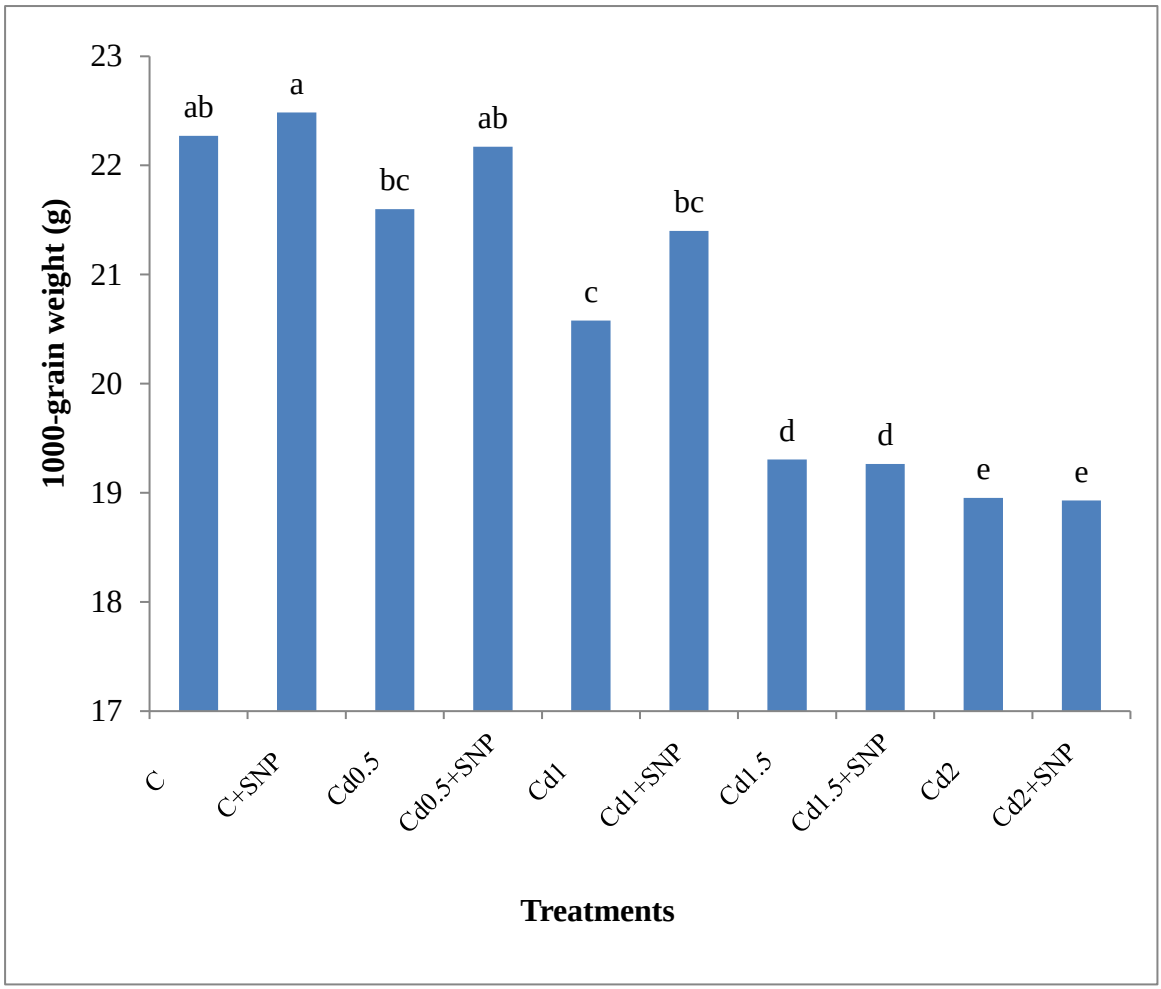

Figure 6. Effect of treatments on 1000-grain weight of rice

4.3.7 Grain yield pot^{-1}

Momentous variation was observed for grain yield due to different cadmium treatments (Figure 7). Grain yield decreased due to cadmium treatment. However, SNP treatment under cadmium stressed condition increased grain yield except 1.5 and 2 mM cadmium stressed condition (5.5 and 3.13% at 0.5 and 1.0 mM stress treatment, respectively) compared to its respective control. Grain yield were statistically similar where plants were treated with only cadmium and cadmium with SNP at 1.5 and 2 mM of cadmium (Figure 7). Under cadmium stress, reduction of grain yield causes from a combination of reductions in plant stand, filled grain number per panicle and harvest index. Among all these contributing components studied, the fertility of grain was found most severely affected which increased unfilled grain and for this reason significant reduction in total yield of grain. Moreover to fertility, panicle length and numbers of panicle were two important factors that influence the grain yield. The increasing of cadmium induced yield losses could not be imposed to a single factor. Several physiological and biochemical factors at different growth stages of rice plants might be engaged. Cadmium is transported to the upper nodes and at last into the panicle (Uraguchi *et al.,* 2011) which inhibits the uptake and translocation of important nutrients in panicle (Li *et al.*, 2012a). For this reduction of fertile grain became higher. Cadmium toxicity deflected leaf and root ultra-structure (Aina *et al.*, 2007) causes reduction of transportation of nutrients and production of food for plants. Lin *et al.* (2016) found that increased cadmium concentration significantly decreased total grain yield in rice.

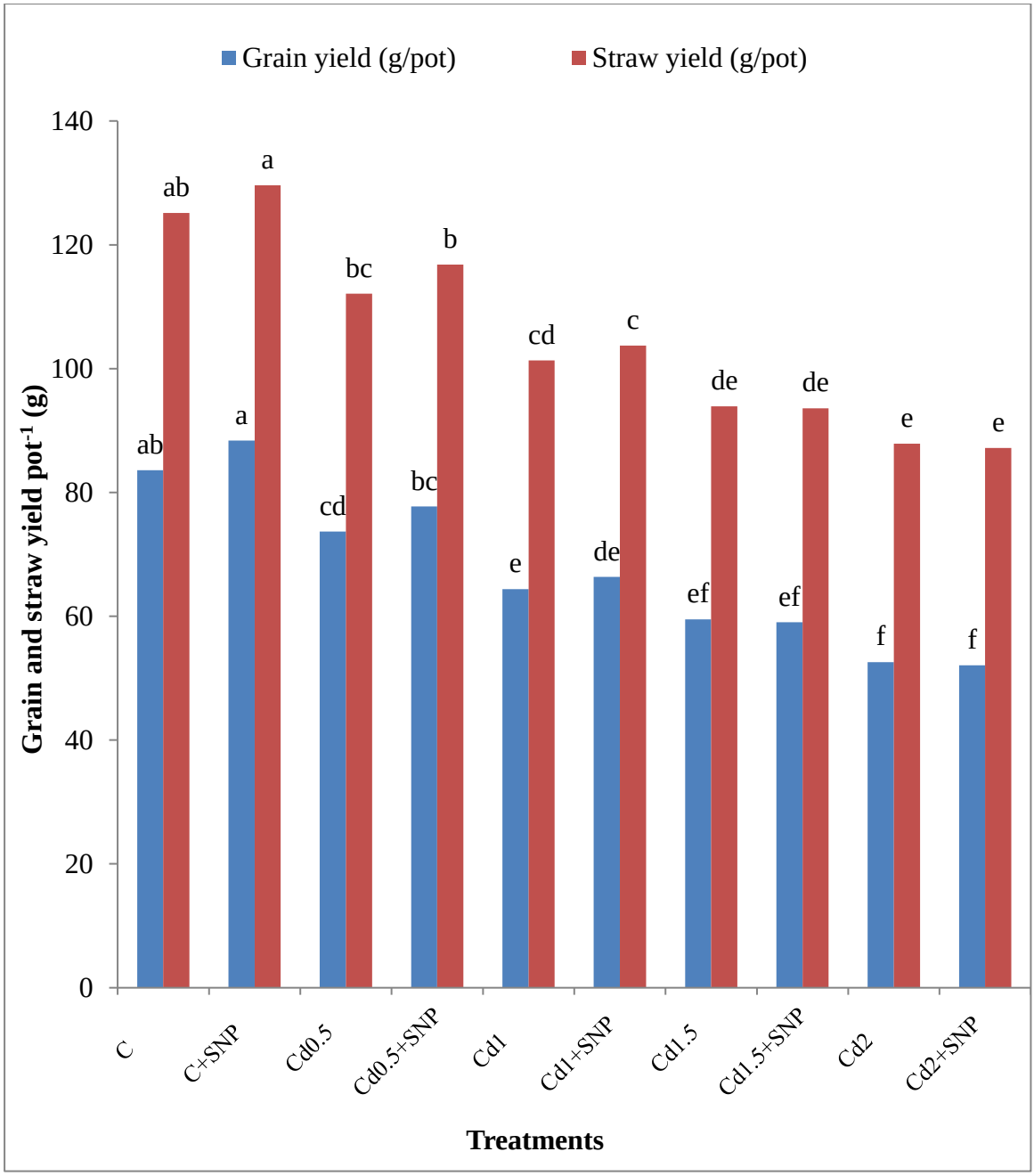

Figure 7. Effect of treatments on grain and straw yield of rice

4.3.8 Straw yield pot^{-1}

Sharp decreases in straw yield were observed (13, 25, 36 and 45% at 0.5, 1.0, 1.5 and 2.0 mM stressed condition, respectively) due to $CdCl_2$ stress (Figure 7). Moreover, SNP treatment increased straw yield under stress condition. The highest straw yield found only SNP treated plant was 129.62g.

4.3.9 Harvest Index

For different cadmium treatments with or without SNP spraying, significant variation was observed for harvest index (Figure 8). Control (39.51%) and only SNP treated plant (40.56%) produced higher harvest index compared to its cadmium stressed condition and SNP treated stressed condition. However, SNP treated cadmium stressed plant produced higher harvest index (40.37 and 39.34% at 0.5 and 1.0 mM cadmium stressed condition, respectively) compared to cadmium stressed condition. But 1.0 and 1.5 mM stressed condition with SNP gave statistically similar harvest index without SNP. 2 mM stressed condition without and with SNP gave the lowest harvest index (37.42%).

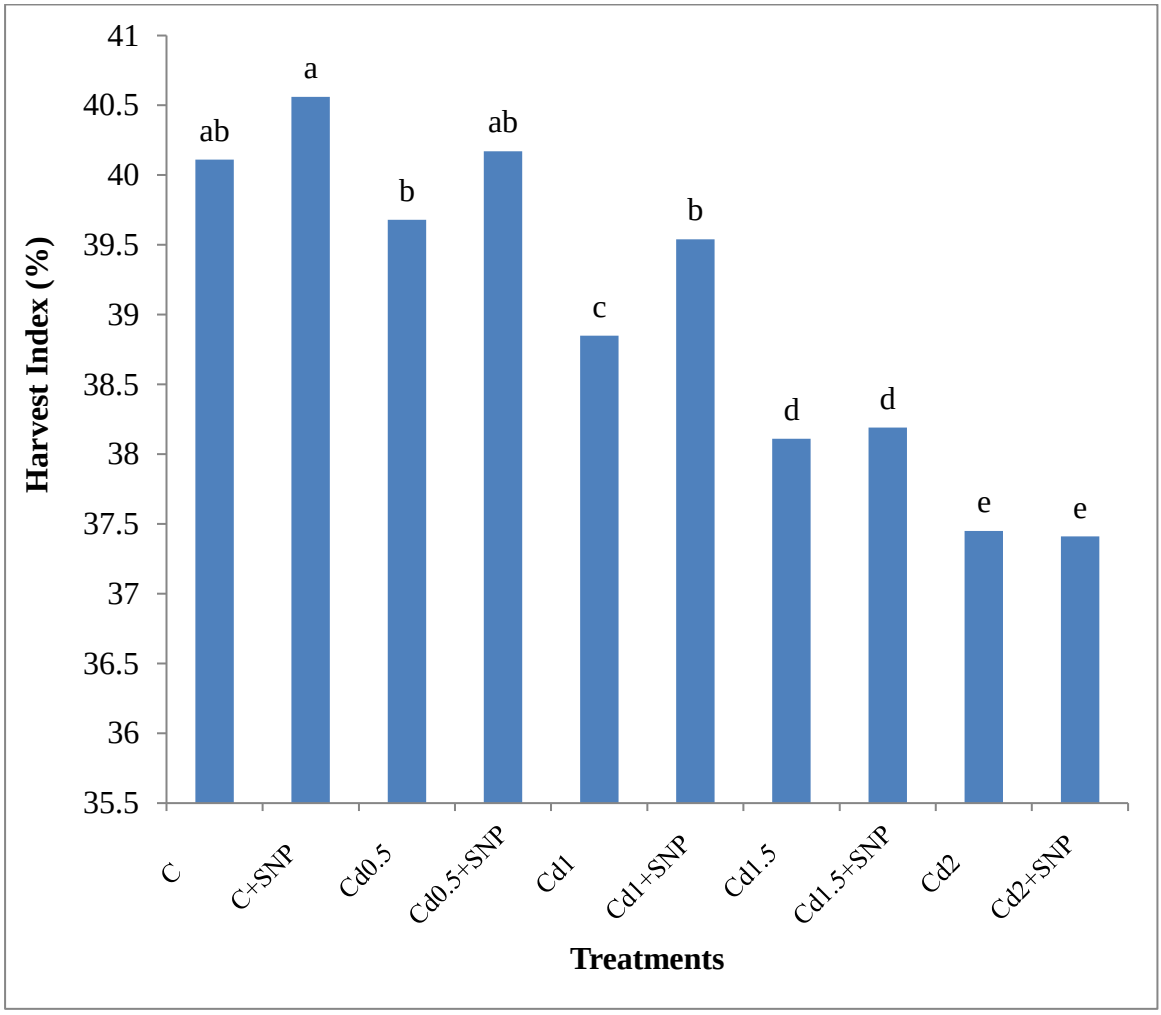

Figure 8. Effect of treatments on harvest index of rice

4.4 Germination parameters

4.4.1 Germination percentage

The data (Figure 9) showed that cadmium also reduced the percentage of germination. On the other hand, germination percentage was higher in SNP treated cadmium stressed condition as compared to without treated cadmium stressed condition. However, germination percentage was higher in control and only SNP treated plant (94.69 and 95%, respectively). Also germination percentage in SNP treated cadmium stressed condition was 88.67, 82.44, 77.34 and 67.67 at 0.5, 1.0, 1.5 and 2.0 mM, respectively. SNP increased germination at 0.5 and 1.0 mM stressed condition (8.3 and 5%, respectively). The lowest germination was found at 2.0 mM cadmium concentration. Ahsan *et al.* (2007) documented that excessive cadmium in the growth medium reduced rice seed germination. Vijayaragavan *et al.* (2011) found that cadmium prevents water uptake and water movement in the embryo axis, this is one of the main reasons for low germination.

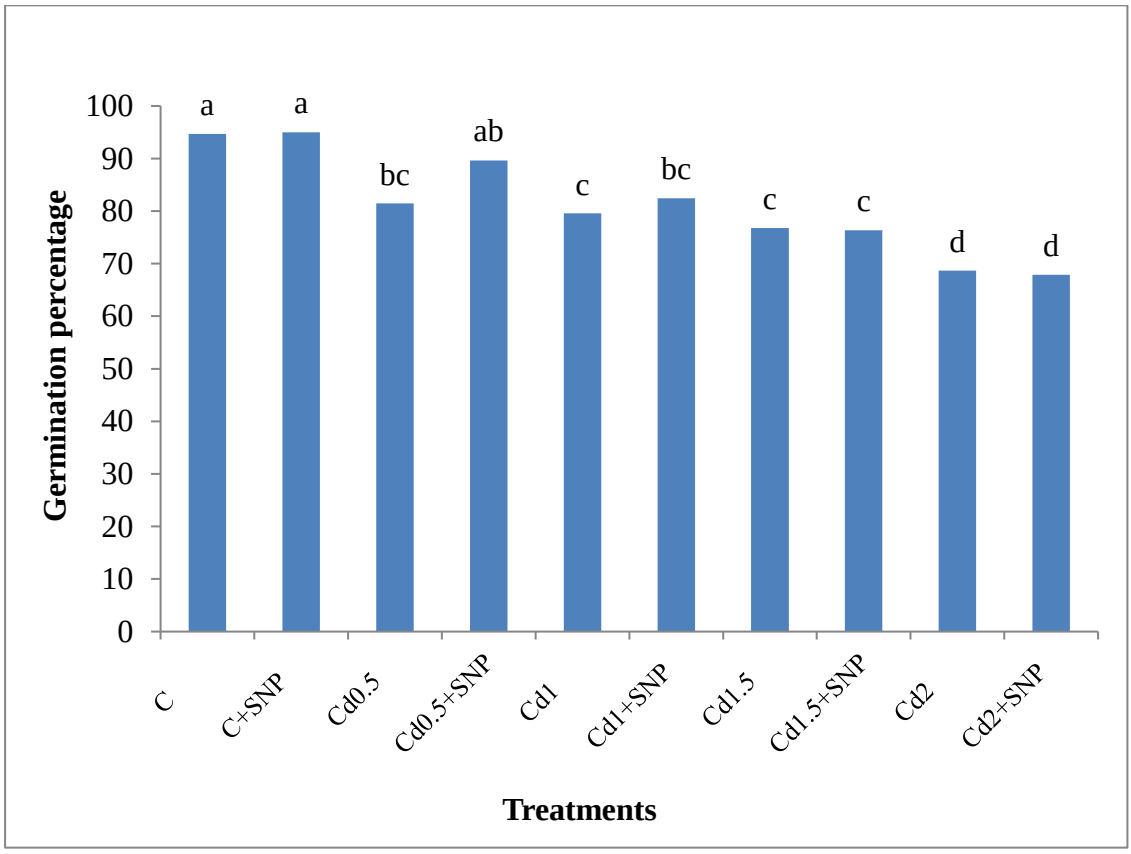

Figure 9. Effect of treatments on germination percentage of rice

4.4.2 Normal seedling

Cadmium caused a significant reduction of normal seedling compared to its control (Figure 10). The highest normal seedling was found in only control, SNP and SNP treated 0.5 mM stressed plant. On the other hand, SNP increased normal seedling number at 1.0 mM stressed condition. Percentage of normal seedling SNP treated plant was (83.89, 76.67, 70.56 and 58% at 0.5, 1.0, 1.5 and 2.0 mM stressed condition, respectively). SNP increased normal seedling 13 and 4% at 0.5 and 1.0 mM stressed condition, respectively.

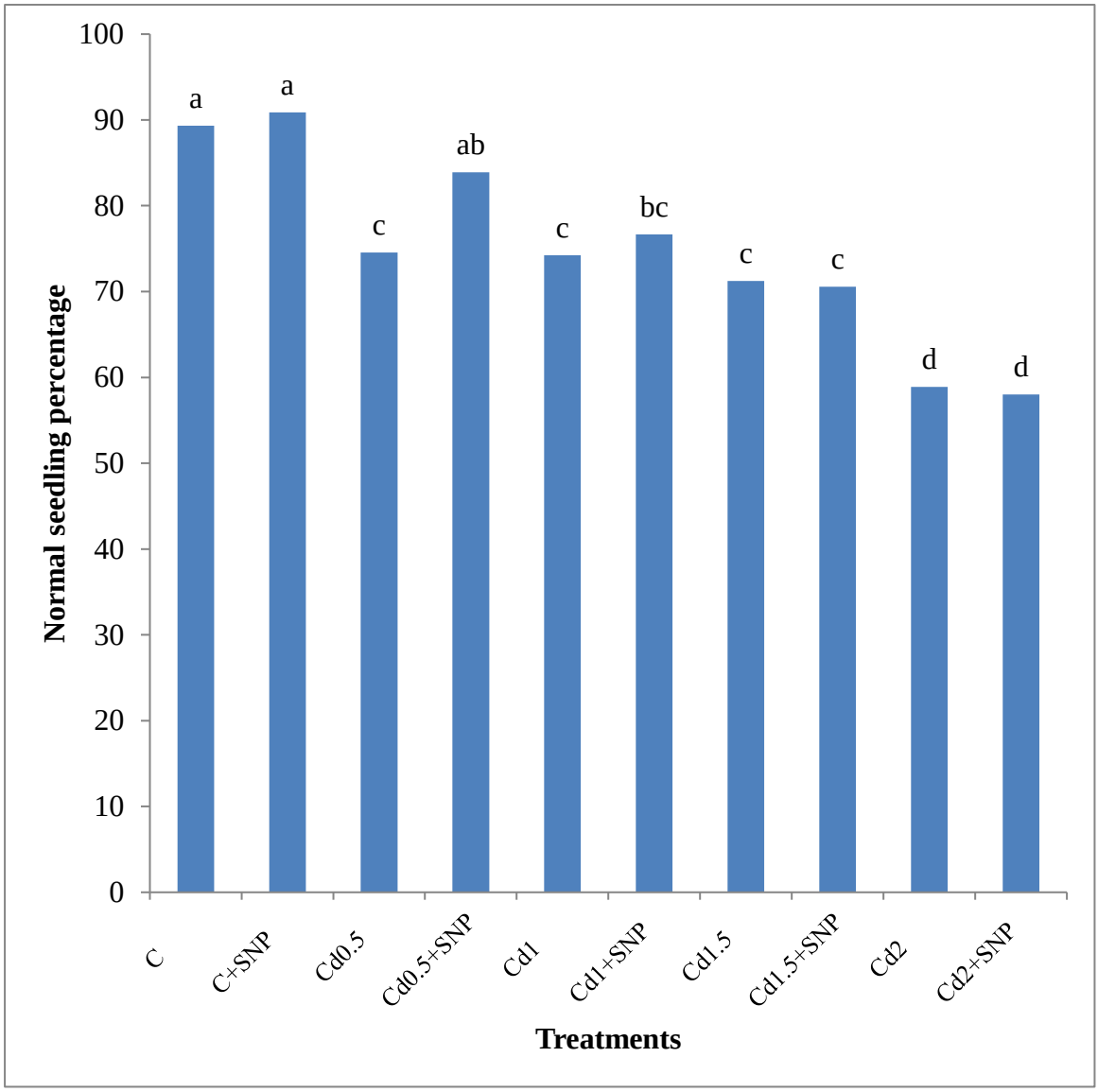

Figure 10. Effect of treatments on normal seedling percentage of rice

4.4.3 Abnormal seedling

On exposure to cadmium stress, abnormal seedling increased significantly compared to their control (Figure 11). The highest abnormal seedling was found at 2 mM cadmium stressed plant (9.87%). Moreover, the lowest abnormal seedling was found in only SNP treated plant (4.1%). Abnormal seedling decreased by SNP application with stressed plant (4.7 and 5.7% at 0.5 and 1.0 mM stressed condition, respectively). Rahoui *et al.* (2010) found that increased cadmium concentration produced abnormal seedling.

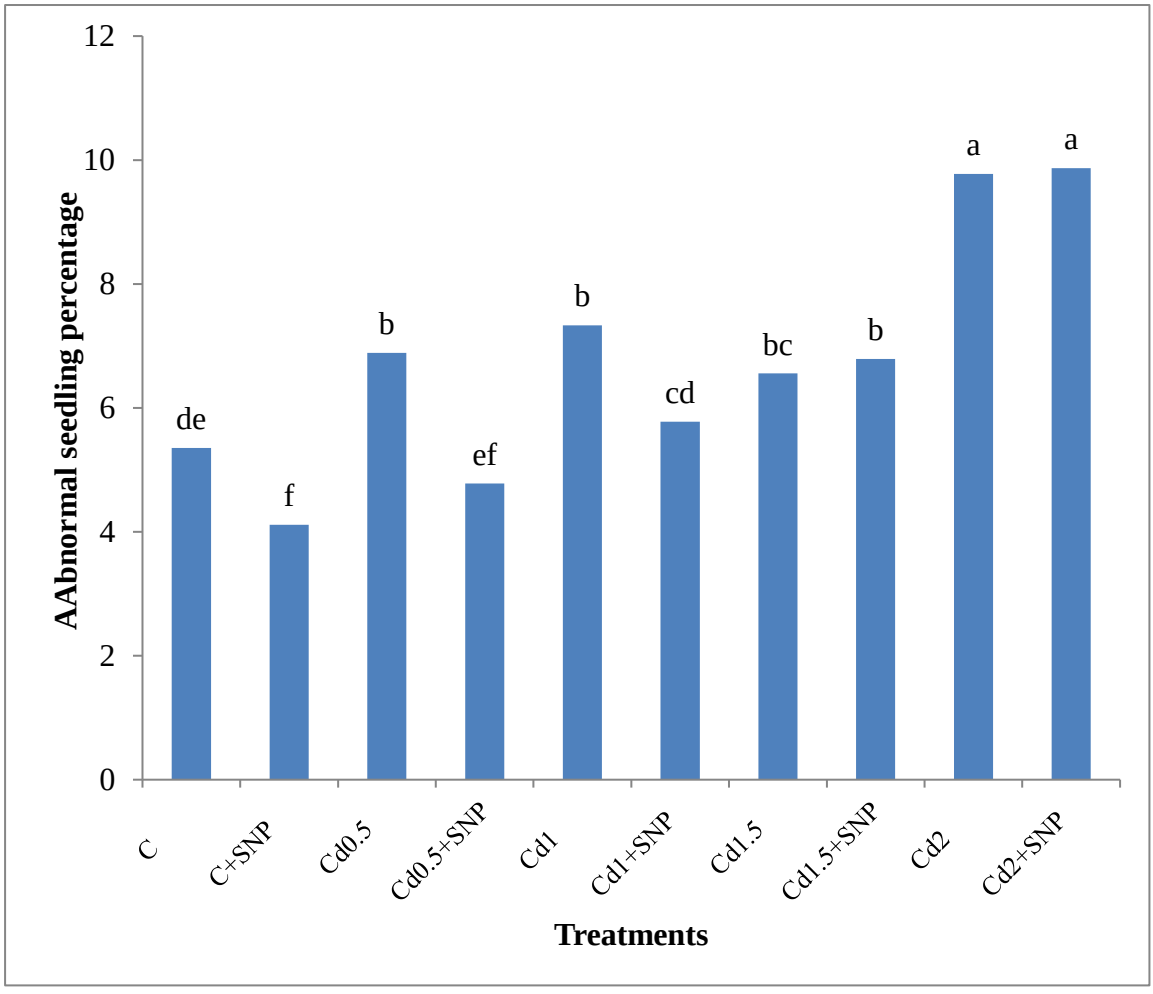

Figure 11. Effect of treatments on abnormal seedling percentage of rice

4.4.4 Length of shoot

Different cadmium treatments affected seedling shoot length significantly. Cadmium treatment decreased length of shoot compared to its control (Figure 12). On the contrary, SNP with cadmium treatments increased shoot length (16 and 4% at 0.5 and 1.0 mM, respectively) where higher level of cadmium treatment did not affect by SNP spraying (2.0 mM cadmium stress). Also 1.5 mM cadmium with SNP spraying increased shoot length which are statistically similar without SNP spraying.

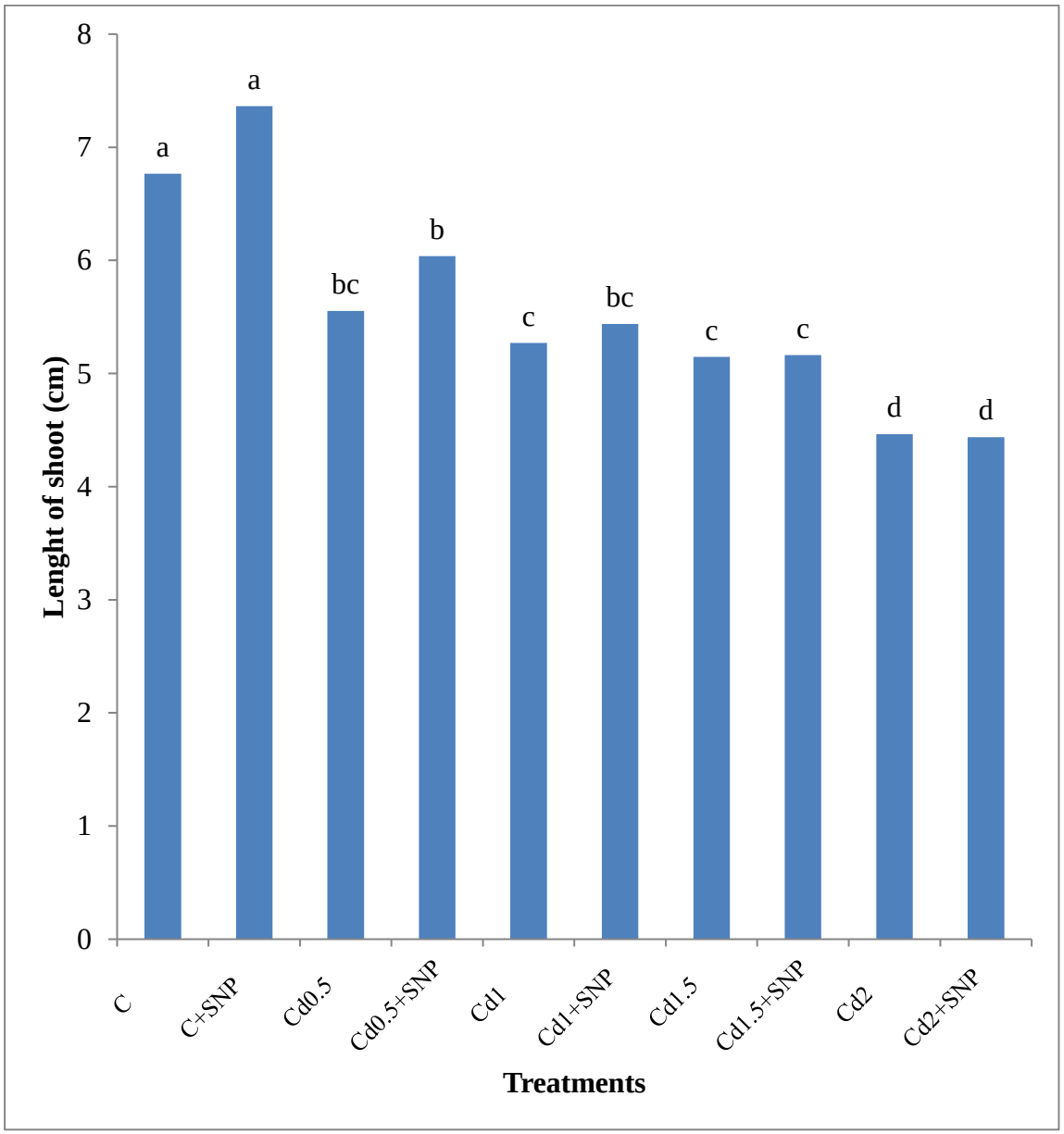

Figure 12. Effect of treatments on seedling shoot length of rice

4.4.5 Length of root

Cadmium caused a significant reduction of seedling root length compared to control (Figure 13). The highest root length was found only in SNP treated plant. On the other hand, SNP increased root length at 0.5 mM was similar with control. Also SNP treated plant at 1.0 mM stressed condition increased the root length 2.5%. 1.5mM and 2.0 mM cadmium stressed condition was not affected by SNP treatment (Figure 13).

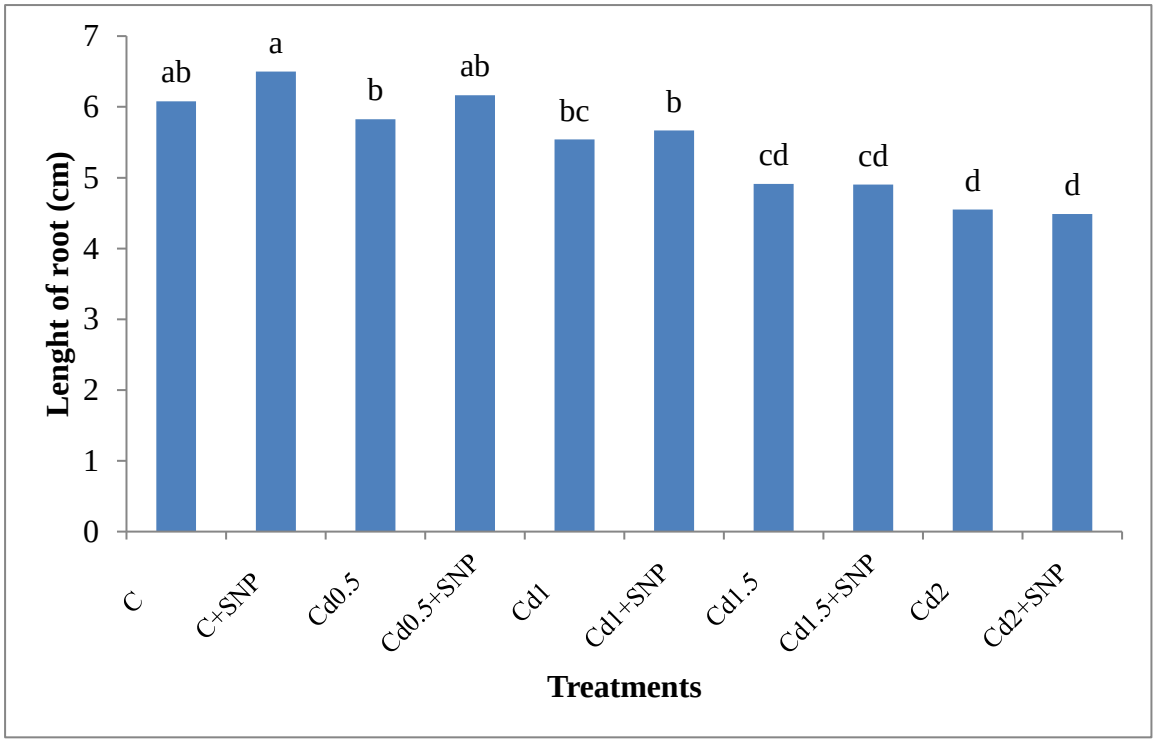

Figure 13. Effect of treatments on seedling root length of rice

4.4.6 Fresh weight of shoot seedling^{-1}

Cadmium caused a significant reduction of fresh weight of shoot compared to their control (Figure 14). The highest (0.0228 and 0.0237 g) seedling^{-1} fresh weight of shoot was found in control and only SNP treated plant, respectively. On the contrary, SNP increased fresh weight of shoot 6.8% at 0.5 mM cadmium stressed condition and also 3.4% increased at 1.0 mM stressed level. At 1.5 and 2.0 mM cadmium plant without and with SNP gave the statistically similar result (Figure 14).

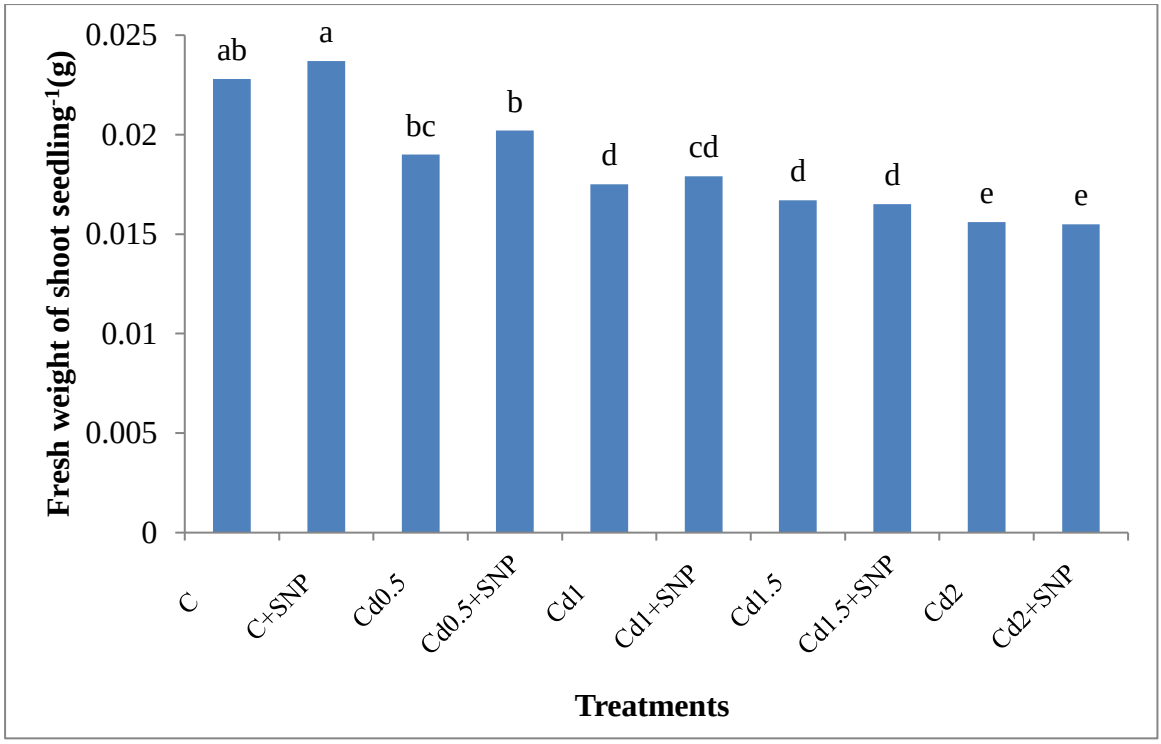

Figure 14. Effect of treatments on fresh weight of shoot seedling $^{-1}$

4.4.7 Fresh weight of root seedling^{-1}

Under cadmium stressed gave significant decreased result in fresh weight of root (18, 52, 68 and 82% at 0.5, 1.0, 1.5 and 2.0 mM stress, respectively). However, SNP with cadmium treatment increased fresh weight up to 1.0 mM cadmium stress (Figure 15). At 1.5 and 2.0 mM cadmium stress with and without SNP spraying produced statistically similar result (Figure 15).

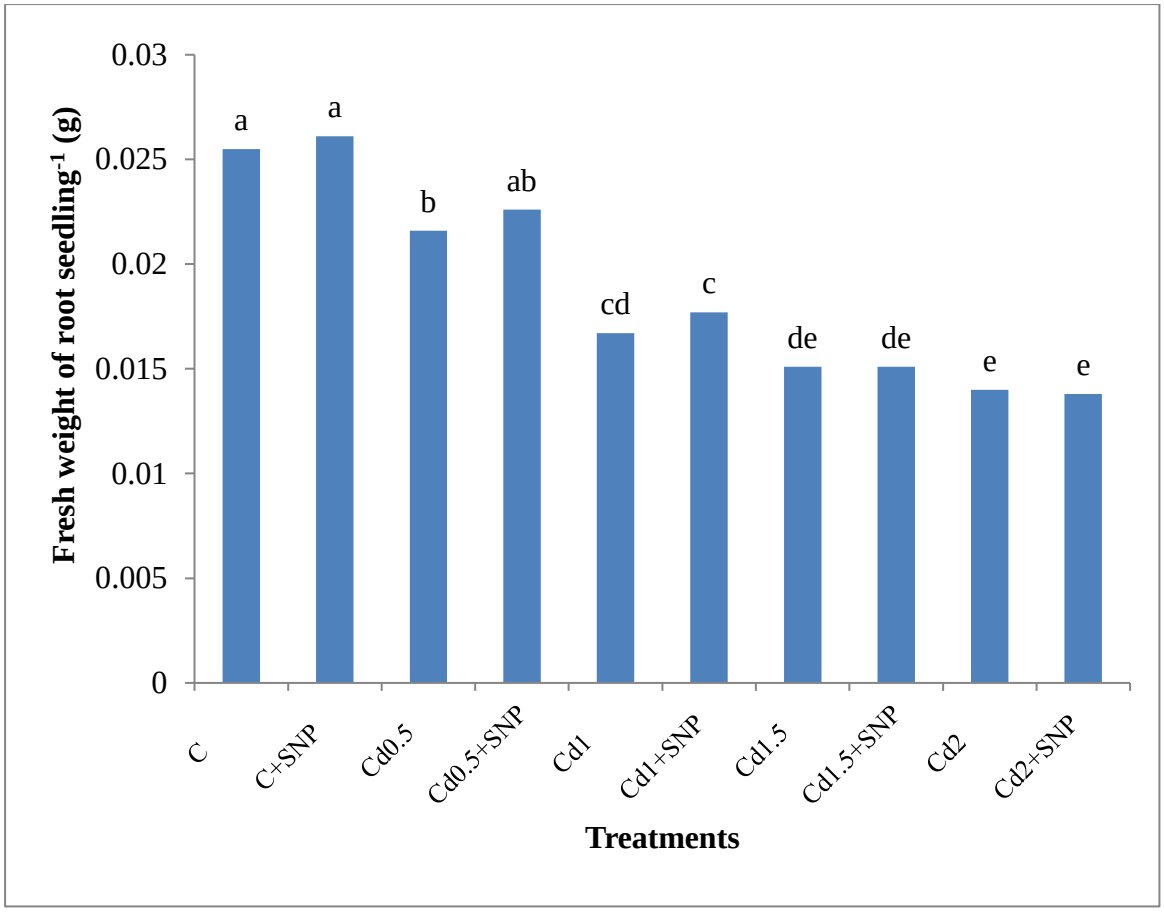

Figure 15. Effect of treatments on fresh weight of root seedling $^{-1}$

4.4.8 Dry weight of shoot seedling^{-1} (g)

For different cadmium treatments with or without SNP spraying, significant variation was observed for dry weight of shoot (Figure 16). Control (0.00339 g) seedling^{-1} and only SNP treated plant (0.00363 g) seedling^{-1} gave the highest dry shoot weight compared to other cadmium treatment and with or without SNP treatment. On the other hand, spraying with SNP gave higher dry weight than cadmium treatment without SNP treatment (4% at 0.5 and 1.0 mM stress.). But 1.5 and 2.0 mM cadmium condition gave statistically similar dry weight with SNP treated 1.5 and 2.0 mM cadmium condition, respectively.

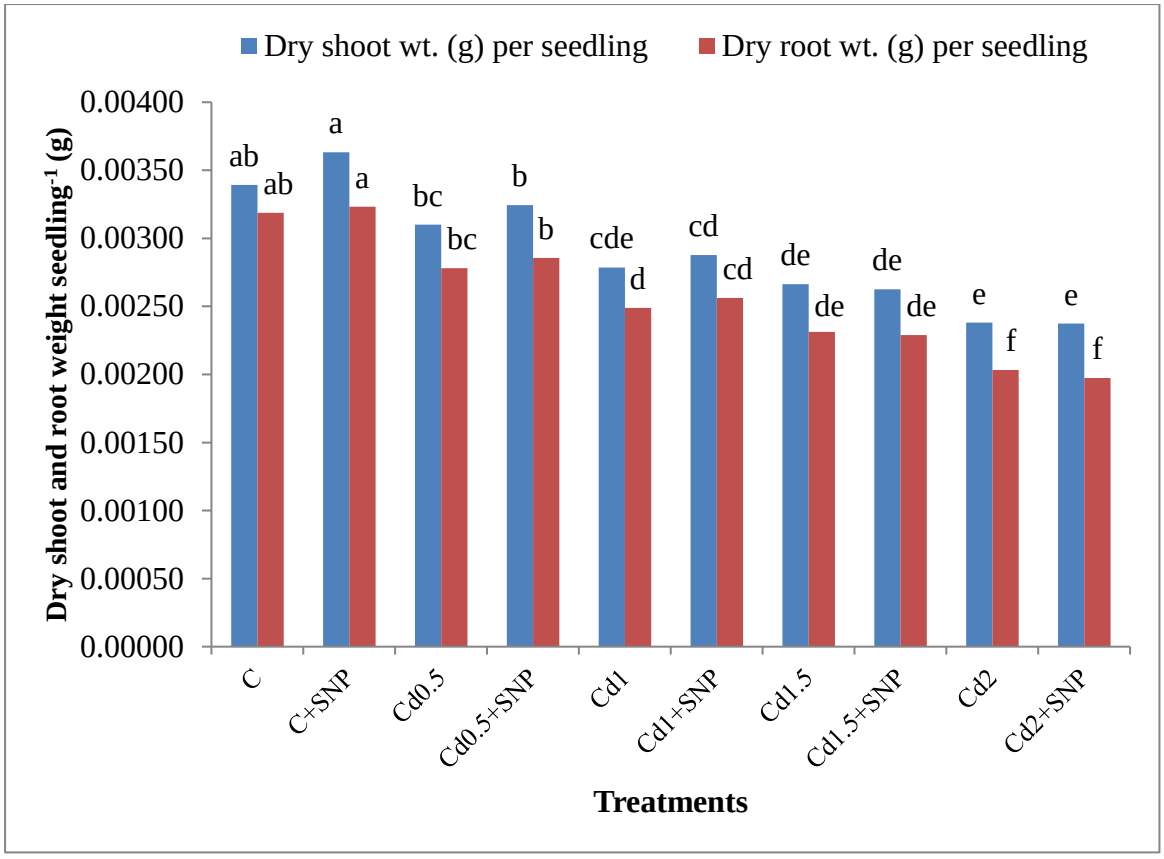

Figure 16. Effect of treatments on dry weight of shoot and root seedling $^{-1}$

4.4.9 Dry weight of root seedling^{-1}

For various cadmium treatments with or without SNP spraying, significant variation was observed for dry root weight (Figure 16). Control (0.00319 g) seedling^{-1} and only SNP treated plant (0.00323 g) seedling^{-1} gave the highest dry root weight compared to all other cadmium treatment and with or without SNP spraying. Moreover, spraying with SNP gave the highest dry weight of root than cadmium treatment without SNP treatment (3% increased at 0.5 and 1.0 mM stressed condition). But 1.5 and 2.0 mM cadmium condition gave slightly higher dry weight with SNP treated 1.5 and 2.0 mM cadmium condition which were statistically similar, respectively (Figure 16).

Chapter 5

SUMMARY AND CONCLUSION

This study was conducted to mitigate cadmium stress in aus rice by exogenous application of SNP (Nitric oxide donor). BRRI dhan48 was used for this experiment which was done at the Experimental shed of the Department of Agronomy, Sher-e-Bangla Agricultural University, in Dhaka during the period of April to August, 2015. BRRI dhan48 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 30 pots all together replication with the given factors. Empty earthen pots with 18 inch depth were used for the experiment. There were 10 treatment combinations. The treatments were control (C), control+SNP (C+0.2 mM SNP), Cd0.5 (0.5 mM cadmium stress), Cd0.5+SNP (0.5 mM cadmium stress with 0.2 mM SNP), Cd1 (1 mM cadmium stress), Cd1+SNP (1 mM cadmium stress with 0.2 mM SNP), Cd1.5 (1.5 mM cadmium stress) and Cd1.5+SNP (1.5 mM cadmium stress with 0.2 mM SNP), Cd2 (2 mM cadmium stress) and Cd2+SNP (2 mM cadmium stress with 0.2 mM SNP). The cadmium treatments were applied instead of irrigation. Germination test was performed of the seeds in the laboratory with treatments. The data were collected from ten days seedlings for three times with some parameters viz. germination (%), number of normal and abnormal seedling, length of shoot and root, fresh weight of shoot and root and dry weight of shoot and root of seedlings.

Different cadmium with or without SNP 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 in control and only SNP treated plant (28.40 and 29.41cm at 30 DAT, 40.32 and 41.25cm at 45 DAT, 52.17 and 52.96cm at 60 DAT, 89.17 and 89.46cm at 75 DAT, 105.86 and 106.83cm at 90 DAT, 103.89 and 104.87cm at harvest, respectively). The highest tillers hill^{-1} was found in control and SNP treated plant (25.50 and 27.60 at 30 DAT, 33.30 and 34.50 at 45 DAT, 39.12 and 40.56 at 60

DAT, 35.06 and 36.74 at 75 DAT, 33.03 and 34.68 at 90 DAT, 28.13 and 29.10 at harvest, respectively).

Cadmium treatments had significant effect on the physiological parameters viz. relative water content was highest in control (95.24%), SNP treated plant (96.20%) and also Cd0.5+SNP give 90.86% which was higher than without SNP. The chlorophyll content was in control (0.05276 mg cm^{-2}), SNP (0.05311 mg cm^{-2}) and Cd0.5+SNP (0.04952 mg cm^{-2}).

Cadmium treatments had significant effect on the yield and yield contributing characters viz. plant height, effective tillers $hill^{-1}$, length of panicle, grain $panicle^{-1}$, number of filled grain $panicle^{-1}$, 1000-grain weight, grain yield, straw yield and harvest index was highest in control and SNP treated plant. Where, non- effective tiller was highest in Cd1.5 and Cd2.

Germination (%) was also affected by the cadmium stress. The highest was found in control and only SNP treated plant (94.69 and 95%, respectively). Also germination (%) was higher in Cd0.5+SNP (88.66%). Only SNP treated plant produced higher number of normal seedling compared to other treatment, where 1.5 mM and 2.0 mM of $CdCl_2$ stress produced higher number of abnormal seedling (7 and 9.87%, respectively). Length of shoot and root was the highest in control and only SNP treated plant. Control and only SNP treatment produced highest fresh and dry weight of shoot and root of seedlings.

Based on result of the present experiment, together with results found in the available literature, we therefore concluded that exogenous SNP solution application is an effective way to overcome the adverse effects of cadmium stress on growth, physiology and yield components of rice effectively. Also SNP increased the Germination (%), normal seedling, fresh and dry weight of shoot and root of seedlings. All parameters decreased significantly from 0.5 mM level of cadmium stress. Exceptions were non-effective tiller $hill^{-1}$, unfilled grain $panicle^{-1}$, abnormal seedling which increased in response to cadmium stress.

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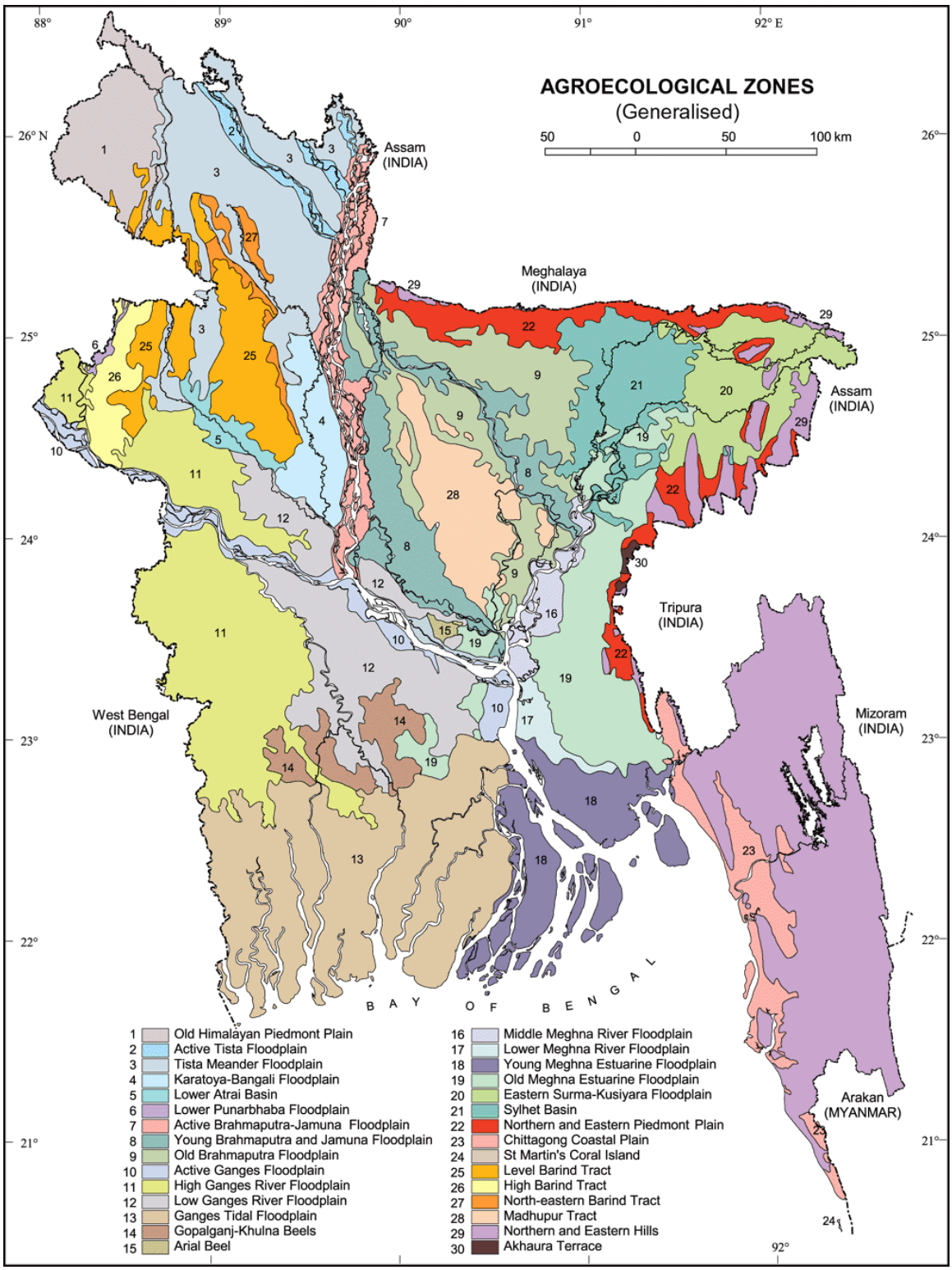
APPENDICES

Appendix I. Experiment was conducted in Sher-e-Bangla Agricultural University, Dhaka (AEZ-28) on the map of Agro-ecological Zones of Bangladesh



Appendix II. Physical and chemical properties of experimental soil analyzed at Soil Resources Development Institute (SRDI), Farmgate, Dhaka

Characteristics	Value
Particle size analysis	
%Sand	26
%Silt	41
%Clay	33
Textural class	Silty-clay
pH	5.7
Organic carbon (%)	0.45
Organic matter (%)	0.72
Total N (%)	0.04
Available P (ppm)	18.00
Exchangeable K (me/100 g soil)	0.12
Available S (ppm)	42

Source: SRDI (Soil Resources Development Institute), Farmgate, Dhaka.

Appendix III. Monthly average air temperature, rainfall and relative humidity of the experimental site during the period from April to August 2015

Months	**Air temperature (°C)**		**Relative humidity (%)**	**Total rainfall (mm)**
	Maximum	**Minimum**		
April, 2015	32.6	23.1	67.5	181.06
May, 2015	35	25.3	70.1	176.27
Jun, 2015	32.7	26.5	77.3	373.38
July, 2015	31.6	25.9	81.5	674.86
August, 2015	32.6	26.9	79.1	352.02

Source: SAU Meteorological Yard , Sher-e-Bangla Nagar, Dhaka-1207.

Appendix IV. Mean square values for plant height of rice

Source of variation	Degrees of freedom	Mean square values for plant height at					
		30 DAT	45 DAT	60 DAT	75 DAT	90 DAT	At harvest
Replication	2	3.21141	7.8699	16.7403	42.5873	59.6134	58.0086
Treatment	9	4.51552	13.2931	20.9726	68.1169	95.9267	82.6083
Error	18	2.89417	5.7462	6.8647	19.9308	27.7656	28.6817
CV (%)		6.34	6.35	5.42	5.48	5.44	5.55

Appendix V. Mean square values for tillers hill^{-1} of rice

Source of variation	Degrees of freedom	Mean square values for tillers hill^{-1} at					
		30 DAT	45 DAT	60 DAT	75 DAT	90 DAT	At harvest
Replication	2	1.701	15.5790	25.3455	13.4027	19.0079	7.6322
Treatment	9	29.988	56.2680	51.1316	48.8957	47.0153	29.2110
Error	18	2.511	3.5190	53.143	2.6758	2.5729	1.6545
CV (%)		7.16	6.78	5.18	5.50	5.77	5.36

Appendix VI. Mean square values for relative water content and chlorophyll content of rice

Source of variation	Degrees of freedom	Mean square values	
		Relative water content	Chlorophyll content
Replication	2	81.9050	30.1761
Treatment	9	80.7187	29.0120
Error	18	12.3360	6.9499
CV (%)		4.00	5.48

Appendix VII. Mean square values for effective tillers and non-effective tillers hill^{-1} of rice

Source of variation	Degrees of freedom	Mean square values	
		Effective tillers	Non-effective tillers hill^{-1}
Replication	2	5.8240	0.50261
Treatment	9	56.9742	5.80190
Error	18	1.6284	0.11066
CV (%)		6.82	6.29

Appendix VIII. Mean square values for panicle length, no. of filled grains and no. of unfilled grains panicle^{-1} of rice

Source of variation	Degrees of freedom	Mean square values		
		Panicle length	No. of filled grains panicle^{-1}	No. of unfilled grains panicle^{-1}
Replication	2	1.7361	150.588	0.8303
Treatment	9	10.3121	302.199	20.7620
Error	18	2.4316	23.446	2.7938
CV (%)		7.45	5.04	7.68

Appendix IX. Mean square values for 1000-grain weight, grain yield pot^{-1}, straw yield pot^{-1} and harvest index of rice

Source of variation	Degrees of freedom	Mean square values			
		1000-grain weight	Grain yield pot^{-1}	Straw yield pot^{-1}	Harvest index
Replication	2	0.21411	21.099	128.372	1.069
Treatment	9	5.69836	480.987	720.754	3.384
Error	18	2.21249	21.159	35.212	2.508
CV (%)		7.23	6.79	5.65	4.05

Appendix X. Mean square values for germination percentage, normal seedling, abnormal seedling, length of seedling shoot and root of rice

Source of variation	Degrees of freedom	Mean square values				
		Germination percentage	Normal seedling	Abnormal seedling	Length of shoot	Length of root
Replication	2	7.4280	7.3843	0.00039	0.13200	0.22902
Treatment	9	23.7036	33.6831	0.99048	2.60908	1.50930
Error	18	1.9440	2.0266	0.02818	0.12459	0.14039
CV (%)		5.70	6.34	8.32	6.34	6.86

Appendix XI. Mean square values for fresh weight of shoot and root seedling^{-1}; dry shoot and root weight seedling^{-1} of rice

Source of variation	Degrees of freedom	Mean square values			
		Fresh weight of shoot	Fresh weight of root	Dry shoot weight	Dry root weight
Replication	2	5.354	5.532	8.143	2.653
Treatment	9	2.218	6.725	5.434	5.804
Error	18	1.079	1.146	4.250	2.717
CV (%)		5.55	5.69	7.09	6.41