MITIGATION OF ARSENIC TOXICITY IN WHEAT WITH SODIUM NITROPRUSSIDE (SNP)

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DHAKA-1207

JUNE, 2016

MITIGATION OF ARSENIC TOXICITY IN WHEAT WITH SODIUM NITROPRUSSIDE (SNP)

BY

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REGISTRATION NO: 10-03782

A Thesis

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 in Agricultural Botany

Semester: January- June, 2016

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CERTIFICATE

This is to certify that the thesis entitled "**MITIGATION OF ARSENIC TOXICITY IN WHEAT WITH SODIUM NITROPRUSSIDE (SNP)**" submitted to the Department of Agricultural Botany, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE** in **AGRICULTURAL BOTANY**, embodies the result of a piece of bona-fide research work carried out by **ISRAT JAHAN**, Registration No. **10-03782** 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.

Dated: June, 2016
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DEDICATED TO

MY BELOVED

PARENTS

ACKNOWLEDGEMENT

First of all the author expresses her admiration and devotion to the almighty Allah-Rabbul Alamin, the most beneficial who has enabled her to perform this research work and to submit this thesis successfully for the degree of Master of Science (M.S.) in Agricultural Botany.

The author feels proud to convey her sincere gratitude to her supervisor **Dr. Mohammad Mahbub Islam, Professor**, Department of Agricultural Botany, Sher-e-Bangla Agricultural University, Dhaka, for his precious guidance, continuous encouragement and support during the total tenure of her research work. The author is grateful to him for introducing her to the intricacies of science.

The author expresses her heartfelt gratitude to her respected Co-Supervisor, **Dr. Md. Ashabul Hoque,Professor**, Department of Agricultural Botany, Sher-e-Bangla Agricultural University, Dhaka for his scholastic guidance, supportive comments and unvarying inspiration, valuable suggestions throughout the research work and in preparation of the thesis.

The author would like to express her deepest respect and boundless gratitude to our Vice Chancellor **Professor Dr. Kamal Uddin Ahamed**, Chaiman **Professor Dr. Nasima Akhter**, and all the respected teachers of the Department of Agricultural Botany, Sher-e-Bangla Agricultural University, Dhaka-1207, for the valuable teaching, sympathetic co-operation and inspirations throughout the course of this study and suggestions and encouragement to research work. The author would like to express her cordial thanks to the departmental and field staffs for their active help during the experimental period.

The author would really thankful the ministry of Science and Technology for the NST fellowship to provide financial support for this research work.

Finally, the author would mention a very special gratefulness for the moral support and sustaining inspiration provided by the members of her family. This dissertation would never been possible without their love and affection.

The Author

ABSTRACT

The arsenic (As) contamination is increasing gradually in agricultural land with climate change. Wheat is the second cereals after rice in Bangladesh. Therefore, wheat production is affecting with As contamination. A pot experiment was conducted in the farm of Sher-e-Bangla Agricultural University, Dhaka, during *rabi* season, November 2015 to March 2016 to examine the role of sodium nitroprusside (SNP) as NO donor on mitigation of As toxicity in wheat. In this experiment, the treatment consisted of four different As levels *viz*. As_0 = without As (control), As_1 = 30 mg As kg^{-1} soil, As_2 = 60 mg As kg^{-1} soil, As_3 = 90 mg As kg^{-1} soil and three different concentrations of SNP *viz*. N_0 = 0 mM SNP, N_1= 0.1 mM SNP and N_2 = 0.2 mM SNP. The experiment was laid out in two factors Completely Randomize Design (CRD) with four replications. The total treatment combinations were 12 (4x3). Results of the experiment showed a significant variation among the treatments in respect of most of the parameters as morphological, physiological, yield contributors and yield characters. The higher levels of As showed greater reduction of growth, development and yield component to control or without As. Separately, exogenous application of SNP improved the morpho-physiology, yield contributing characters and yield of wheat. The different parameters such as plant height, leaf number $plant^{-1}$, number of tiller and effective tiller $plant^{-1}$, length of spike, membrane stability, number of spikelet $spike^{-1}$, number of effective spikelet $spike^{-1}$ number of grains $plant^{-1}$, 100 grains weight and grain yield pot^{-1} of wheat significantly decreased with As contamination whereas SNP significantly increased these grain yield pot^{-1} by improving the morphological, physiological and yield characters. The interaction between different levels of As and SNP showed significant difference in wheat by altering the morpho-physiological and yield contributing characters and grain yield per pot. The maximum grain yield (12.60 g pot^{-1}) was obtained from 0.1 mM SNP along without As (As_0N_1) treatment combination which did not show any difference with 30 mg As kg^{-1} soil and 0.1 mM SNP (As_1N_1). The grain yield pot^{-1} of wheat was improved from 10.30 g to 12.35 g, 8.50 g to 9.82 g and 5.69 g to 6.88 g at 30 mg, 60 mg and 90 mg As kg^{-1} soil, respectively along with 0.1 mM SNP and are suggesting that exogenous foliar application of SNP mitigates the adverse effects of As toxicity in wheat.

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ACRONYMS

AEZ = Agro- Ecological Zone

AO = Ascorbate oxidase

APX = Ascorbate peroxidase

As = Arsenic

AsA = Ascorbic acid

BARI = Bangladesh Agricultural Research Institute

BBS = Bangladesh Bureau of Statistics

CA = Carboninc anhydrase

CAT = Catalage

Chl = Chlorophyll

cm = Centimeter

Conc. = Concentration

CRD = Completely randomize design

CV (%) = Percentage of coefficient of variance

DAS = Days after sowing

DHA = Dehydroascorbate

DHAR = Dehydroascorbate reductase

EDTA = Ethylenediaminetetraacetic acid

et al. = And others

FAO = Food and Agriculture Organization

Gly I = Glyoxalase I

Gly II = Glyoxalase II

GR = Glutathione Reductase

GSH = Reduced Glutathione

GSSG = Oxidized Glutathione

H_2O_2 = Hydrogen peroxide

IAA = Indole Acetic Acid

Kg = Kilogram (g)

Lsd = Least Significant Difference

MDA = Malondialdehyde

mg = Milligram

mm = Millimeter

mM = Millimolar

NO = Nitric Oxide

No. = Number

NS = Non Significant

PGRs = Plant Growth Regulators

POD = Peroxidase

POX = Guaiacol Peroxidase

Pro = Proline

ROS = Reactive Oxygen Species

SAU = Sher-e-Bangla Agricultural University

SNP = Sodium Nitroprusside

SOD = Superoxide Dismutase

CHAPTER ONE
INTRODUCTION

CHAPTER 1
INTRODUCTION

Agriculture is the main source of income for a majority of the people of Bangladesh and agricultural development plays a major role in improving food security safety and nutrition. Wheat (*Triticum aestivium* L.) is second most important cereal after rice in Bangladesh. It is under Poaceae family and is sensitive to temperature. In addition, it was also reported in 2016 that the world production of wheat was 749 million tons, making it the second most-produced cereal after maize (1.03 billion tons), with more than rice (499 million tons) (FAOSTAT, 2016). Average yield and total production of wheat in Bangladesh has been estimated 3.09 t/ha and 1,347,926 metric tons, respectively (BBS, 2015). It grows generally in the winter season, October-March. The main constituent of wheat is carbohydrates protein.

The northern and north-western zone of Bangladesh is used mainly for wheat cultivation. Bangladesh had become highly dependent on wheat imports while dietary preferences were changing such that wheat is becoming a highly desirable food supplement to rice. According to consumption importance, it occupies 4 % of the total cropped area and 11 % of the area cropped in *rabi* season and contributes 7 % to the total output of food cereals (Anonymous, 2008). Corresponding with biotic stresses, abiotic stresses conditions such as drought, salinity, metal toxicity, extreme temperatures, low nutrient availability, and high ultraviolet radiation exposition also impose restrictions to growth and development of wheat plant. Separately, these adverse effects of environment can be mitigated by improving defense functions in wheat. Therefore, the production of wheat is gradually decreasing due to lack of suitable techniques for improving stress tolerance with climate change in Bangladesh.
Arsenic (As) is a heavy metal, environmental toxicant and a ubiquitous trace element is found in ground water and agricultural land. It has been reported that Bangladesh is extremely high As contaminated regions where As concentration in water has been reported up to 3200 $\mu g L^{-1}$ against the safe limit of 10 $\mu g L^{-1}$ recommended by WHO (McCarty*etal*., 2011). Numerous sites are identified as As contaminant area in Bangladesh such as Jessore, Faridpur, Madaripur, Chapainouabgonj, Chadpur etc. Arsenic causes skin lesions, cancer of lungs, bladder, disease of cardiovascular system, diabetes, reproductive failure etc. in human beings (Smith, 1992; Tseng *et al.*,

2003; Navas-Acien *et al.*, 2008; Hendryx, 2009). In addition, the rate of As contamination increasing day by day and the flora and fauna become vulnerable to As contaminated environment.

Arsenic exists in three major forms, arsenate (AsV), arsenite (AsIII) and as methylated species. These forms are taken up by different mechanism and mobilized inside the plants. The causes of As toxicity are mainly due to interference in ATP synthesis and alteration in protein structure and catalytic properties (Sinha *et al*., 2013). The toxicity of As decreased the germination (%), biomass, chlorophyll and carotenoid content whereas enhanced the MDA content and proline content in plant tissue. Li *et al.* (2007) reported that wheat seedling growth was also stimulated at low and inhibited at high As concentrations of wheat. As exposure induces reactive oxygen species (ROS) production which leads to cellular membrane damage (Kumar *et al*., 2013, 2014). As induces the synthesis of phytochelatins (PCs) that bind to As III and sequester it into vacuole and reduces the free As in cytoplasm (Dixit *et al*., 2015). Arsenite decreased all the endpoints like seed germination, relative root length andshoot height, arsenic accumulation in young seedling and total amylolytic activity in wheat more remarkably than arsenate (Liu *et al.,* 2005). Therefore, to my knowledge information is not enough about the adverse effects of arsenite on changes in morphophysiology and yield of wheat.

Previous reports stated that numerous organic & inorganic substances such as PGRs, proline, sugar, sugar alcohol, H_2O_2, NO mitigate the adverse effects in plants from both biotic and abiotic stresses. Nitric oxide (NO) is a gaseous free radical molecule and serves as an effective signaling molecule in plant system. It develops an immune response against pathogen attack in plants (Bellin *et al.*, 2013). As a NO donar, SNP (Sodium nitroprusside) also involves in regulating many physiological and biochemical process in plant to become effective under stress environment. Plant physiologists reported that SNP increases the activity of IAA against oxidized condition (Garcia-Mata and Lamattina, 2001). NO can neutralize heavy metal induce ROS in two ways, first being a free radical it can directly react with ROS and neutralize them and second being a signaling molecule, it may stimulate antioxidant system to abate oxidative stress (Laspina *et al*., 2005). Ascorbate peroxidase (APX), catalase (CAT), and superoxide dismutase (SOD) are good candidates for NO regulated antioxidants in plants (Groß *et al.*, 2013*)*.

Many previous reports stated that the exogenous application of NO enhances relative water content, chlorophyll, and proline contents as well as the yield of wheat plants under As-induced oxidative stress. SNP increased the AsA and GSH contents and the GSH/GSSG ratio as well as the activities of MDHAR, DHAR, GR, GPX, CAT, Gly I and Gly II in the seedlings subjected to As stress (Hasanuzzaman *et al.,* 2012; Hasanuzzaman and Fujita, 2013). Singh *et al.* (2013) also reported that H_2O_2 as well as NO can improve plant fitness to Astoxicity. Tan *et al.* (2008) enhancing the evidence of NO has ability to alleviate oxidative damage in leaves of wheat seedlings subjected to osmotic stress. Supplementation of cadium (Cd) treatment with SNP significantly reduced the Cd-induced lipid peroxidation, H_2O_2 content and electrolyte leakage in wheat roots. It indicated a reactive oxygen species (ROS) scavenging activity of NO (Singh *et al.,* 2013). The exogenous NO provides resistance to rice against As-toxicity and has an ameliorating effect against As (Singh *et al.,* 2009). However, to my knowledge no study has conducted on the role of NO on improvement of morphophysiology and yield of wheat.

In perspective to this scenario, the present study was carried out to evaluate the effectiveness of sodium nitroprusside (SNP) to mitigate the adverse effect of As toxicity for wheat production.

Objectives of the research

According to the above discussion, the present work was based to evaluate the following objectives-

1. To investigate the adverse effects of arsenic on changes in morphophysiological and yield characteristics of wheat.
2. To investigate the mitigating ability of sodium nitroprusside (SNP) on arsenic toxicity in wheat.
3. To find out a method of mitigating the arsenic toxicity in wheat with SNP.

CHAPTER TWO
REVIEW OF LITERATURE

CHAPTER 2
REVIEW OF LITERATURE

Wheat (*Triticum aestivum* L.) is one of the important cereal crops in Bangladesh. A good number of research works have been done on this crop in different countries of the world. In perspective of Bangladesh, arsenic (As) an abiotic stress is one of the serious problems in agricultural sector. Arsenic is a highly toxic metalloid and its contamination in soil and water has become an important environmental concern. As is taken up by plants and subsequently transformed in plant tissue is therefore essential for estimating the risks posed to human and wildlife populations by As contaminated soils (Meharg and Hartley-Whitaker, 2002). The scientists of Bangladesh are conducting different experiments to adopt different crops in the As induced soil; wheat is one of them. Very limited research works have been conducted to adapt wheat in the As stressed condition of Bangladesh. An attempt has been made to find out the performance of wheat at different levels of As as well as to find out the possible mitigation ways by using SNP in the As induced wheat plants. SNP, a nitric oxide (NO) donor is one kind of phytohormone used in plants to enhance growth ability. To facilitate the research works different literatures have been reviewed in this chapter under the following headings:

2.1 Effects of arsenic (As) toxicity in plants

2.1.1 Morphological characteristics

Akhtar and Shoaib (2014) conducted a pot experiment and observed damaging influence of arsenate (V) on germination and early growth of wheat (*Triticum aestivum* L.) in Pakistan. Four concentrations of As (V) i.e. 0.013, 0.025, 0.038 and 0.05 mg pot^{-1} were used to spike sandy loam soil (sand: 76%, silt: 15%, clay: 9%). Different As (V) treatments in the soil resulted in significant reduction in germination rate (G %), germination index (GI) and relative germination rate (RGR), while increased in arsenic response index (ARI). Shoot growth and biomass were significantly declined by 40-90%, and that of roots were decreased by 50-99% in 2-week and 4- weeks old plants. Morphological symptoms like yellowing of leaf margins, stunt roots and stems were observed at higher dose (0.05 mg) of As (V) in 4-week seedling. The tolerance index (Tindex) of seedling was decreased and metal accumulation was increased that revealed increase in the seedling sensitivity with increasing concentrations of As(V). Root accumulated more metal than the shoots.

Das *et al.* (2014) conducted a pot culture experiment graded doses of arsenic (As) in order to determine the toxicity symptoms in rice plants. The arsenic accumulation by the plant parts generally followed the order: root>stem>leaf>economic produce. Yield reduction of 80.8% was observed in treatment applying more than 60 mg kg^{-1} As. The arsenic toxicity symptoms in leaf and root were observed with treatment above 40 mg kg^{-1}As, while the symptoms were apparent in economic produce above 60 mg kg^{-1}. The phytotoxicity threshold levels were observed with yield reduction to 55.5% in tillering and 54.8% at harvesting. In field experiments of rice-green manure–rice cropping sequence, no toxicity symptoms developed but a greater extent of As accumulation in rice crops was observed.

Tripathi *et al.* (2012) reported that arsenic (As) is a wide-spread toxic and carcinogenic metalloid. As can induce growth inhibition, low productivity, and poor grain quality by inducing oxidative stress in crop plants (Gunes *et al.*, 2009).

Norton *et al.* (2010) observed in field trails in Bangladesh and China between phosphorus (P) and As and silicon (Si), and As were established in a wide range of cultivars grown in As contaminated soil. No correlations were observed between shoot and grain speciation, with the inorganic form comprising 93.0−97.0% of As in the shoot and 63.0−83.7% in the grains. The percentage of dimethylarsinic acid (DMA) was between 1.4 and 6.6% in the shoot and 14.6 and 37.0% in the grains; however, the concentrations were comparable, ranging from 0.07 to 0.26 mg kg^{-1} in the shoots and 0.03 to 0.25 mg kg^{-1} in the grains. A positive correlation was observed between shoot As and shoot Si, however, no correlation was observed between shoot Si and grain Arsenic. A significant negative correlation was observed between shoot P and grain As concentrations. These results suggest that the translocation of As into the grain from the shoots is potentially using P rather than Si transport mechanisms.
Norton *et al.* (2009) has confirmed that excess As accumulation in rice plants threatens human health as almost 50% of the world population consumes rice as their staple food. Arsenic is a crystalline metalloid that exists in several forms and

oxidation states. Its toxicity and mobility in the environment depend on both its chemical form and species (Pongratz, 1998).

Luan *et al*. (2008) showed that seed germination was significantly influenced only when concentrations of Cd, Pb and As in the soil were relatively high (> 800 mg/kg) indicating some resistance of seed germination to toxicity of these metals. The seed germination does not seem to be a sensitive indicator of the phytotoxic effects of Cd, Pb and As in most experimental treatments.

Chaturvedi (2006) observed that by using two Brassica juncea genotypes (Varuna and DHR-9504) in a green house experiment highly affected by arsenic. Arsenic extraction by plants increased significantly with increasing arsenic concentrations in soils. Uptake of arsenite by Indian mustard genotypes was higher than that of arsenate. Stunted growth of the plants was also observed.

According to Evans *et al.* (2005), As in low doses (100 μg/l) can be even beneficial to growth and development of maize.

Abedin *et al.* (2002b) observed on long-term use of As contaminated groundwater to irrigate crops, especially paddy rice (*Oryza sativa* L.). A greenhouse pot experiment was conducted to evaluate the impact of As-contaminated irrigation water on the growth and uptake of As into rice grain, husk, straw and root. There were altogether 10 treatments which were a combination of five arsenate irrigation water concentrations (0–8 mg As L^{-1}) and two soil phosphate amendments. Use of arsenate containing irrigation water reduced plant height, decreased rice yield and affected development of root growth. Arsenic concentrations in all plant parts increased with increasing arsenate concentration in irrigation water. However, As concentration in rice grain did not exceed the maximum permissible limit of 1.0 mg As kg^{-1}. Arsenic accumulation in rice straw at very high levels indicates that feeding cattle with such contaminated straw could be a direct threat for their health and also, indirectly, to human health.

In pot experiments rice was grown by Kang *et al*. (1996) on loam paddy soil with available arsenic contents of 1.3, 6.0, 7.8 or 10.3 mg/kg and total arsenic contents of

1.3, 27.7, 36.6 and 56.0 mg/kg, respectively. Increasing the level of As decreased plant height, number of effective tillers, dry weight of above ground parts and 1000-grain weight. Yields decreased from 48.7 g/pot with the lowest rate of arsenic to 17.9 g with the highest rate. Content of arsenic was higher in roots than in stems plus leaves or in grain, but in all parts the content increased as soil arsenic increased. The contents of arsenic in stems plus leaves were more closely related to soil total and available arsenic than those of roots or grain.

Onken and Hossner (1995) set an experiment to study on determined the species and concentrations of As present in soil solution of flooded soils and correlated them to As concentration, P concentration, and growth rate of plants grown in treated soils. Rice (*Oryza sativa* L.) was grown in two soils treated with 0, 5, 15, 25, 35, and 45 mg As kg^{-1} soil added as either Na-arsenate or Na-arsenite. Soil solution samples and plant samples were collected over a period of 60 d. The As concentration of rice plants best correlated to the mean soil solution arsenate concentration in a Beaumont clay (fine, montmorillonitic, thermic Entic Pelludert) and to the mean soil solution arsenite concentration in a Midland silt loam (fine, montmorillonitic, thermic Typic Ochraqualf). In both soils, plant P concentration was best correlated to the amount of As added to the soil rather than any soil solution As concentration. Plant weight was best correlated to the mean soil solution arsenate concentration in both soils. The rate of As uptake by plants increased as the rate of plant growth increased. Plants grown in soils treated with As had higher rates of As uptake for similar rates of growth when compared with plants in untreated soils. However, growth 12 per unit of As uptake was higher for plants in untreated soils than plants in As treated soils.

Pot and field experiments were conducted by Chen and Liu (1993) to investigate the effect of pH in the movement of arsenic (As) in the plant-soil system. Increasing the soil pH decreased As adsorption and thereby increased the As concentration in the soil solution. Therefore as the soil pH rose As availability to rice increased and toxicity problems became more serious. They also reported that from a pot experiments, low levels of arsenite (As^{3+}) added to the soil increased growth due to inhibition of photorespiration unnecesary for growth, which led to less depletion of photosynthates. High levels of arsenite caused phytotoxicity due to inhibition of necessary respiration.

Marin *et al*. (1992), Carbonell *et al*., (1995), Abedin *et al.* (2002a, 2002b), Johan *et al.* (2003) has confirmed that, when plants were exposed to excess arsenic either in soil or in solution culture, they exhibited toxicity symptoms such as: inhibition of seed germination, decrease in plant height, depress in tillering, reduction in root growth, decrease in shoot growth, lower fruit and grain yield, and sometimes, leads to death.

2.1.2 Physiological characteristics

Rahman *et al*. (2015) recorded that arsenic-induced stress decreased chl content in the rice seedlings. Chlorophyll content decreased with increasing As concentration. Application of 0.5 and 1mMAs in the rice seedlings decreased chl ($a + b$) content by 33 and 44%, respectively, compared with control. Dry weight of the rice seedlings also decreased by 28 and 35%with 0.5 and 1mM As exposure respectively. Arsenic in the growth medium negatively changed other physiological conditions, including RWC, Pro accumulation, and the glyoxalase system. Excess As in the growth medium also caused higher As accumulation in the plants along with other physiological changes that ultimately arrested plant growth.

Upadhyaya *et al.* (2014) reported that arsenic is a great hazard to the environment since it is a major source of soil and water contamination leading to phytotoxicity. It caused reduction in seed germination and growth of plants including mung bean (*Vigna radiata*) causing loss of crop productivity. Arsenic induced reduction in fresh, dry mass, length of root and shoot and pigment content.

Srivastava and Sharma (2013) observed that arsenic significantly enhanced lipid peroxidation, electrolyte leakage, and level of proline showing oxidative stress in black gram (*Vigna mungo* L.). Arsenic toxicity was associated with an increase in the activities of antioxidative enzymes like superoxide dismutase, peroxidase, and ascorbate peroxidase whereas catalase activity decreased at higher arsenic dose.

Fayiga *et al.* (2007) recorded that plants ability to solubilize soil arsenic as chemical fractionation of an arsenic contaminated soil spiked with 50 or 200 mg kg^{-1} Ni, Zn, Cd or Pb was performed before and after growing the arsenic hyperaccumulator *Pteris vittata L.* for 8 weeks using NH4Cl (water-soluble plus exchangeable, WE-As), NH_4F

(Al-As), NaOH (Fe-As), and H_2SO_4 (Ca-As). Arsenic in the soil was present primarily as the recalcitrant forms with Ca-As being the dominant fraction (45%). Arsenic taken up by *P. vittata* was from all fractions though Ca-As contributed the most (51e71% reduction). After 8 weeks of plant growth, the Al-As and Fe-As fractions were significantly ($p < 0.01$) greater in the metal-spiked soils than the control, with changes in the WE-As fraction being significantly (p¼ 0.007) correlated with plant arsenic removal. It enhanced its ability to hyperaccumulate arsenic. It has been reported that superoxide dismutase, catalase, ascorbate peroxidase, glutathione peroxidase and glutathione reeducates were stimulated in crops upon exposure to arsenic (Hartley-Whitaker *et al.*, 2001; Singh *et al.*, 2007).

Li *et al*. (2007) reported that wheat the second most cereal crop in Bangladesh is also cultivated in badly arsenic contaminated regions like Jessore and Faridpur. There is a general perception that upland crop like wheat may contain low amount of Asthan that of rice. Physiological activities of wheat seedlings changed under As stress.

Claussen (2005) stated that Proline accumulation under stress conditions may be caused by induction of proline biosynthesis enzymes, reduction the rate of proline oxidation conversion to glutamate, decrease utilization of proline in proteins synthesis and enhancing proteins turnover.

Jha and Dubey (2004) observed that in rice seedlings arsenic toxicity causes changes in carbohydrate metabolism leading to the accumulation of soluble sugars by altering enzyme activity. Sucrose synthase possibly plays a positive role in synthesis of sucrose under As-toxicity.

The visual phytotoxicity symptoms observed by Fecht-Christoffers *et al.* (2003) were slight chlorosis, necrosis of leaf tips in case of barley and pea treated with As, blackening of roots and basal parts of shoots of beans treated with Cd and As. Blackening was appearing on faba bean roots which can indicate metal-induced oxidation of different phenols in roots.

Nguyen *et al*. (2003) reported that the electron transport processes are impeded developing toxic systems. Several toxic reactive oxygen species (ROS) are generated in the cell wall region as well as inside the cell during the process, which affects membrane permeability, enzyme activity, metabolic pool, plant biomass, leaf chlorosis and necrosis.

Ducsay (2000) conducted an experiment in laboratory conditions to investigate the impacts of upward doses of As, cadmium (Cd) and lead (Pb) on quality of cultivated biomass wheat (*Triticum aestivum* L.) during 21 days and production of chlorophyll a and chlorophyll b. The most expressive decrease of growth crops were found in As, Cd and Pb. The increase of their concentrations in soil caused increasing of their concentration in biomass.

Matschullat (2000) reported that the limited accumulation of As by roots and its limited translocation to the shoots, is usually used by most plants such as carrot, tomato and grass. These plants contain relatively low As and accumulate As primarily in their root systems (O’Neill, 1995).

Pickering *et al.* (2000) also observed a large accumulation of As by mustard plants, in roots as compared to stems.

Meharg (1994) conducted that the conversion of arsenate to arsenite, a process that usually occurs in plants by synthesizing both enzymatic and non-enzymatic antioxidants. Arsenate acts as a phosphate analogue and is transported across the plasma membrane via phosphate transport systems. Inside the cytoplasm it reacts with phosphate, replacing ATP to form ADP-As, precisely because of disruption of energy flow in cells.

Marin *et al.* (1992) stated that, at higher concentration, arsenic was toxic to most plants. It interfered with metabolic processes and inhibited plant growth and development through arsenic induced phytotoxicity.

Arsenic is generally considered phytotoxic and is expected to negatively affect plant growth (Kabata- Pendias and Pendias, 1991).

Bertolero *et al*. (1987) reported that arsenate reduced to arsenite in plant tissue, does not normally have enough cytoplasmic concentrationsto exert toxicity.

2.1.3 Yield and yield contributing characters

Noor *et al*. (2016) showed the performance of different wheat varieties on arsenic contaminated soil and irrigation water at field trials of Jessore (Chowgacha and Sharsha), Faridpur (Poranpur) and also at low contaminated Shatkhira (Benerpota) during 2010-2011 and 2011-12. Five varieties of wheat viz. Shatabdi, Bijoy, Prodip, BARI Gom-25 and BARI Gom-26 were tested. Total arsenic contents in the soils were 36.4, 32.8, 28.5 and 6.8 mg kg^{-1} for Sharsha, Chowgacha, Poranpur and Benerpota, respectively. Irrigation waters contained 0.346, 0.272, 0.238 and 0.140 mg L^{-1} arsenic for Sharsha, Chowgacha, Poranpur and Benerpota, respectively. No significant variations in yield and yield components among the tested wheat varieties were observed despite of arsenic contaminations in the irrigation water and soil. The variety, Prodip contains 0.043 and 0.028 mg kg^{-1} arsenic in straw and grain, respectively, which was lower than the other tested varieties. But arsenic contents in all of the tested wheat varieties were found much lower than that of the permissible limit (1 mg kg^{-1}). The transfer coefficient (TC) of arsenic from soil to above ground parts (straw + grain) of wheat varied slightly among the tested varieties where Prodip showed the lowest TC (0.0015-0.0018).

Liu *et al.* (2012) conducted a pot experiment with the soil As bioavailability at different growth stages of wheat and rape. The results indicated that winter wheat was much more sensitive to As stress than rape. Wheat yields were elevated at low rates of As addition (< 60 mg/kg) but reduced at high rates of As concentrations (80–100 mg/kg); while the growth of rape hadn’t showed significant responses to As addition. When soil As concentration was less than 60 mg/kg, both wheat and rape could grow satisfactorily without adverse effects; when soil As concentration was 80–100 mg/kg, rape was more suitable to be planted than wheat.

Arsenic (As) is now regarded as one of the most serious contaminants as a typical noxious element-especially inorganic arsenic reported by Wang *et al*. (2010). Indeed, arsenic has a chronic poisoning effect in human body. Recent studies have shown that rice is much more efficient in assimilating arsenic into its straw and grains than other staple cereal crops, and consumption of rice constitutes a large proportion of dietary intake of arsenic. Therefore, scientists pay a high degree of attention to arsenic in rice. In rice total As content varies from 0.005 to 0.710 mg/kg. Arsenic speciation in rice grain is dominated by inorganic As (III+V) and dimethylarsinic (DMA). The inorganic As content in rice varies from 10% to 90% of total As.

Liu *et al.* (2009) studied various amounts of poultry litter contaminated with roxarsone in pot experiments to evaluate the effect of roxarsone on rice agronomic parameters and the bioaccumulation of total and inorganic As in rice-plant tissues. Rice-grain yield decreased significantly with increasing As content of the soil, and the critical threshold that killed rice was 200 mg roxarsone (kg^{-1} soil). The As concentrations in root, straw, leaf, husk, and grain increased with increasing soil As ($p < 1\%$). At 100 mg roxarsone per kg of soil, the As concentration in the rice grain exceeded the statutory permissible limit of 1.0 mg As (kg dry weight)$^{-1}$ and at 25 mg roxarsone (kg^{-1} soil), the inorganic As concentrations in grains exceeded the statutory limit of 0.15 mg of inorganic As kg^{-1} in China. Arsenite was the predominant species in root, straw, and grain, while arsenate was the predominant species in leaf and husk. No significant difference existed between the amounts of arsenite and arsenate when various amounts of poultry litter were applied. This result illustrates that large amounts of added roxarsone are not only toxic to rice but also accumulate in grains in the inorganic As forms, potentially posing a threat to human health *via* the food chain.

Zhang *et al.* (2009) conducted a field experiment on soil contamination on four wheat varieties (Jimai, Gaoyou, Weimai and Wennong) in Eastern China, using 50 or 100 mg arsenic/kg soil. Results showed that addition of arsenic significantly ($p<0.05$) reduced root, stem and spike dry weight and yield components, which resulted in the decrease of grain yield per plant. Arsenic concentrations in plant tissues increased significantly ($p<0.05$) with treatments, and its uptake varied considerably among wheat varieties, plant tissues and arsenic treatments. Arsenic concentrations in plant tissues were as follows: roots > stems > leaves and rachises > grains > glumes > awns. In the arsenic treatments, arsenic concentrations in bran were about 2-3 times higher than those in flour. Most of the arsenic contaminated flour exceeded the Chinese tolerance limit. Arsenic contents of grain parts were dependent on variety and treatment level in polluted soils. Weimai and Wennong showed highest amounts of arsenic in flour than the other varieties at 50 or 100 mg/ kg soil treatment, respectively. Weimai possessed significantly lower ($p<0.05$) amount of arsenic in bran than any other wheat variety.

Gulz *et al.* (2005) observed that As had increased wheat yield significantly with 20.1% higher than the control when As addition was 60 mg/kg. When soil As concentration was ≥ 80 mg/kg, the wheat yield significantly decreased. And the same tendency occurred with the weight of 1000 grains.

In grain, As concentrations of wheat and rape increased with arsenic added in soil but did not exceed the maximum permissible limit for food stuffs of 1.0 mg/kg (National Food Authority 1993).

Jiang and Singh (1994) stated that arsenic is a nonessential element for plants, and inorganic As species are generally highly phytotoxic. Biomass production and yields of a variety of crops are reduced significantly at elevated arsenic concentrations (Carbonell- Barrachina et al., 1997), with application of only 50 mg As kg^{-1} to soil significantly decreasing the yields of barley (Hordeum vulgare L.) and ryegrass (*Lolium perenne* L.).

2.2 Effects of sodium nitroprusside (SNP) in plants

Habib *et al.* (2016) evaluated that salt stress induced a significant reduction in biomass and grain yield while increased the plant proline, ascorbic acid, H_2O_2 and MDA contents in all studied cultivars of rice (*Oryza sativa* L.). SOD, POD and CAT activities also significantly increased in salt stressed plants; however, the total phenolics content decreased. Application of SNP as seed priming reduced the adverse effects of salinity on plant biomass production and grain yield, while the accumulation of MDA and H_2O_2 decreased. Of different SNP levels, 0.1 mM regime was more effective in reducing the negative effects of salinity. Among fine rice cultivars, Shaheen basmati performed better, while among coarse rice cultivars the performance of IRRI-6 was better to exogenously applied 0.1 mM SNP.

Bai *et al.* (2015) used SNP in rice seedlings at different concentrations in the presence of lead (Pb) and they found that Pb- induced oxidative damage was reduced with 50, 100 and 200 μM of SNP however, 400 μM of SNP had no obvious alleviating effect in Pb toxicity.

Nitric oxide (NO), a multifunctional gaseous molecule, mediates a variety of responses to biotic and abiotic stresses which reported by Chen *et al.* (2015) in rice (Oryza sativa cv. 'Zhonghua 11') plant growth under mercuric chloride ($HgCl_2$) stress. The results showed that 60 lM Hg significantly inhibited the root elongation of rice plantlets after seed germination. While 100 lM or 200 lM sodium nitroprusside (SNP, a donor of NO) could increase the root length by attenuating the effects of 2,3,5-triiodobenzoic acid (TIBA) and Hg, which indicated the role of NO in auxin transport-promoting in roots. On the other hand, SNP decreased the absorption and transportation of Hg in roots and shoots of rice seedlings at five-leaf stage. Moreover, the levels of superoxide radical (O^{-2}) and hydrogen peroxide (H_2O_2) in leaves were also decreased significantly. However, the activities of antioxidant enzymes were not enhanced by SNP. Moreover, NO promoted the growth of rice plantlets under Hg stress even when superoxide dismutase (SOD, EC 1.15.1.1) or catalase (CAT, 1.11.1.6) activity was inhibited by diethyldithiocarbamate (DDC, an inhibitor of SOD) or 3-amino-1,2,4- triazole (AT, an inhibitor of catalase), respectively.

Jaiswal and Srivastava (2015) identified one water logging resistant (HUZM-265) and a susceptible (HUZM-55) Out of 28 genotypes (inbred lines) of maize (*Zea mays* L.). Selected genotypes were further grown in pots and after 20 days subjected to root zone waterlogging with or without 50, 500 and 2000 μmol L^{-1} sodium nitroprusside (SNP) as a donor of NO in the flooding water. Waterlogging caused reduction inleaf number, leaf area and dry weights of plants in both genotypes. Flooding root zone with 50 μmol L^{-1} SNP, alleviated the stress effects or sensitivity (not tolerance), but to a greater magnitude in susceptiblegenotype. Stomatal conductance, transpiration rate, chlorophyll decreased as the water loggingduration increased. Nitrogen content in roots and shoot of waterlogged plants also declined significantly. 500 μmol L^{-1} SNP treatment tend to alleviate the deleterious effect of water logging. Cellmembrane injury in roots of waterlogged plant was higher in genotype HUZM-55 than in HUZM-265 and500 μmol L^{-1} SNP was found to have mitigating role in combating it.

500 μmol L^{-1} SNP was foundeffective for alleviating transpiration rate, chlorophyll content and nitrogen content in both genotypes while 50 and 2000 μmol L^{-1} SNP increased stomatal conductance in HUZM-265 and HUZM-55, respectively.

Esim *et al.* (2014) reported that NO (Nitric Oxide) was suppressed the chilling induced condition in wheat (*Triticum aestivum* L.) plant. NO treatment was carried out through spraying of sodium nitroprusside (SNP), which is a donor of NO. To do this, SNP concentrations of 0.1 and 1 mM were applied on the leaves of 11-day plants and the plants were then exposed to chilling conditions (5/2 C) for 3 days. The chilling stress treatment increased both the activities of antioxidant enzymes and the levels of MDA, H_2O_2 and O^{-2}. Similarly, NO treatment enhanced SOD, POX and CAT activities under chilling stress, whereas it decreased H_2O_2 and O^{-2} contents as well as MDA level. The most effective concentration was determined as 0.1 mM SNP. Exogenous SNP application as a donor of NO was found to have an important ameliorative effect on cold tolerance of seedling exposed to chilling stress by stimulating antioxidant enzyme activity.

Chen *et al.* (2013) observed that the treatment of Cd-stressed barley seedlings with SNP (250 μM) increased chlorophyll content and net photosynthesis and ameliorated Cd-induced damage to leaf and root ultrastructure.

Esim and Atıcı (2013) reported that the effects of exogenous NO as sodium nitroprusside (SNP) on boron (B)-induced oxidative damage and growth in maize (*Zea mays* L.) were investigated. The addition of B significantly reduced the growth of plants and increased the values of electrolyte leakage, malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents. SNP (100 μM) that was applied to seeds before germination significantly increased plant height (respectively, 8 and 5%), fresh weight (respectively, 9 and 6%), and dry weight (respectively, 15 and 12%) of both 11 and 15 day old maize. Furthermore, the measured B-induced oxidative stress increased MDA, electrolyte leakage, H_2O_2 content when compared to a supplementation of NO. SNP application also increased activities of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX). The results suggest that the increased NO, resulting from SNP application, improved the antioxidant capacity of maize plants against B-induced oxidative stress.

Gill *et al.* (2013) founded most effective compound Nitric Oxide (NO) that alleviate Cd stress and is a hydrophobic gaseous molecule involved in various physiological processes such as germination, root growth, stomatal closure, control of the flowering timing etc. NO also functions as cell signaling molecule in plants and play important roles in the regulation of plant responses to both abiotic and biotic stress conditions. At the molecular level, NO signaling includes protein modification by binding to critical cysteine residues, heme or ironesulfur centers and tyrosine residue nitration via peroxynitrite formation ($ONOO^-$), mobilization of secondary messengers (Ca^{2+}, cyclic GMP and cyclic ADP-Rib) and modulation of protein kinase activities.

Kausar *et al.* (2013) was observed in pot experiment that exogenous application of NO as foliar spray markedly increased morphological plant growth, yield per plant, number of seeds per plant, and 100-seed weight of wheat cultivar S-24 under no stressed conditions, while only 0.1 mM NO enhanced grain yield per plant under saline conditions. Without nitric oxide (NO) application did not show significant effect on yield attributes of wheat under saline condition.

Siddiqui *et al.* (2013) carried out an experiment about combined effects of NO and salicylic acid (SA) with nickel (Ni) stress in wheat (*Triticumaestivum* L.) plant. The results showed Ni-fed plants exhibited moderate reduction in growth characteristics (PH, FW, DW and LA). Also, the application of Ni inhibited CA activity, content of essential elements (N, P, K) and Chl. Plants showed higher accumulation of Ni and MDA under Ni stress. Under Ni stress, the combined application of NO and SA induced the activities of enzymes (CA, SOD, POD, CAT) and accumulation of osmoprotectant proline (Pro). Also, combined application of SA and NO improved the photosynthetic pigments (Chl *a* and Chl *b*) and maintained nutrients homeostasis in plants under Ni-stress. Application of NO and SA together was more effective in suppression of deleterious effect of Ni stress by reducing MDA formation in plants.

Bavita *et al.* (2012) conducted an experiment with two wheat (*Triticum aestivum* L.) cultivars, C 306 (heat tolerant) and PBW 550 (comparatively heat susceptible) and SNP(NO donar) to study the extend of oxidative injury and activities of antioxidant enzyme in relation to high temperature(HT) stress. HT stress resulted in a marked decrease in membrane thermostability (MTS) and 2, 3, 5-triphenyl tetrazolium chloride (TTC) cell viability whereas content of lipid peroxide increased in both the cultivars. The tolerant cultivar C 306 registered less damage to cellular membranes compared to PBW 550 under heat temperature stress. Activities of antioxidant enzymes viz, superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase increased with HT stress in both the cultivars. Following treatment with SNP, activities of all antioxidant enzymes further increased in correspondence with an increase in MTS and TTC. Apparently, lipid peroxide content was reduced by SNP more in shoots of heat tolerant cultivar C 306 indicating better protection over roots under HT stress.

Wang *et al.* (2011) brought a shed of light on the effects of sodium nitroprusside (SNP), a NO donor, on both morphological and physiological characteristics of maize under waterlogging stress. Results showed that SNP could increase height, dry weight and activities of catalase, peroxidase and superoxide dismutase, and decrease MDA content and ion leakage ratio under water logging stress.

Application of sodium nitroprusside (SNP, a donor of NO) was able to reduce root to shoot translocation of Ni in *Brassica napus* (Kazemi *et al.*2010).

Zhang *et al.* (2008) reported that generation of endogenous NO was positively associated with the proline level in Cu treated algae. Furthermore, NO could alleviate phytotoxicity by directly regulating accumulation and translocation of heavy metals in plants.

Hsu and Kao (2004) observed that metal toxicity can affect plasma membrane permeability and there is protective evidence to show that the NO donor protects plasma membrane integrity (Wang *et al.*, 2005; Hu *et al.*, 2007).

Tian and Lei (2007) investigated that physiological and biochemical responses of wheat seedlings to drought, UV-B radiation, and combined stress were seriously mitigated by foliar application of SNP. Drought, UV-B, and combined stresses retarded seedling growth by 26.5, 29.1, and 55.9%, respectively. One reason for growth retardation may be the oxidative damage indicated by an increase in the H_2O_2 content and lipid peroxidation degree. catalase activity decreased under the combined stress as compared to the control. The combination of drought and UV-B caused more severe damage to wheat seedlings than stress factors applied separately. Thus, the combined application of drought and UV-B had more strong adverse effects on wheat seedlings. The addition of 0.2 mM sodium nitroprusside (SNP) enhanced wheat seedling growth under drought, UV-B, and combined stress, likely, due to decreasing the accumulation of H_2O_2 and lipid peroxidation as well as activating the antioxidant enzymes. However, SNP treatment also decreased the proline content.

2.3 Mitigation of arsenic toxicity with sodium nitroprusside (SNP) in plants

Singh *et al.* (2016) evaluated the protective role of NO against arsenate (AsV) toxicity in rice plants. AsV exposure has hampered the plant growth, reduced the chlorophyll content, and enhanced the oxidative stress, while the exogenous NO supplementation has reverted these symptoms.The NO donor SNP has been applied to plants submitted to arsenic (As) stress. In these study with hydroponically-grown rice plants, the treatment with 25 or 50 μM arsenate (Na_2HAsO_4: AsV) led to an augment of root and shoot H_2O_2 and MDA contents, but the levels of both oxidative stress biomarkers were decreased when AsV-treated plants were supplemented with 100 μM SNP. In addition to reduce oxidative stress, exogenous NO supplementation prevented the deleterious effects of AsV in plant growth through the reduction of AsVaccumulation in the tissues, the reversion of the AsV -induced iron deficiency and the modulation of thiol metabolism. Conclusively, exogenous application of NO could be advantageous against AsV toxicity and could confer the tolerance to AsV stress in rice.

Shukla *et al.* (2015) studied on the mitigation effects of nitric oxide (NO) in Astoxicity. The altered expression of stress responsive gene alternative oxidase (Aox1) in seedlings of barley (*Hordeum vulgare* L.) was found in response to Astoxicity. As toxicity decreased the germination percentage, biomass, chlorophyll and carotenoid content whereas, As toxicity enhanced the MDA content and proline content in a dose dependent manner. Other enzyme activities like catalase and superoxide dismutase increased with the increase in concentrations but it fell down at higher concentration of As. Pretreatment of nitric oxide results in the enhanced expression of alternative oxidase which showed the adaptation of alternative pathway during the As stress and it also enhances the growth ability and adaptability towards the As stress.

Singh *et al.* (2013) observed that the exogenous NO supply by SNP (100 μM) also alleviated the toxic effects of As (V) on photosynthetic parameters of *Luffa acutangula* seedlings, as well as it induced the antioxidant response. The involvement of direct ROS scavenging by NO in the alleviation of As (V) toxicity has been also shown in rice plants treated with 50 μM SNP.

Ismail (2012) observed that in mung bean (*Vigna radiata*), exogenous application of NO as SNP (75 μM) significantly improved seed germination, growth and CAT activity and decreased As accumulation and activity of SOD.

Studies analyzing function of NO under As toxicity in higher plants are limited. In roots of rice (*O. sativa*), exogenous application of SNP provided tolerance against As toxicity and ameliorated the As-induced decrease in length. Furthermore, SNP reduced As-induced accumulation of malondialdehyde (MDA), superoxide ion (O^{-2}.), and H_2O_2 (Singh *et al*., 2009), and an increase in the activities of SOD, CAT and APX was recorded in the presence of SNP under As stress (Jin *et al.,* 2010).

CHAPTER THREE
MATERIALS AND METHODS

CHAPTER 3
MATERIALS AND METHODS

A pot experiment was conducted at the Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the period from November 2015 to March 2016 to study the mitigation of arsenic toxicity in wheat with sodium nitroprusside (SNP). The materials and methods those were used and followed under the experiment have been presented below:

3.1 Experimental site

This was a pot experiment conducted in the net house at the field laboratory of Agricultural Botany Department, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka. The location of the site is 23º74´N latitude and 90º35´E longitude with an elevation of 8.2 meter from sea level (Anon., 2004), which have been in the Appendix I.

3.2 Characteristics of the Soil

The soil of the experimental area that used in the pot for wheat cultivation belongs to "The Modhupur Tract", AEZ – 28(FAO, 1988). The characteristics of the soil under the experiment were analyzed in the Laboratory of Soil Science Department, SAU, Dhaka and details of the pot soil have been presented in Appendix II.

3.3 Climatic condition of the experimental site

The experimental site is situated in the subtropical monsoon climatic zone, which is characterized by heavy rainfall during the months from mid March to mid October, *Kharif* season and scanty of rainfall during rest of the year, mid October to mid March, *Rabi* season which are suitable for growing of wheat in Bangladesh. The weather information regarding temperature prevailed at the experimental site during the cropping season October 2015 to May 2016 have been presented in Appendix VII.

3.4 Planting material

The variety BARI Gom 24 is known as Prodip was used as a planting material. The seeds of wheat were collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur, Bangladesh. It is high yielding but sensitive to heat. The total crop duration is almost 102-110 days. In addition, this variety is also tolerant to *Bipolaris* leaf blight along with leaf rust (BARI, 2012).

3.5 Treatments of the experiment

The two factorial experiments was carried out in Completely Randomize Design (CRD) with four replications having –

Factor (A) Different concentration of Arsenic (As)

1. As_0 =0 mg As kg^{-1}soil (Control)
2. As_1 = 30 mg As kg^{-1} soil
3. As_2 = 60 mg As kg^{-1} soil
4. As_3 = 90 mg As kg^{-1} soil

Factor (B) Different concentration of Sodium Nitroprusside (SNP) as nitric oxide donor:

1. N_0 = 0 mM SNP(Control)
2. N_1 = 0.1 mM SNP
3. N_2 = 0.2 mM SNP

Total 12 treatment combinations were as followed:

As_0N_0: Control (Without As and SNP)
As_0N_1: Zero As + 0.1 mM SNP
As_0N_2: Zero As + 0.2 mM SNP
As_1N_0: 30 mg As kg^{-1} soil + No SNP
As_1N_1: 30 mg As kg^{-1} soil + 0.1 mM SNP
As_1N_2: 30 mg As kg^{-1} soil + 0.2 mM SNP
As_2N_0: 60 mg As kg^{-1} soil + without SNP
As_2N_1: 60 mg As kg^{-1} soil + 0.1 mM SNP
As_2N_2: 60 mg As kg^{-1} soil + 0.2 mM SNP
As_3N_0: 90 mg As kg^{-1} soil + No SNP
As_3N_1:90 mg As kg^{-1} soil + 0.1 mM SNP
As_3N_2: 90 mg As kg^{-1} soil + 0.2 mM SNP

3.6 Design and layout of the experiment

The two factors experiment was laid out in Completely Randomize Design (CRD) with four levels of As and three levels of SNP. Four replications were maintained in this experiment. The total number of unit pots was 48 (12×4). Each earthen pot was 35 cm in diameter and 30 cm in height. The experiment was set in the net house which was made by bamboo with nylon net and each pot was kept on the individual earthen plate to reuse the deposited water properly for analyzing the As toxicity at different concentration.

3.7 Application of the treatments and pot preparation

Wheat plants were treated with 0, 30, 60 and 90 mg of arsenic (As) per kg soil @ 0, 6.24, 12.48, and 18.73 g sodium arsenite salt, respectively. In the experiment, As used in the form of sodium arsenite ($NaAsO_2$) and SNP in the form of $C_5FeN_6Na_2O.2H_2O$ which collected from local market. According to treatment, the total amounts of As were mixed in soil and covered with polythene sheet for three (3) days to mix with the soil correctly. The soil was fully rotated once to mixture the arsenic well. Then the treated soil was put into the pot which contains 10 kg soil per pot. All 48 pots were filled on 26 November 2015. Weeds and stubbles were completely removed from the soil. Then SNP solution at 0, 0.1 mM and 0.2 mM concentrations which were maintained by adding 0, 0.03 g and 0.06 g SNP, respectively per liter of water. The 0.1% of Tween-20 was used as an adhesive material. At 35, 45 and 55 day after sowing (DAS) the SNP solution was sprayed by a hand sprayer at 1.00 -3.00 pm.

3.8 Seed sowing

Seeds were sown on 26th November 2015. Ten (10) seeds were sown in each pot. Seeds were placed in 2 cm depth, and then covered with soil properly.

3.9 Fertilizers and manure application

The fertilizers N, P, K, S, Zn and B in the form of urea, triple super phosphate (TSP), murate of potash (MP), gypsum, zinc sulphate and boric acid were applied @ 1.26 g, 1.05 g, 0.56 g, 0.49 g, 0.07 g and 0.07 g pot $^{-1}$, respectively. Cow dung was applied @ 500 g pot $^{-1}$ at the pot soil. The entire amount of TSP, gypsum, zinc sulphate and boric acid were applied during in the soil before potting. Two- third of urea and half of MP were applied in the final preparation of pot land (BARI, 2015).

3.10 Intercultural operations

After the germination of seeds, various intercultural operations such as irrigation, weeding, top dressing of fertilizer and plant protection measures were accomplished for better growth and development of the wheat seedlings.

3.10.1 Irrigation

Light watering was provided with water cane immediately after sowing of seeds and this technique of irrigation was used as required. The amount of irrigation water was limited up to that quantity which does not leached out through the bottom. The water was deposited on the earthen plate which was further poured into the pot again for maintaining the arsenic toxicity level as treatment.

3.10.2 Thinning

Thinning was done as required and finally eight plants kept in each pot.

3.10.3 Weeding

Weeding was done whenever it was necessary, mostly in vegetative stage.

3.10.4 Top dressing

After basal dose, the remaining dose of urea and MP was top-dressed in tillering stage and was applied on both sides of seedlings rows in the soil.

Plant Protection Measures

Marshal 20 EC was applied @ 2ml/ L water against jab insect. Melathion 57 EC and Dursban 20 EC were applied @ 2 ml/ L of water against the cutworm and aphid when needed.

3.11 Harvesting

Maturity of crop was determined when 90% of the spike became golden yellow in color. Five plants per pot were preselected randomly from which different growth and yield attributes data were collected and then each pot was harvested separately and bundled, properly tagged and then brought to the threshing floor for recording grain yield. The grains were cleaned and sun dried to a moisture content of 12%.

3.12 Data Collection

Experimental data were recorded from 60 days of sowing and continued up to harvest. The following data were recorded during the experimentation.

A. Morphological characters

1. Plant height (cm)
2. Number of leaves plant $^{-1}$
3. Number of tillers plant^{-1}
4. Number of effective tillers plant^{-1}

B. Physiological characters

5. SPAD value
6. Membrane stability (%)

C. Yield contributing and yield characters

7. Length of spike (cm)
8. Number of spikelet spike^{-1}
9. Number of effective spikelet spike^{-1}
10. Number of grains plant^{-1}
11. Hundred grains weight (g)
12. Grain yield (g pot^{-1})
13. Mitigation (%)

3.13 Detailed Procedures of Recording Data

A brief outline of the data recording procedure followed during the study is given below:

A. Morphological characters

1. Plant height (cm)

Plant height was measured at 60 days after sowing (DAS) and at harvest. The height of the plant was determined by measuring the distance from the soil surface to the tip of the leaf. The collected data were finally averaged.

2. Number of leaves plant^{-1}

Leaf number was counted at 60 DAS. The number of leaves plant^{-1} was counted five plants of each pot and then averaged.

3. Number of tillers plant^{-1}

Number of tillers plant^{-1} were counted at 60 DAS from five plants of each pot and then averaged.

4. Number of effective tillers plant^{-1}

Number of effective tillers plant^{-1} were counted at harvest from five plants of each pot and then averaged.

B. Physiological characters

5. SPAD value

Leaf chlorophyll content was analyzed as measured in SPAD value using a hand-held SPAD meter (CCM-200, Opti-Science, USA). At each evaluation the value was measure 5 times from five leaves at different positions plant^{-1} and the average was used for analysis.

6. Membrane stability (%)

The plasma membrane intactness was estimated through the leakage of electrolytes, described by Sun et al. (2006). Fresh leaves (0.30 g) were placed in tubes; containing 30 ml distilled water and kept for 2 h in water bath at 30 °C for measuring the initial conductivity (EC_1). The final electrolyte conductivity (EC_2) was measured after boiling the plant samples for 15 min. The leakage percentage was calculated as (EC_1/EC_2) x 100 %.

C. Yield contributing and yield characters

7. Length of spike (cm)

Length of spike was measured at harvest from five plants of each pot and then averaged.

8. Number of spikelet spike^{-1}

Total number of spikelet in a spike was counted. It included both sterile and non-sterile spikelet.

9. Number of effective spikelet spike^{-1}

Number of effective spikelet spike^{-1} was counted from sterile spikelet of spike in each plant. Total five plants are counted in each pot and then averaged.

10. Number of grains spike^{-1}

Number of grains spike^{-1} was counted from sterile spike of five plants in each pot and then averaged.

11. Hundred grains weight (g)

One hundred grains were counted randomly from the total cleaned harvested grains of each individual pot and then weighed and recorded which was expressed in grams.

12. Grain yield (g pot^{-1})

Grain yield pot^{-1} of wheat was calculated by counting the whole grains in the pot and then weighted and recorded which was expressed in grams.

13. Mitigation (%)

Mitigation percent was measured by the following formula:

$$\text{Mitigation (\%)} = \frac{\text{Yield value of treatment combination}}{\text{Yield value of control treatment}} \times 100$$

3.14 Statistical Analysis

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

CHAPTER FOUR

RESULTS AND DISCUSSIONS

CHAPTER 4
RESULTS AND DISCUSSION

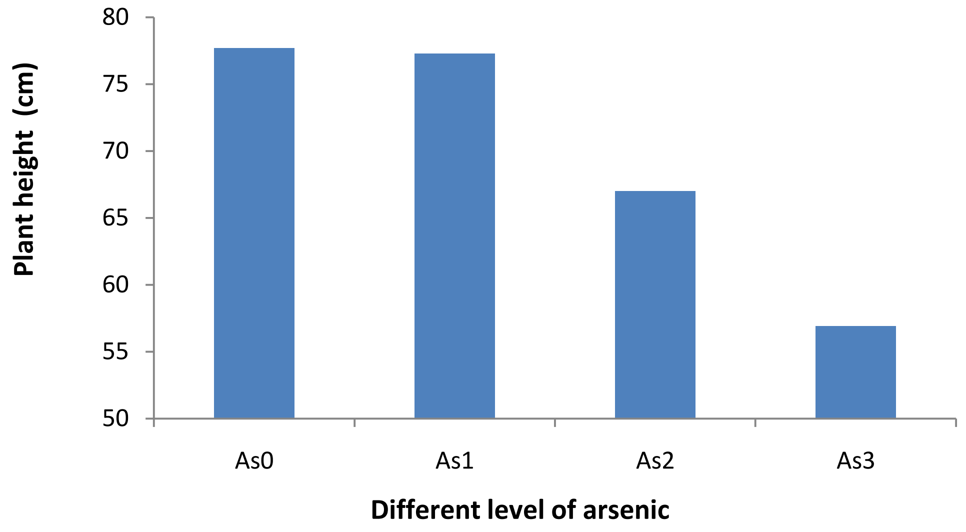
The results obtained with different level of arsenic (As) and sodium nitroprusside (SNP) and their combinations are presented and discussed in this chapter. Data on morpho-physiological parameters, yield contributing characters and grain yield of wheat have been presented in both Tables and Figures and analyzes of variance and corresponding degrees of freedom have been shown in Appendices.

4.1 Plant height

4.1.1 Effect of arsenic

4.1.2

It is well known that plant height is the most common promising characteristic of plant to analyze its growth. In this study, I investigated the adverse effect of arsenic (As) on change of plant height (cm) of wheat. The experimental results showed that As significantly reduced the plant height (Figure 1 and Appendix III). I measured plant height at 60 days after sowing (DAS). The highest plant height 77.67 cm was found from As_0 (control) which was statistically similar as 77.29 cm found from As_1 or 30 mg kg^{-1} soil. The lowest plant height 56.90 cm was observed with As_3 level or soil contaminated with 90 mg As kg^{-1} soil. These results are agreed with the findings of Chen and Liu, (1993); Liu *et al.* (2012) who reported that plant height of wheat did not affect with lower concentration of As but higher concentration of As affect to increase the plant height in cereals including maize. In addition, Kang *et al*. (1996) reported that Arsenic generally provides a slow growth and development of cells by uptaking As through roots to stem, leaves and grain and suggesting that root absorbed more As than shoot. All together, these results suggest that As impede plant height of wheat as other cereals.

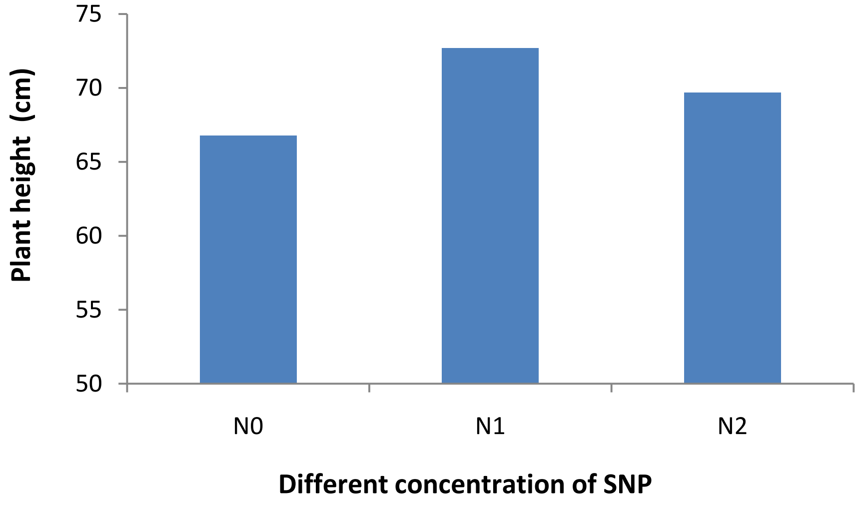


As_0 = Control, As_1 = 30 mg As kg^{-1} soil, As_2 = 60 mg As kg^{-1} soil, As_3 = 90 mg As kg^{-1} soil

Figure 1: Effect of different level of arsenic (As) on plant height of wheat ($Lsd_{0.05}$ = 0.63) at 60 DAS

4.1.3 Effect of SNP

It has been reported that nitric oxide (NO) is an oxidizing agent which have many functions in plant thus regulates plant growth and generates defense to both abiotic and biotic stresses. Since NO is a small molecule which crosses cell membrane and acting as an important signaling messenger (Seabra*et al.*, 2013). However, so far I know little study has conducted whether the role of NO on changes in plant height of wheat under our environmental conditions. In this study, I used sodium nitroprusside (SNP) as a source of NO donor. These experimental results showed significant difference to increase the plant height with SNP (Figure 2 and Appendix III).The highest plant height 72.69 cm was observed from N_1 or 0.1 mM SNP at 60 (DAS) whereas lowest value was 66.80 cm from N_0 or zero SNP at the same DAS. The higher concentration of SNP 0.2 mM failed to increase plant height as 0.1 mM SNP. Numerous previous reports showed that exogenous NO improve the plant physiology by enhancing the activities of antioxidant enzymes such as superoxide dismutase [SOD], peroxidase [POD], and catalase [CAT] and levels of soluble proteins and proline, which ultimately increased plant growth as shoot height in wheat (Hasanuzzaman *et al*., 2011, 2012; Kausar *et al.*, 2013). Taken together, these results indicate that NO increase plant height of wheat.



N_0 = 0 mM SNP (control), N_1 = 0.1 mM SNP, N_2 = 0.2 mM SNP

Figure 2: Effect of different concentration of sodium nitroprusside (SNP) on plant height of wheat ($Lsd_{0.05}$ =0.54) at 60 DAS

4.1.4 Interaction effect of As and SNP

In this experiment, I used four different level of As and three concentration of NO. To my knowledge, little study has conducted on the mitigation of As toxicity in wheat with NO. The NO reduces As toxicity in wheat in view of plant height. Interaction effect of As and SNP showed significant variation on plant height of wheat at 60 DAS (Table 1 and Appendix III). At 60 DAS, the highest plant height 80.60 cm was observed from the As_0N_1 treatment which was statistically similar with As_1N_1 (80.38 cm) and the lowest 55.08 cm plant height was observed from As_3N_0 treatment combination. These results are agreed with Singh *et al.* (2016) who reported that exogenous NO supplementation prevented the deleterious effects of As in plant growth through the reduction of As accumulation in the tissues that could be advantageous against As toxicity and could confer the tolerance to As stress in rice. The positive effect of exogenous application of SNP on the growth parameters has also been reported under stress conditions (Wang *et al.*, 2011; Esim and Atıcı, 2013; Siddiqui *et al.*, 2013). All together, these results indicate that 0.1 mM SNP increases maximum plant height with zero As contamination of wheat.

Table 1: Interaction effect of different level of arsenic (As) and sodium nitroprusside (SNP) on plant height (cm) of wheat at 60 DAS

Treatment	Plant height (cm)
As_0N_0	75.10 c
As_0N_1	80.60 a
As_0N_2	77.30 b
As_1N_0	74.40 c
As_1N_1	80.38 a
As_1N_2	77.10 b
As_2N_0	62.63 f
As_2N_1	70.97 d
As_2N_2	67.43 e
As_3N_0	55.08 i
As_3N_1	58.80 g
As_3N_2	56.83 h
$Lsd_{0.05}$	1.08
Significant level	**
CV (%)	6.08

As_0 = Control, As_1 = 30 mg As kg^{-1} soil, As_2 = 60 mg As kg^{-1} soil, As_3 = 90 mg As kg^{-1} soil

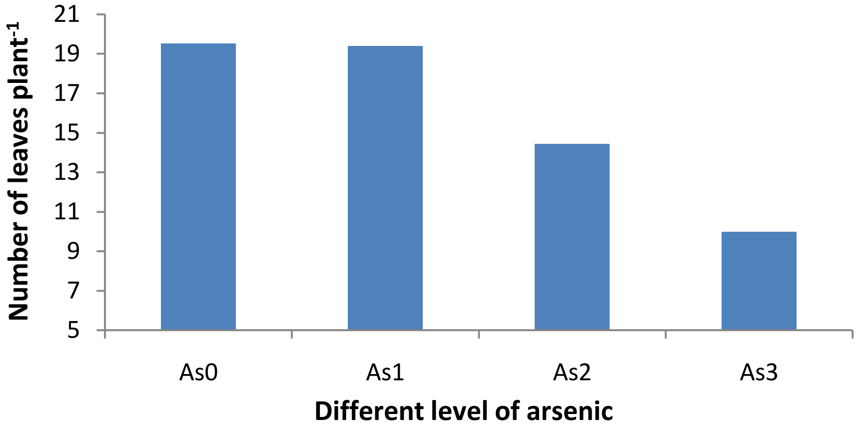
N_0 = 0 mM SNP (control), N_1 = 0.1 mM SNP, N_2 = 0.2 mM SNP

** Significant at 1% level of probability

4.2 Number of leaves plant^{-1}

4.2.1 Effects of arsenic

The leaf number is a fundamental morphological character for plant growth and development as leaf is the main photosynthetic organ. To investigate the effects of different concentration of As toxicity of wheat on changes in the number of leaves plant^{-1} up to 60 DAS, the number of leaves was counted. In this experiment, different concentration of As showed a significant reduction on the formation of leaves plant^{-1} of wheat (Figure 3 and Appendix III). The highest number of leaves plant^{-1} (19.52) was found from As_o or control which was statistically similar to the result of 19.39 that found from the As_1 or 30 mg As kg^{-1} soil, where as the lowest number of leaves plant^{-1} (9.98) was observed at As_3 or 90 mg As kg^{-1} soil. These results are co-related with Das *et al.* (2014) who reported that the As accumulation by the plant parts generally followed the order: root>stem>leaf>economic produce. The As toxicity symptoms in leaf and root were observed with treatment above 40 mg kg^{-1} As, while the symptoms were apparent in economic produce above 60 mg kg^{-1}. Taken together, these results suggest that number of leaves is declining gradually with the increases of As concentration of wheat and also finds that 30 mg arsenic kg^{-1} soil has no detrimental effects on formation on leaves which is consistent with the plant height of wheat (Figure 1).

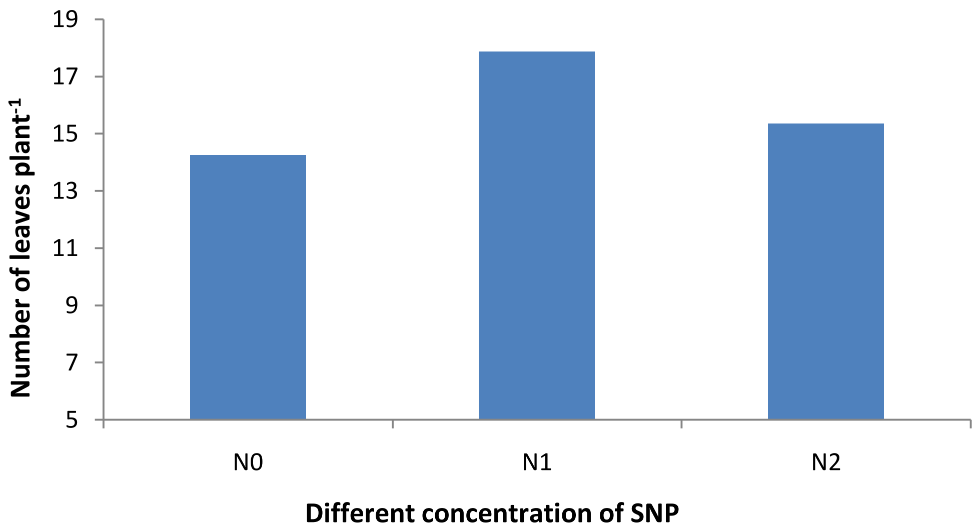


As_0 = Control, As_1 = 30 mg As kg^{-1} soil, As_2 = 60 mg As kg^{-1} soil, As_3 = 90 mg As kg^{-1} soil

Figure 3: Effect of different level of arsenic (As) on number of leaves plant^{-1} of wheat ($Lsd_{0.05}$ = 0.39) at 60 DAS

4.2.2 Effect of SNP

It has been reported that NO is a highly reactive, membrane-permeable free radical and a highly toxic compound and plays a vital role in diverse physiological functions in plants like regulation of plant metabolism and senescence (Guo and Crawford, 2005). It showed an effective variation in the number of leaves plant^{-1}. This experimental result showed significant difference to increase the number of leaves plant^{-1} with SNP (Figure 4 and Appendix III). The highest leaves number (17.17) were observed from N_1 or 0.1 mM SNP at 60 days after sowing (DAS). And lowest value was 14.26 from N_0 or zero SNP at the same DAS. Jaiswal and Srivastava (2015) mentioned that SNP as a donor of NO in the flooding water alleviated the stress caused reduction in leaf number, green leaf number and leaf area of maize plant. All together, these results indicate that NO improves the morphological characters including number of leaves which is consistent with the plant height of wheat (Fig 2).



N_0 = 0 mM SNP (control), N_1 = 0.1 mM SNP, N_2 = 0.2 mM SNP

Figure 4: Effect of different concentration of sodium nitroprusside (SNP) on number of leaves plant^{-1} of wheat ($Lsd_{0.05}$ = 0.34) at 60 DAS

4.2.3 Interaction effect of As and SNP

The results of the present study showed that the interaction effect of As and SNP showed a significant variation on leaves number plant^{-1} of wheat at 60 DAS (Table 2 and Appendix III). The highest number of leaves plant^{-1} (22.19) was observed from the As_0N_1 treatment which was statistically similar to As_1N_1 (21.92), whereas the lowest number of leaves plant^{-1} (9.08) was observed from the As_3N_0 treatment which was statistically similar to As_3N_2 (9.15) treatment. These results are supported by Wang *et al.* (2011); Jaiswal and Srivastava (2015) who stated that exogenous NO supplementation prevented deleterious effects of stress condition of rice and maize. All together, these results indicate that 0.1 mM SNP increases maximum number of leaves plant^{-1} in As contaminated soil which is also similar with the other result of this study including plant height (Table 1).

Table 2: Interaction effect of different level of arsenic (As) and sodium nitroprusside (SNP) on number of leaves plant^{-1} of wheat at 60 DAS

Treatment	Number of leaves plant^{-1}
As_0N_0	17.25 c
As_0N_1	22.19 a
As_0N_2	19.14 b
As_1N_0	17.20 c
As_1N_1	21.92 a
As_1N_2	19.05 b
As_2N_0	13.52 e
As_2N_1	15.68 d
As_2N_2	14.13 e
As_3N_0	9.08 g
As_3N_1	11.70 f
As_3N_2	9.15 g
$Lsd_{0.05}$	0.67
Significant level	*
CV (%)	3.94

As_0 = Control, As_1 = 30 mg As kg^{-1} soil, As_2 = 60 mg As kg^{-1} soil, As_3 = 90 mg As kg^{-1} soil

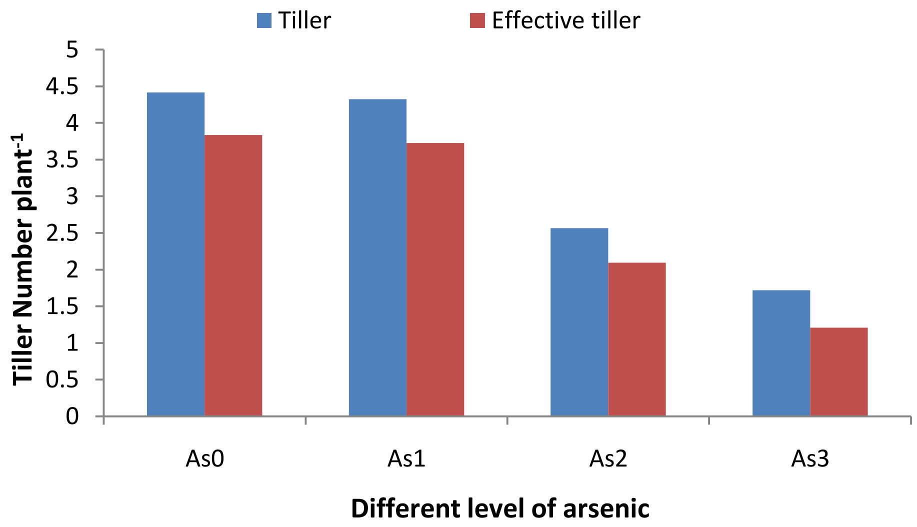
N_0 = 0 mM SNP (control), N_1 = 0.1 mM SNP, N_2 = 0.2 mM SNP

** Significant at 1% level of probability

4.3 Number of tillers plant^{-1}

4.3.1 Effect of arsenic

Arsenic (As) is one of the most harmful toxic metals which is wide-spread in nature. Application of different concentrations of As showed significant variation on number of tillers plant^{-1} at 60 DAS (Figure 5 and Appendix III). At 60 DAS, the highest number of tillers plant^{-1} (4.42) was found in As_0 or 0 mg As kg^{-1} soil which was statistically similar (4.33) with As_1 or 30 mg As kg^{-1} soil. On the other hand, while the lowest number of tillers plant^{-1} (1.72) in As_3 or 90 mg As kg^{-1} soil. Liu *et al.* (2012) confirmed that As decreased number of tiller plant^{-1} in higher concentration. Other researchers also reported that when plants were exposed to excess As either in soil or in solution culture, they exhibited toxicity symptoms such as: inhibition of seed germination, decrease in plant height, depress in tillering, reduction in root growth, decrease in shoot growth, lower fruit and grain yield, and sometimes, leads to death (Marin *et al*., 1992; Carbonell *et al*., 1995; Abedin *et al.*, 2002a, 2002b; Johan *et al.*, 2003; Das *et al.*, 2014). Although As is not directly involved inspecific metabolic reactions, and is not connected with enzyme system, the higher concentration of As in soil or in nutrient solutions is taken up by the phosphate transport system in the plants thus interferes with metabolic process and inhibits plant growth and finally led to death (Stoeva *et al.*,2005). Taken together, these results suggest that high concentration of As reduces tiller number of wheat as other cereals crops including maize and rice.

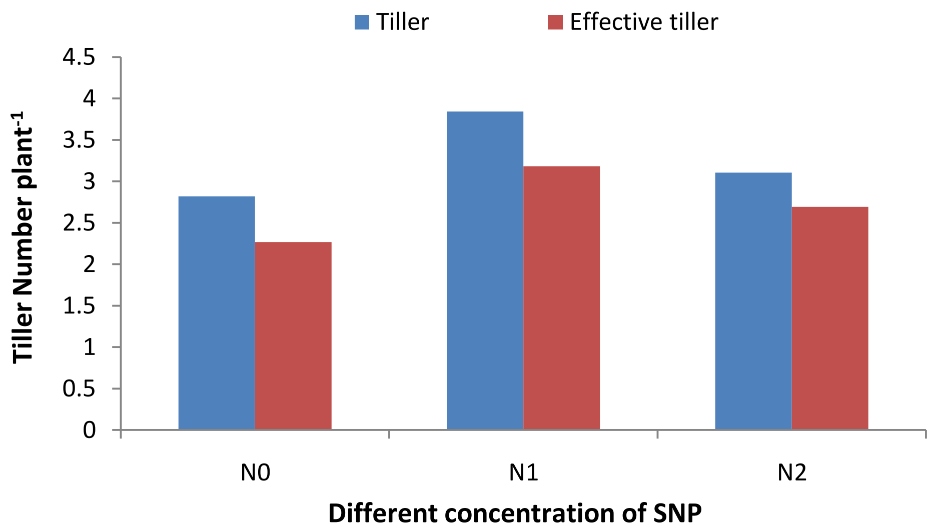


As_0 = Control, As_1 = 30 mg As kg^{-1} soil, As_2 = 60 mg As kg^{-1} soil, As_3 = 90 mg As kg^{-1} soil

Figure 5: Effect of different level of arsenic (As) on number of tillers plant^{-1} and number of effective tillers plant^{-1} of wheat ($Lsd_{0.05}$ = 0.16 and $Lsd_{0.05}$ = 1.55 for tiller and effective tiller, respectively) at 60 DAS

4.3.2 Effect of SNP

It has been reported that NO has emerged as an important signaling molecule and numerous reports indicated that the application of exogenous NO donors confers tolerance to various abiotic stresses (Hasanuzzaman *et al*. 2010, 2011). In this experiment, application of SNP showed significant reduction in number of tiller plant^{-1} of wheat at 60 DAS (Table 6 and Appendix III). The maximum number of tiller plant^{-1} (3.84) was found from N_1 or 0.1 mM SNP whereas the minimum number of tiller plant^{-1} (2.82) was observed from A_0 or control. These results are supported by Habib *et al.* (2016) who reported that number of tillers was also significantly reduced due to rooting medium salinity in all rice cultivars. A significant ameliorative effect of seed priming with SNP was found on number of tillers under salt stress in all rice cultivars and both SNP levels (0.1 and 0.2 mM) were found equally effective in this regard. Taken together, these results suggest that NO increases tiller number of wheat.



N_0 = 0 mM SNP (control), N_1 = 0.1 mM SNP, N_2 = 0.2 mM SNP

Figure 6: Effect of different concentration of sodium nitroprusside (SNP) on number of tillers plant^{-1} and number of effective tillers plant^{-1} of wheat ($Lsd_{0.05}$ = 0.14 and $Lsd_{0.05}$ = 0.09 for tiller and effective tiller, respectively) at 60 DAS

4.3.3 Interaction effect of As and SNP

Interaction of As and SNP showed significant variation on number of tillers plant^{-1} of wheat at 60 DAS (Table 3 and Appendix III). The combination of As_0N_1 gave the maximum number of tiller plant^{-1} 5.25 which was statistically similar to As_1N_1 (5.10) whereas the minimum number of tiller plant^{-1}`(1.50) was recorded from As_3N_0 which is statistically similar with As_3N_2 (1.65). These results are showed same evaluation by Habib *et al.* (2016) in salt stressed condition with SNP. These factors indicated that maximum tiller produced in 0.1mM SNP condition with zero As. 30 mg As kg^{-1} soil provided same as control condition. And lowest tiller number observed from 90 mg As kg^{-1} soil with zero SNP as well as 0.2 mM SNP. However, it is obvious that NO emerges number of tiller in As contamination in view of salt stress.

Table 3: Interaction effect of different level of arsenic (As) and sodium nitroprusside (SNP) on number of tillers plant^{-1} and number of effective tillers plant^{-1} of wheat at 60 DAS

Treatment	Number of tillers plant^{-1}	Number of effective tillers plant^{-1}
As_0N_0	3.83 c	3.20 c
As_0N_1	5.25 a	4.50 a
As_0N_2	4.18 b	3.80 b
As_1N_0	3.75 c	3.13 c
As_1N_1	5.10 a	4.33 a
As_1N_2	4.13 b	3.73 b
As_2N_0	2.20 ef	1.75 f
As_2N_1	3.03 d	2.51 d
As_2N_2	2.48 e	2.03 e
As_3N_0	1.50 g	1.00 h
As_3N_1	2.00 f	1.40 g
As_3N_2	1.65 g	1.23 g
$Lsd_{0.05}$	0.28	0.18
Significant level	**	**
CV (%)	5.89	4.48

As_0 = Control, As_1 = 30 mg As kg^{-1} soil, As_2 = 60 mg As kg^{-1} soil, As_3 = 90 mg As kg^{-1} soil

N_0 = 0 mM SNP (control), N_1 = 0.1 mM SNP, N_2 = 0.2 mM SNP

** Significant at 1% level of probability

4.4 Number of effective tillers plant^{-1}

4.4.1 Effect of arsenic

Application of different concentration of As showed significant variation on number of effective tillers plant^{-1} at 60 DAS (Figure 5 and Appendix III). At 60 DAS the highest number of effective tillers plant^{-1} (3.83) was found in As_0 or 0 mg As kg^{-1} soil which was statistically similar (3.73) with As_1 or 30 mg As kg^{-1} soil. On the other hand, while the lowest number of effective tillers plant^{-1} (1.21) in As_3 or 90 mg As kg^{-1} soil. These results agreed with Liu *et al.* (2009) who stated that the amount of roxarsone (an organic- arsenic compound used as additive of poultry feed) added significantly reduced effective tiller number and As-induced reduction was most pronounced in tiller number plant^{-1}. Taken together, these results suggest that As decreases effective tiller number which is consistent with total tiller number of wheat (Figure 5).

4.4.2 Effect of SNP

Number of effective tillers plant^{-1} was significantly influenced by different concentrations of SNP of wheat at 60 DAS (Figure 6 and Appendix III). The maximum number of effective tiller plant^{-1} 3.18 was found from the N_1 and the lowest number of effective tiller plant^{-1} 2.27 was found from N_0 level SNP. However, so far I know no study has conducted whether the role of NO on changes in effective tiller number under environmental condition of Bangladesh. Taken together, these results suggest that 0.1 mM SNP increases maximum tiller number plant^{-1} and zero SNP showed lowest number which is consistent with total tiller number of wheat (Figure 6).

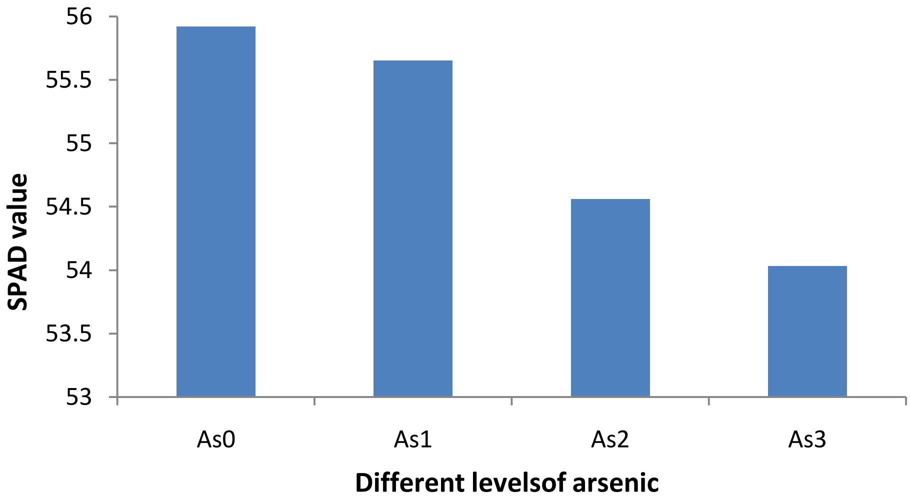
4.4.3 Interaction effect of arsenic and SNP

The results of the present study showed that the interaction effect of As and SNP showed a significant variation on number of effective tillers plant^{-1} of wheat at 60 DAS (Table 3 and Appendix III). The highest number of effective tillers plant^{-1} (4.50) was observed from the As_0N_1 treatment which was statistically similar to As_1N_1 (4.33), whereas the lowest value (1.00) was observed from the As_3N_0 treatment. These results co-related with number of tiller (Figure 3). Esim *et al.* (2014) reported that stress treatment increased both the activities of antioxidant enzymes and the levels of MDA, H_2O_2 and O^{-2}. Similarly, NO treatment enhanced SOD, POX and CAT activities under stress condition, whereas it decreased H_2O_2 and O^{-2} contents as well as MDA level. These indicated that antioxidant improved plant growth and also affected on effective tiller number plant^{-1} positively. Taken together, these results indicate that 0.1 mM SNP with zero As imputes maximum effective tiller as well as 0.1 mM SNP with 30 mg As kg^{-1} soil and minimum effective tillers observes in 90 mg As kg^{-1} soil with zero SNP.

4.5 SPAD value

4.5.1 Effect of arsenic

Photosynthesis is the most important biochemical event on earth. It serves as the world's largest solar battery. Photosynthesis converts massive amount of sunlight into electrical and then chemical energy (Hall and Rao, 1999). The effect of As, one of the fatal phytotoxic tress elements, on chlorophyll content in wheat leaf was studied and showed statistically non- significant variations at 60 DAS (Figure 7 and Appendix IV). But numerically the highest SPAD value 55.92 was found from $As_{0,}$ whereas the lowest SPAD value 54.03 was found from As_3 or addition of 90 mg As kg^{-1} soil. These results are agreed with Kausar *et al.* (2013) and Shahbaz *et al*. (2011) who observed a non-significant effect of saline stress on chlorophyll contents in wheat and sunflower respectively. Rahman *et al*. (2015) reported that As-induced stress decreased chl content in the rice seedlings. Chlorophyll content decreased with increasing As concentration, which was disagreed with this result. The impacts of upward doses of As, cadmium (Cd) and lead (Pb) on quality of cultivated biomass wheat (*Triticum aestivum* L.) during 21 days and production of chlorophyll a and chlorophyll b positively decreased (Ducsay, 2000). All together, these results suggest that As does not show any detrimental effect on SPAD value of wheat.

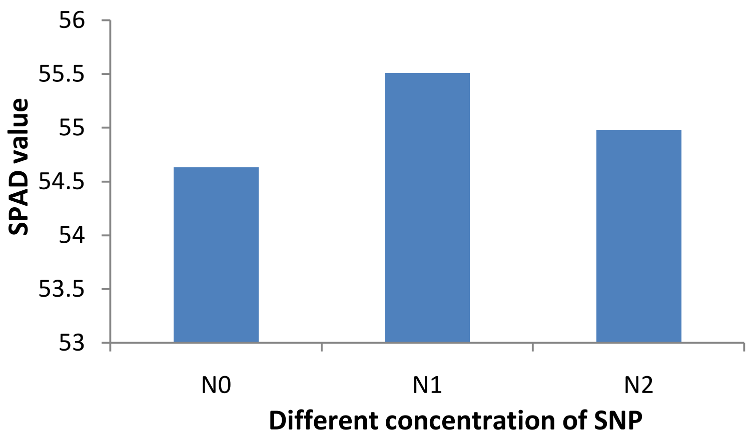


As_0 = Control, As_1 = 30 mg As kg^{-1} soil, As_2 = 60 mg As kg^{-1} soil, As_3 = 90 mg As kg^{-1} soil

Figure 7: Effect of different level of arsenic (As) on SPAD value of wheat ($Lsd_{0.05}$ = NS)

4.5.2 Effect of SNP

The role of NO in plants is highly diverse because of its involvement in different physiological mechanisms. Foliar spray of NO has been reported to improve growth. It also enhances chlorophyll contents (Ruan *et al.,* 2004) and its exogenous application protects plants from oxidative damage by enhancing antioxidant system capacity (Tuncz- Ozdemir *et al.,* 2009). In this experiment, SPAD value of wheat on the application of different concentrations of SNP was observed statistically insignificant at 60 DAS (Figure 8 and Appendix IV). But numerically the highest value (55.51) was found from N_1 or 0.1 mM SNP, whereas the lowest value (54.63) was identified from N_0 or zero SNP concentration. Although NO application was found effective in enhancing chlorophyll contents in wheat plants (Ruan *et al.*, 2004), such a prominent effect was not observed in my study. Jaiswal and Srivastava (2015) also reported that SNP was found effective for alleviating chlorophyll content in stomatal conductance in HUZM-265 and HUZM-55, respectively. This might have been due to many factors, such as the type of wheat cultivar, NO concentration, and time of application used (Wu *et al.*, 2010). Altogether, these results indicate that NO has no effects on changes in SPAD value of wheat.



N_0 = 0 mM SNP (control), N_1 = 0.1 mM SNP, N_2 = 0.2 mM SNP

Figure 8: Effect of different concentration of sodium nitroprusside (SNP) on SPAD value of wheat ($Lsd_{0.05}$ = NS)

4.5.3 Interaction effect of As and SNP

Interaction effect of As and SNP on SPAD value showed statistically insignificant variations at harvest (Table 4 and Appendix IV). But numerically the maximum SPAD value 56.63 was observed from As_0N_1 treatment and the minimum SPAD value 53.55 was recorded from As_3N_0 treatment. These results are agreed by Kausar *et al.* (2013) and Shahbaz *et al.* (2011) in stressed condition. In contrast, Shukla *et al.* (2015) who observed that the effects of NO mitigated As toxicity in in seedlings of barley (*Hordeum vulgare* L.). Numerous previous reports also stated that exogenous application of NO has been reported to effectively reduce salt-induced ion toxicity by protecting the membrane of the cell organelle containing chlorophyll, as earlier observed in many crops like rice, lupin, and maize (Uchida *et al.*, 2002; Wu *et al.*, 2010). As toxicity decreased the chlorophyll content whereas, As toxicity enhanced the MDA content and proline content in a dose dependent manner. Pretreatment of NO results in the enhanced expression of alternative oxidase which showed the adaptation of alternative pathway during the arsenic (Hasanuzzaman and Fujita, 2013). Taken together, these results indicate that NO does not affect chlorophyll content in wheat measured in SPAD value under various level of As toxicity.

Table 4: Interaction effect of different concentration of arsenic (As) and sodium nitroprusside (SNP) on SPAD value of wheat

Treatment	SPAD value
As_0N_0	55.53
As_0N_1	56.63
As_0N_2	55.63
As_1N_0	55.35
As_1N_1	56.05
As_1N_2	55.55
As_2N_0	54.10
As_2N_1	55.05
As_2N_2	54.53
As_3N_0	53.55
As_3N_1	54.30
As_3N_2	54.22
$Lsd_{0.05}$	NS
Significant level	---
CV (%)	16.97

As_0 = Control, As_1 = 30 mg As kg^{-1} soil, As_2 = 60 mg As kg^{-1} soil, As_3 = 90 mg As kg^{-1} soil

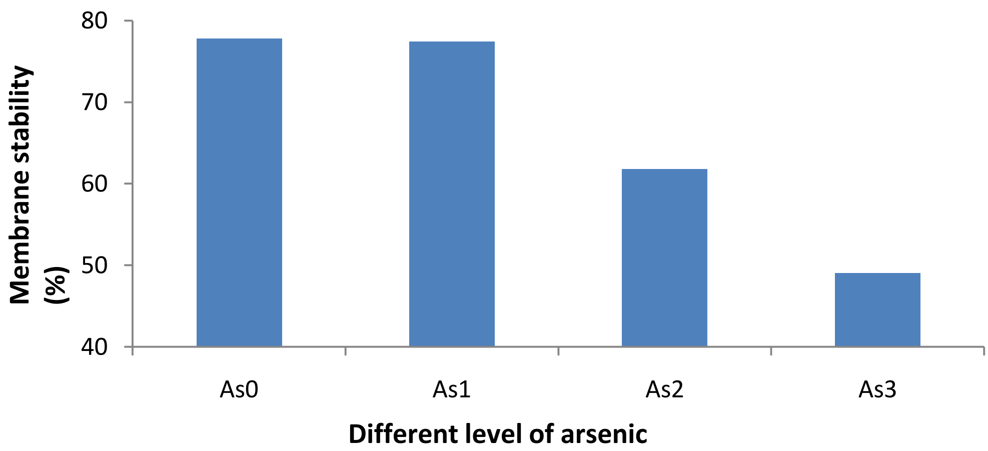
N_0 = 0 mM SNP (control), N_1 = 0.1 mM SNP, N_2 = 0.2 mM SNP

NS = Not significant

4.6 Membrane stability (%)

4.6.1 Effect of arsenic

It has been reported that As disrupts the biochemical function of cells reacting with proteins and enzymes, which severely hampers photosynthesis, transpiration, respiration, plant metabolism, and other physiological activities and finally arrests plant growth (Meharg and Hartley-Whitaker, 2002). In this study, As showed significant effect on the membrane stability (%) at higher (90 mg As kg^{-1} soil) concentration (Figure 9 and appendix IV). A linear decrease occurred in the membrane stability (%) with the increase in the concentration of As. The maximum membrane stability 77.81 % was recorded from As_0 treatment which was statistically similar to As_1 (77.44 %) treatment or 30 mg As kg^{-1} soil, whereas the minimum 49.08 % was observed from As_3 or addition of 90 mg As kg^{-1} soil. These results are agreed by Nguyen *et al*. (2003) who reported that the electron transport processes are impeded developing toxic systems. Several toxic reactive oxygen species (ROS) are generated in the cell wall region as well as inside the cell during the process, which affects membrane permeability. So, these results suggest that at lower concentration of As (30 mg) has certain no effect in the percentage of membrane stability of wheat while at higher concentration (90 mg) shows maximum detrimental effect of wheat.

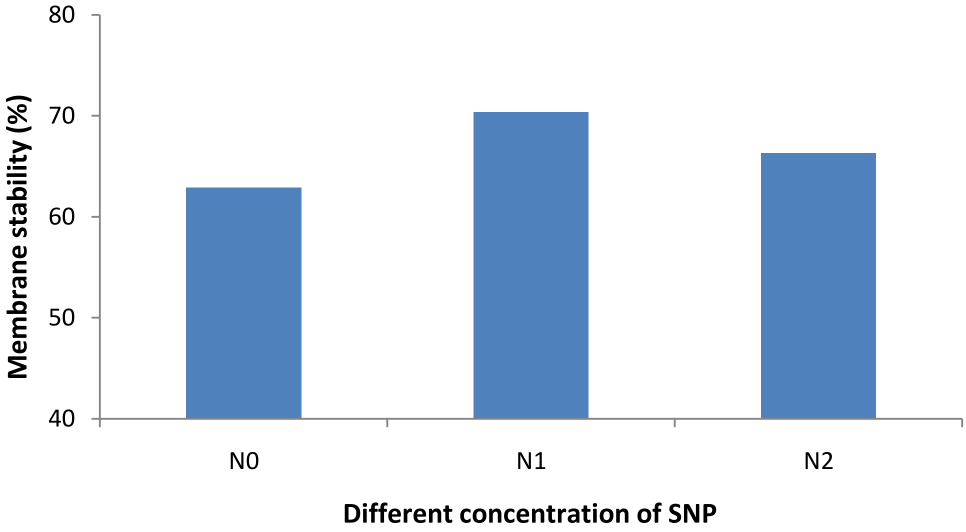


As_0 = Control, As_1 = 30 mg As kg^{-1} soil, As_2 = 60 mg As kg^{-1} soil, As_3 = 90 mg As kg^{-1} soil

Figure 9: Effect of different level of arsenic (As) on membrane stability (%) of wheat ($Lsd_{0.05}$ = 0.45)

4.6.2 Effect of SNP

The percentage of membrane stability was significantly affected by different concentration of SNP (Figure 10 and Appendix IV). The highest membrane stability 70.40 % was observed from N_1 or 0.1 mM SNP whereas the lowest mitigation 62.91% was observed from N_0 or zero SNP. MDA is the product of biomembrane lipid peroxidation, its content in plants reflects the degree of membrane injury. Ion leakage ratio also reflects the degree of plant cell membrane injury under stress treatment (Pérez-Tornero *et al.*, 2009). The membrane stability percentage of wheat increased with increasing the application of SNP. Jaiswal and Srivastava (2015) reported that SNP as NO donor has mitigating role in combating cell membrane injury in roots of waterlogged plant. The increased NO, resulting from SNP application, improved the antioxidant capacity of maize plants against oxidative stress which increased electrolyte leakage (Esim and Atıcı, 2013). Others reports also reported that the protective effect of NO against abiotic stress is closely related to the NO-mediated reduction of ROS in plants (Hao and Zhang, 2010; Corpas *etal.*,2011). All together, these results indicate that NO increases membrane stability as well as electrolyte leakage is decreased.



N_0 = 0 mM SNP (control), N_1 = 0.1 mM SNP, N_2 = 0.2 mM SNP

Figure 10: Effect of different concentration of sodium nitroprusside (SNP) on membrane stability (%) of wheat ($Lsd_{0.05}$ = 0.39)

4.6.3 Interaction effect of As and SNP

Interaction effect of As and SNP on membrane stability (%) of wheat was showed a significant variation (Table 5 and Appendix IV). In this work, the highest membrane stability 80.69 % was observed from As_0N_1 which was statistically similar with As_1N_1 (80.15 %). The lowest membrane stability 45.50 % was recorded from As_3N_0 treatment. Wang *et al.* (2011) reported that the effects of SNP, a NO donor, on physiological characteristics of maize under stressed condition as well as it decreased ion leakage ratio. Pretreatment of SNP improved the membrane stability index significantly even at higher concentration (Shukla *et al.*, 2015). Other researchers also agreed this results and stated that metal toxicity can affect plasma membrane permeability and there is protective evidence to show that the NO donor protects plasma membrane integrity (Hsu and Kao, 2004; Wang *et al*., 2005; Hu *et al*., 2007; Bavita *et al.*, 2012). Taken together, these results indicate that 0.1 mM SNP provides maximum membrane stability (%) of leaf in zero As which similar to 30 mg As kg^{-1} soil of wheat.

Table 5: Interaction effect of different level of arsenic (As) and sodium nitroprusside (SNP) on membrane stability (%) of wheat

Treatment	Membrane stability (%)
As_0N_0	75.47 c
As_0N_1	80.69 a
As_0N_2	77.25 b
As_1N_0	75.15 c
As_1N_1	80.15 a
As_1N_2	77.03 b
As_2N_0	55.50 f
As_2N_1	68.50 d
As_2N_2	61.50 e
As_3N_0	45.50 i
As_3N_1	52.25 g
As_3N_2	49.50 h
$Lsd_{0.05}$	0.78
Significant level	**
CV (%)	4.81

As_0 = Control, As_1 = 30 mg As kg^{-1} soil, As_2 = 60 mg As kg^{-1} soil, As_3 = 90 mg As kg^{-1} soil

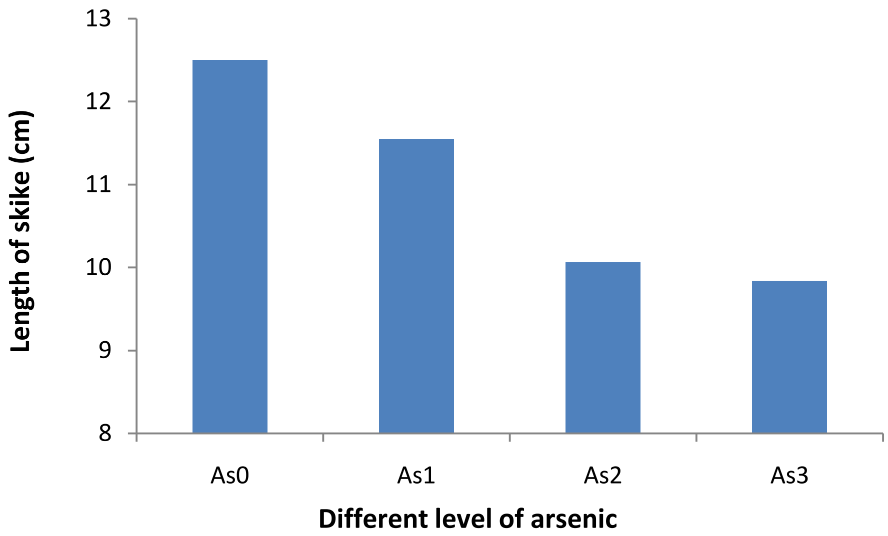
N_0 = 0 mM SNP (control), N_1 = 0.1 mM SNP, N_2 = 0.2 mM SNP

** Significant at 1% level of probability

4.7 Length of spike

4.7.1 Effect of arsenic

Length of spike is the primary condition of the yield stage. It indicates an assumption of how much grains are found. Application of different concentrations of As showed significant variation in length of spike under the present trial at harvest (Figure 11 and Appendix IV). The maximum length of spike (12.50 cm) was recorded in As_0 or control which was statistically similar with As_1 (11.55 cm) or 30 mg As kg^{-1} soil, whereas the minimum length (9.84 cm) found in As_3 or 90 mg As kg^{-1} soil which was statistically similar with As_2 (10.06 cm) or 60 mg As kg^{-1} soil. Liu *et al.* (2012) reported that exposed to high levels of As (≥80 mg/ kg soil) in wheat, the length of spike decreased significantly. Therefore, these results indicate that As reduces the morphological character as length of spike which is consistent with plant height of wheat (Figure 1).

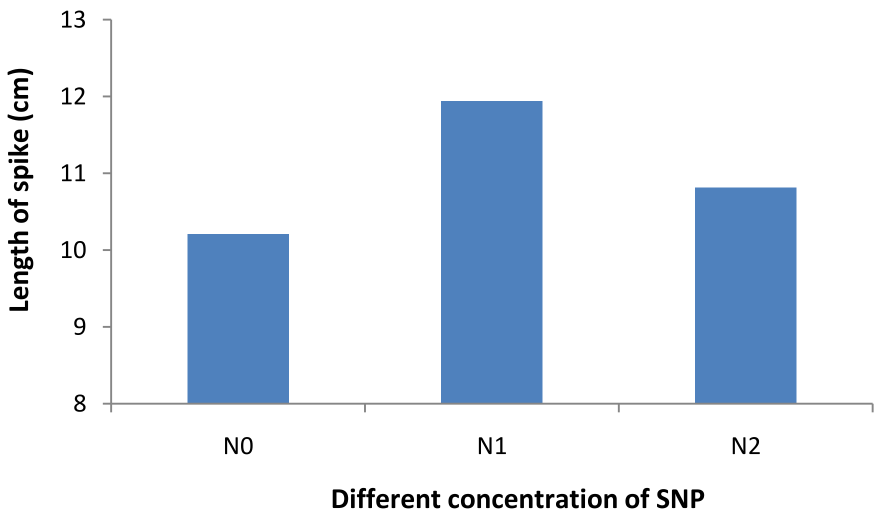


As_0 = Control, As_1 = 30 mg As kg^{-1} soil, As_2 = 60 mg As kg^{-1} soil, As_3 = 90 mg As kg^{-1} soil

Figure 11: Effect of different level of arsenic (As) on length of spike plant^{-1} of wheat ($Lsd_{0.05}$ = 0.96)

4.7.2 *Effect of SNP*

Different concentrations of SNP showed statistically significant variation in terms of length of spike at harvest (Figure 12 and Appendix IV). The maximum length of spike (11.9 cm) was recorded in N_1 or 0.1 mM SNP, whereas the minimum length of spike (10.21 cm) was found in N_0 (zero SNP). It has been reported that NO can neutralize heavy metal induce ROS in two ways, first being a free radical it can directly react with ROS and neutralize them and second being a signaling molecule, it may stimulate antioxidant system to abate oxidative stress (Lamattina *et al.*, 2003; Laspina *et al.*, 2005). So far, it contributed on increasing biomass as well as length of spike. Taken together, these results provide that SNP increases length of spike which is consistent with plant height of wheat (Figure 2).



N_0 = 0 mM SNP(control), N_1 = 0.1 mM SNP, N_2 = 0.2 mM SNP

Figure 12: Effect of different concentration of sodium nitroprusside (SNP) on length of spike plant of wheat ($Lsd_{0.05}$ = 0.29)

4.7.3 *Interaction effect of As and SNP*

Interaction effect of As and SNP showed statistically significant differences on length of spike at harvest (Table 6 and Appendix IV). The maximum length of spike (14.38 cm) was attained in As_0N_1 or 0 mg As kg^{-1} soil in 0.1 mM SNP and the minimum length of spike (9.56 cm) in As_3N_0 or 90 mg As kg^{-1} soil in zero SNP which was statistically similar to As_2N_0 (9.65 cm), As_3N_2 (9.87 cm), As_2N_2 (10.10 cm), As_3N_1 (10.11 cm) and As_2N_1 (10.43 cm) treatment combination. This result agreed with Jin *et al.*, (2010) who stated that exogenous application of SNP in rice provided tolerance against As toxicity and ameliorated the As-induced decrease in length. Furthermore, SNP reduced As-induced accumulation of malondialdehyde (MDA), superoxide ion (O^{-2}.), and H_2O_2 (Singh *et al*., 2009), and an increase in the activities of SOD, CAT and APX was recorded in the presence of SNP under As stress. All together, these results suggest that SNP increases the length of spike of wheat with or without As contaminations.

Table 6: Interaction effect of different level of arsenic (As) and sodium nitroprusside (SNP) on length of spike plant^{-1} of wheat

Treatment	**Length of spike plant^{-1}**
As_0N_0	10.98 de
As_0N_1	14.38 a
As_0N_2	12.15 c
As_1N_0	10.65 def
As_1N_1	12.82 b
As_1N_2	11.18 d
As_2N_0	9.65 h
As_2N_1	10.43 efg
As_2N_2	10.10 fgh
As_3N_0	9.56 h
As_3N_1	10.11 fgh
As_3N_2	9.87 gh
$Lsd_{0.05}$	0.58
Significant level	**
CV (%)	4.64

As_0 = Control, As_1 = 30 mg As kg^{-1} soil, As_2 = 60 mg As kg^{-1} soil, As_3 = 90 mg As kg^{-1} soil

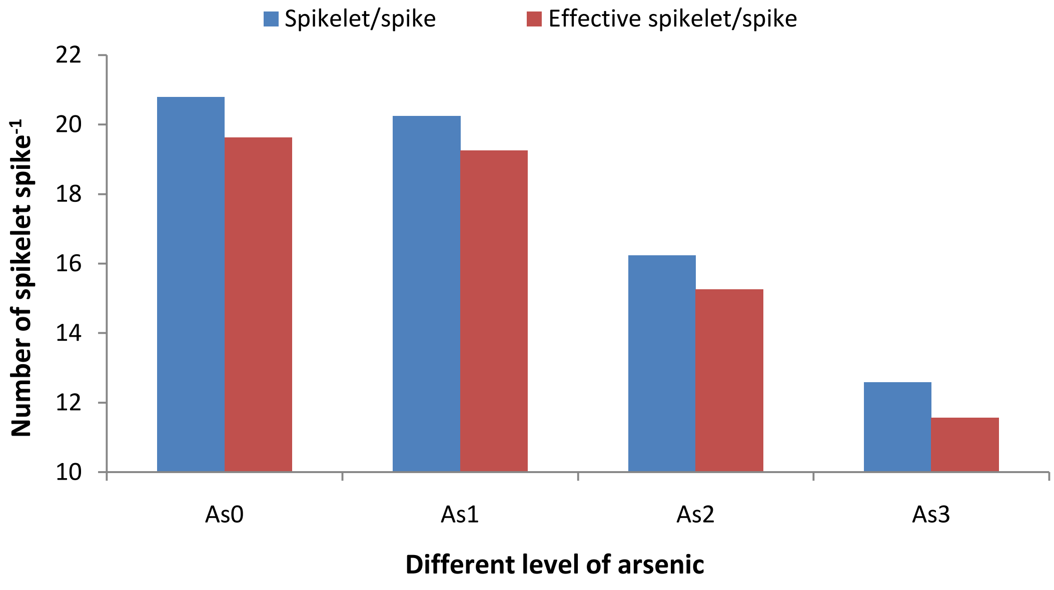
N_0 = 0 mM SNP (control), N_1 = 0.1 mM SNP, N_2 = 0.2 mM SNP

** Significant at 1% level of probability

4.8 Number of spikelet spike^{-1}

4.8.1 Effect of arsenic

High concentration of As in soils have been phytotoxic in plants: decreases in plant growth and fruit yields. Effect of As on the number of spikelet spike^{-1} of wheat is shown in Figure 13 and Appendix IV. It is noticed that the As stress provided significant variations in this experiment. The maximum number of spikelet spike^{-1} 20.79 was recorded from the As_0 treatment or control which was statistically similar to the As_1 (20.24) treatment or 30 mg As kg^{-1} soil and the lowest number of spikelet spike^{-1} 12.58 was observed from As_3 or addition of 90 mg As kg^{-1} soil. Zhang *et al.* (2009) reported that in the 100 mg As kg^{-1} soil treatment, the number of spikelet spike^{-1} was showed lower result than the control. However, such decreases seldom reached significant levels. This experiment showed that As had an adverse effect on spike number per plant, which resulted from less tillering and weak tillers (Figure 5). All together, these results suggest that the number of spikelet spike^{-1} of wheat decreases with increasing the As concentration.

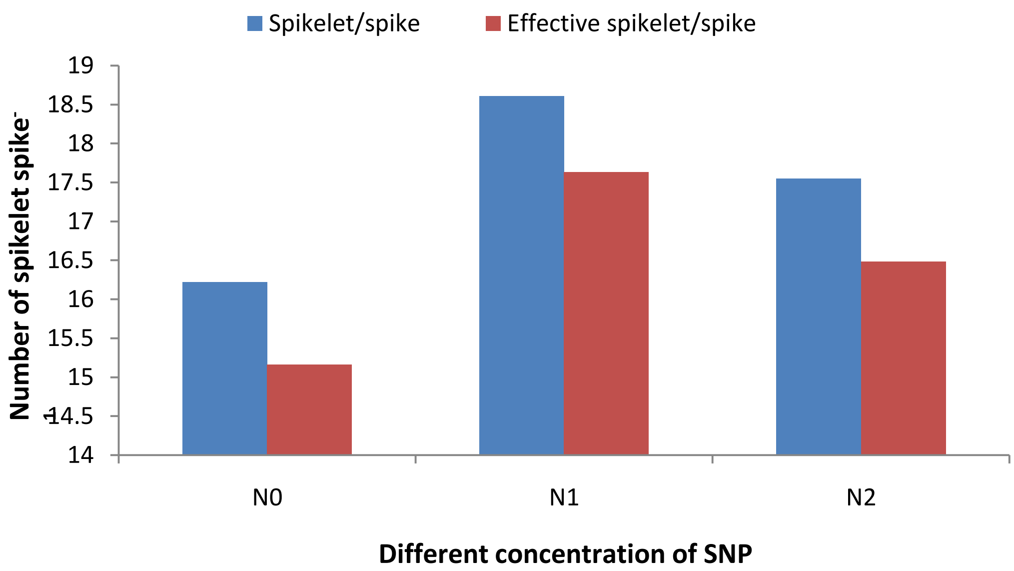


As_0 = Control, As_1 = 30 mg As kg^{-1} soil, As_2 = 60 mg As kg^{-1} soil, As_3 = 90 mg As kg^{-1} soil

Figure 13: Effect of different level of arsenic (As) on number of spikelet spike^{-1} and number of effective spikelet spike^{-1} of wheat ($Lsd_{0.05}$ = 1.02 and $Lsd_{0.05}$ =0.27 for spikelet and effective spikelet, respectively)

4.8.2 Effect of SNP

A significant variation was recorded due to the different concentrations of SNP for the number of spikelet spike^{-1} of wheat (Figure 14 and Appendix IV). The maximum number of spikelet spike^{-1} 18.61 was observed from N_1 or 0.1 mM SNP, whereas the minimum number of spikelet spike^{-1} 16.22 was observed from N_0 or zero SNP. It is consistent result of tiller number (Figure 6). Siddiqui *et al.* (2013) reported that the application of NO induced the activities of enzymes (CA, SOD, POD, CAT) and accumulation of osmoprotectant proline (Pro). These improved the photosynthetic pigments (Chl *a* and Chl *b*) and maintained nutrients homeostasis in plants under stressed condition. Ultimately this factor provided good result in increasing yield characters. So, these results suggest that 0.1 mM concentration of SNP increases the number of spikelet spike^{-1} of wheat, whereas 0.2 mM SNP has no much mitigation ability.



N_0 = 0 mM SNP (control), N_1 = 0.1 mM SNP, N_2 = 0.2 mM SNP

Figure 14: Effect of different concentration of sodium nitroprusside (SNP) on number of spikelet spike^{-1} and number of effective spikelet spike^{-1} of wheat ($Lsd_{0.05}$ = 0.32 and $Lsd_{0.05}$ = 0.23 for spikelet and effective spikelet, respectively)

4.8.3 *Interaction effect of As and SNP*

Interaction of As and SNP showed statistically significant variation on the number of spikelet spike^{-1} of wheat (Table 7 and Appendix IV). The highest number of spikelet spike^{-1} 22.20 was observed from As_0N_1 which was statistically similar to As_1N_1 (21.58) and As_0N_2 (20.95) while the lowest 12.00 was recorded from As_3N_0 treatment which was statistically similar to As_3N_2 (12.05) treatment. The maximum number of spikelet spike^{-1} identified in 0.1 mM SNP in zero As treatment, whereas it was similar to the treatment of 0.1 mM SNP in 30 mg As kg^{-1} soil and 0.2 mM SNP in zero As conc. The minimum number of spikelet spike^{-1} came from 90 mg As kg^{-1} soil in zero SNP as well as 0.2 mM SNP concentration. Numerous previous reports stated that number of spikelet had negative and positive effects in different conc. of As and SNP (Zhang *et al.*, 2009; Siddiqui *et al.*, 2013). However, these results suggest that NO increases spikelet number of wheat under As contamination.

Table 7: Interaction effect of different level of arsenic (As) and sodium nitroprusside (SNP) on number of spikelet spike^{-1} and number of effective spikelet spike^{-1} of wheat

Treatment	Number of spikelet spike^{-1}	Number of effective spikelet spike^{-1}
As_0N_0	19.23 d	18.05 c
As_0N_1	22.20 a	21.05 a
As_0N_2	20.95 bc	19.75 b
As_1N_0	18.50 e	17.45 d
As_1N_1	21.58 ab	20.67 a
As_1N_2	20.65 c	19.65 b
As_2N_0	15.15 g	14.15 g
As_2N_1	16.98 f	16.15 e
As_2N_2	16.55 f	15.48 f
As_3N_0	12.00 i	10.98 i
As_3N_1	13.70 h	12.65 h
As_3N_2	12.05 i	11.05 i
$Lsd_{0.05}$	0.63	0.46
Significant level	**	**
CV (%)	5.51	3.94

As_0 = Control, As_1 = 30 mg As kg^{-1} soil, As_2 = 60 mg As kg^{-1} soil, As_3 = 90 mg As kg^{-1} soil
N_0 = 0 mm SNP (control), N_1 = 0.1 mM SNP, N_2 = 0.2 mM SNP
** Significant at 1% level of probability

4.9 Number of effective spikelet spike^{-1}

4.9.1 Effect of arsenic

Statistically significant variation was recorded in terms of the number of effective spikelet spike^{-1}due to different levels of As under the present trial (Figure 13 and Appendix V). This result is correlated with spikelet number spike^{-1} (Figure 13).The maximum number of effective spikelet spike^{-1}(19.62) was recorded from As_0or control and they were statistically identical, while the minimum number of effective spikelet spike^{-1} (11.56) was obtained from As_3 or 90 mg As kg^{-1}.These results considered by some researchers who stated that As is a nonessential element for plants, and inorganic As species are generally highly phytotoxic. Biomass production and yields of a variety of crops are reduced significantly at elevated As concentrations (Carbonell- Barrachina *et al.*, 1997), with application of only 50 mg As kg^{-1} to soil significantly decreasing the yields of barley (*Hordeum vulgare* L.) and ryegrass (*Lolium perenne* L.) (Jiang and Singh, 1994). All together, these results suggest that As decreases the number of effective spikelet spike^{-1} of wheat as other cereals including barley.

4.9.2 Effect of SNP

Different concentrations of SNP showed statistically significant difference on the number of effective spikelet spike^{-1} (Figure 14 and Appendix V). Data revealed that the maximum number of effective spikelet spike^{-1}(17.63) was found from N_1 or 0.1 mM SNP. On the other hand, the minimum number of effective spikelet spike^{-1}(15.16) was recorded from N_0 or zero SNP. These results indicate that SNP as NO donor increases effective spikelet number in a certain concentration (0.1mM) than higher concentration (0.2 mM) of SNP as well as total spikelet number of wheat (Figure 14).

4.9.3 Interaction effect of As and SNP

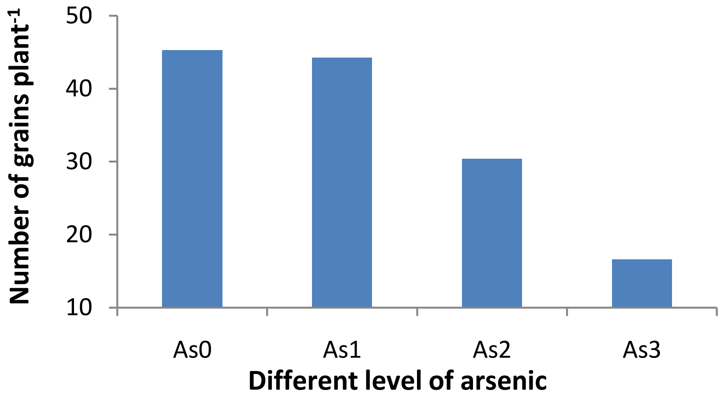
Significant variation was observed for the number of effective spikelet spike^{-1} influenced by the interaction effect of As and SNP (Table 7 and Appendix V). Results revealed that the maximum number of effective spikelet spike^{-1}(21.05) was achieved from As_0N_1 treatment which was statistically similar to As_1N_1 (20.67) treatment and the minimum number of effective spikelet spike^{-1} (10.98) were obtained from As_3N_0

treatment which was significantly similar to As_3N_2 (11.05) treatment. The number of effective spikelet spike^{-1} increased with zero As and the increasing rates of SNP up to 0.1 SNP. Many scientists mentioned that sodium nitroprusside (SNP) as nitric oxide (NO) donor increased antioxidant activity which provided a good plant growth in stressed condition (Singh *et al.,* 2009; Hasanuzzaman and Fujita, 2013). All together, these results stated that NO has a contribution on effective spikelet spike^{-1}at 0.1 mM SNP which is consistent with total spikelet number (Table 7).

4.10 Number of grains plant^{-1}

4.10.1 Effect of arsenic

Arsenic (As) is a wide-spread toxic and carcinogenic metalloid. Grain number of wheat is dependent on plant growth. The influence of As on the number of grains plant^{-1} was statistically significant (Figure 15 and Appendix V). Under the present study, it was found that the maximum number of grains plant^{-1} (45.25) was achieved from the treatment As_0 or control. Again, the minimum number of grains plant^{-1} (16.58) was obtained from the treatment of As_3 or 90 mg As kg^{-1} soil which was significantly different from all other treatments. Number of grains decreased with the increase of As levels (0 to 90 mg As kg^{-1} soil) which was correlated with effective spikelet number (Figure 13). Noor *et al*. (2016) reported that different wheat varieties such as Shatabdi, Bijoy, Prodip, BARI Gom-25 and BARI Gom-26 on As contaminated soil up to 36.4 mg kg^{-1} soil in different region in Bangladesh had no significant variations in number of grains plant^{-1}. Fifty or 100 mg As kg^{-1} soil resulted in the decrease of grain yield per plant (Zhang *et al.*, 2009). Taken together, these results suggest that As reduces the grain number per plant in high concentration which is consistent with the number of effective spikelet spike^{-1} of wheat (Figure 13).

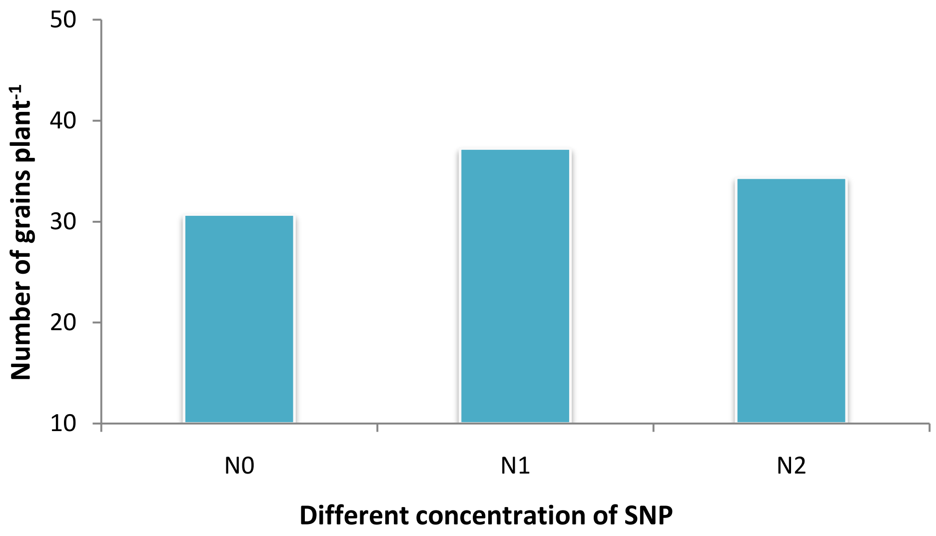


As_0 = Control, As_1 = 30 mg As kg^{-1} soil, As_2 = 60 mg As kg^{-1} soil, As_3 = 90 mg As kg^{-1} soil

Figure 15: Effect of different level of arsenic (As) on number of grains plant^{-1} of wheat ($Lsd_{0.05}$ = 0.84)

4.10.2 Effect of SNP

It has been reported that NO is a gaseous free radical, which acts as inter- as well as intracellular signaling molecule and affects many physiological and biochemical processes under normal as well as in stress condition (Xiong *et al.*, 2010).t In this study, the number of grains plant^{-1} was significantly affected by different concentration of SNP of wheat (Figure 16 and Appendix V). The maximum number of grains plant^{-1} 37.25 was observed from N_1 or 0.1 mM SNP whereas the minimum number of grains plant^{-1} 30.71 was observed from N_0 or control. Exogenous application of NO can be used to improve crop growth under various abiotic stresses (Zhang *et al*., 2009; Hasanuzzaman *et al*., 2012). All together, these results indicate that the number of grains plant^{-1} of wheat is increased with increasing the application of SNP, but high concentration of SNP has little effectiveness than control in view of effective spikelet number spike^{-1} (Figure 14).



N_0 = 0 mM SNP (control), N_1 = 0.1 mM SNP, N_2 = 0.2 mM SNP

Figure 16: Effect of different concentration of sodium nitroprusside (SNP) on number of grains plant^{-1} of wheat ($Lsd_{0.05}$ = 0.73)

4.10.3 Interaction effect of As and SNP

Exogenous application of NO was earlier reported to enhance stress tolerance in wheat seedlings. Combined effect of As and SNP showed significant variation on the number of grains plant^{-1} of wheat in this experiment (Table 8 and Appendix V). The highest number of grains plant^{-1} 48.25 was observed from As_0N_1 which was statistically similar to As_1N_1 treatment whereas the lowest number of grains plant^{-1} 12.50 was recorded from As_3N_0 treatment. Higher concentration of As decreased grain yield of wheat reported by Zhang *et al.* (2009). However, the application of SNP (0.1 mM) enhanced the yield and yield components of the wheat. These results are confirmed by Kausar *et al.* (2013) who reported that exogenous application of NO as a foliar spray significantly increased number of grains of wheat cultivar S-24 under nonstressed conditions, while NO enhanced grain yield per plant under saline conditions of seeds per plant (Hasanuzzaman *et al*., 2012). Taken together, these results suggest that NO increases number of grains plant^{-1} of wheat which is consistent with effective spikelet number spike^{-1} under As concentration (Table 7).

Table 8: Interaction effect of different level of arsenic (As) and sodium nitroprusside (SNP) on number of grains plant^{-1} of wheat

Treatment	Number of grains plant^{-1}
As_0N_0	41.88 c
As_0N_1	48.25 a
As_0N_2	45.63 b
As_1N_0	40.22 d
As_1N_1	47.50 a
As_1N_2	45.00 b
As_2N_0	28.23 g
As_2N_1	32.50 e
As_2N_2	30.33 f
As_3N_0	12.50 j
As_3N_1	20.75 h
As_3N_2	16.50 i
$Lsd_{0.05}$	1.45
Significant level	*
CV (%)	5.96

As_0 = Control, As_1 = 30 mg As kg^{-1} soil, As_2 = 60 mg As kg^{-1} soil, As_3 = 90 mg As kg^{-1} soil

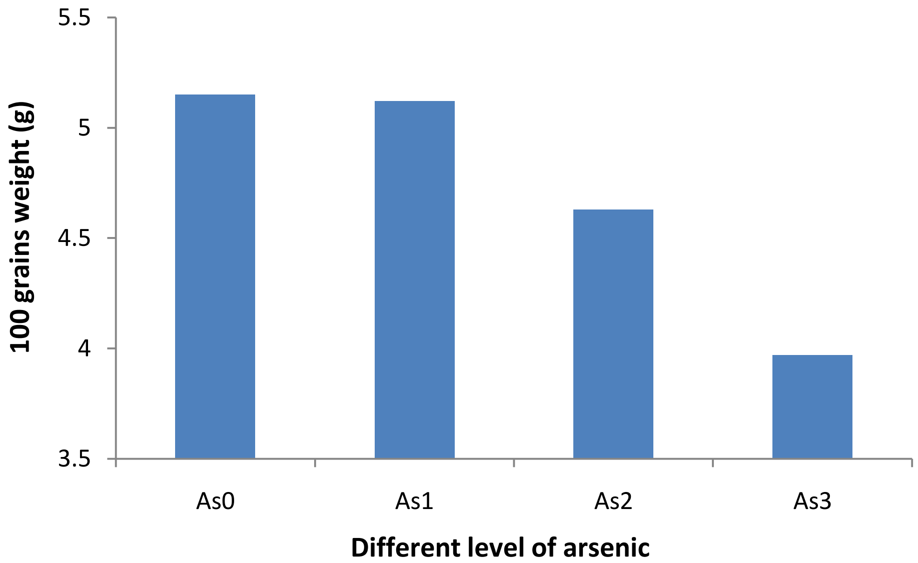
N_0 = 0 mM SNP (control), N_1 = 0.1 mM SNP, N_2 = 0.2 mM SNP

* Significant at 5% level of probability

4.11 Hundred grains weight (g)

4.11.1 Effect of arsenic

Hundred grains weight (g) is a unique and important yield contributing character of crop plant. Higher 100 grain weight indicates more healthy seeds and resulted higher grain yield ha^{-1}. Here, 100 grains weight (g) was significantly influenced by different As concentration. 100 grains weight decreased with increasing As toxicity in wheat (Figure 17 and Appendix V). The highest 100 grains weight 5.15 g was recorded from control, As_0 or without As treated plant which was statistically similar to As_1 or 30 mg As kg^{-1} soil, whereas the lowest 3.97 g was recorded from As_3 or 90 mg As kg^{-1} soil. Liu *et al.* (2009) reported that the amount of roxarsone (As contaminated) added significantly reduced 1000-grain weight, and grain yield ($p < 5\%$) of rice. This As-induced reduction was most pronounced in tiller number and grain yield. This fact is also confirmed by Noor *et al*. (2016) who stated that 1000- grains weight was in significant in As concentration up to 32.4 mg As kg^{-1} soil. This 1000- grains weight is evaluated as 100 grains weight (g). All together, these results suggest that As mitigates 100 grains weight of wheat as other cereals.

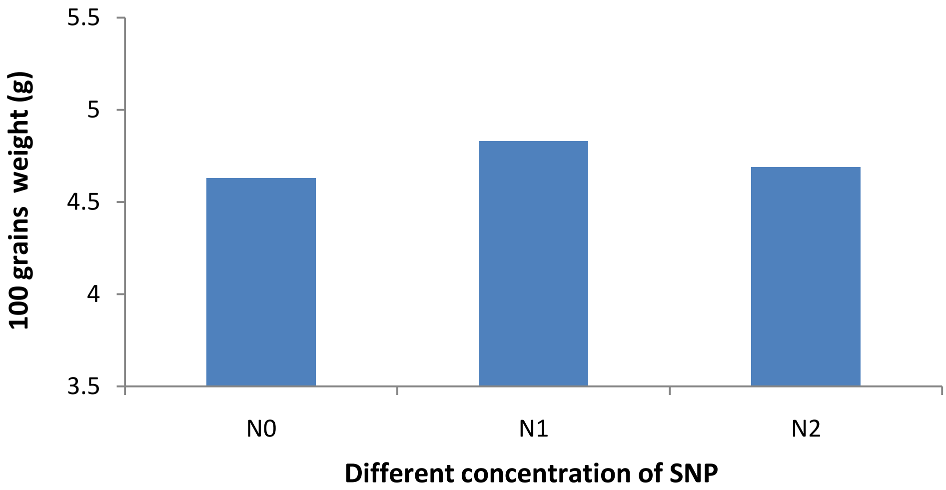


As_0 = Control, As_1 = 30 mg As kg^{-1} soil, As_2 = 60 mg As kg^{-1} soil, As_3 = 90 mg As kg^{-1} soil

Figure 17: Effect of different level of arsenic (As) on 100 grains weight (g) of wheat ($Lsd_{0.05}$ = 0.07)

4.11.2 Effect of SNP

Statistically significant variation was recorded in terms of hundred grains weight (g) due to different concentration of SNP of wheat (Figure 18 and Appendix V). The maximum 100 grains weight 4.83 g was observed from N_1 or 0.1 mM SNP whereas the minimum 100 grains weight 4.63 g was observed from N_0 as well as N_2 or 0.2 mM SNP. And that indicated statistically similar. These results are agreed by Kausar *et al.* (2013) who mentioned that exogenous application of NO as foliar spray markedly increased yield per plant, number of seeds per plant, and 100-seed weight of wheat. It has also been reported that NO is effective in enhancing growth and development from seed germination to the adult stage (Desikan *et al.*, 2004). So, these results suggest that 100 grains weight (g) of wheat is increased mostly with the application of 0.1 mM SNP and 0.2 mM SNP is caused sometimes negative effects of wheat.



N_0 = 0 mM SNP (control), N_1 = 0.1 mM SNP, N_2 = 0.2 mM SNP

Figure 18: Effect of different concentration of sodium nitroprusside (SNP) on 100 grains weight (g) of wheat ($Lsd_{0.05}$ = 0.06)

4.11.3 Interaction effect of As and SNP

The hundred grains weight (g) of wheat showed a non-significant variation due to the combined effect of As and SNP (Table 9 and Appendix V). But numerically the highest 100 grains weight 5.30 g was observed from As_0N_1 while the lowest 100 grains weight 3.91 g was recorded from As_3N_0 treatment. This result was not agreed by Kausar *et al.* (2013) who mentioned that exogenous application of NO as foliar spray markedly increased yield per plant, number of seeds per plant, and 100-seed weight of wheat cultivar S-24 under no stressed conditions, while only 0.1 mM NO enhanced grain yield per plant under saline conditions. In addition, Habib *et al.* (2016) reported that stress induced a significant reduction in biomass and grain yield while increased the plant proline, ascorbic acid, H_2O_2 and MDA contents in all studied cultivars of rice (*Oryza sativa* L.). Application of SNP as seed priming reduced the adverse effects of salinity on plant biomass production and grain yield, while the accumulation of MDA and H_2O_2 decreased. Of different SNP levels, 0.1 mM regime was more effective in reducing the negative effects of stress. In this study, these results do not show any collaborating difference.

Table 9: Interaction effect of different level of arsenic (As) and sodium nitroprusside (SNP) on 100 grains weight (g) of wheat

Treatment	100 grains weight(g)
As_0N_0	5.04
As_0N_1	5.3
As_0N_2	5.12
As_1N_0	5
As_1N_1	5.26
As_1N_2	5.11
As_2N_0	4.59
As_2N_1	4.7
As_2N_2	4.62
As_3N_0	3.91
As_3N_1	4.08
As_3N_2	3.93
$Lsd_{0.05}$	NS
Significant level	---
CV (%)	3.86

As_0 = Control, As_1 = 30 mg As kg^{-1} soil, As_2 = 60 mg As kg^{-1} soil, As_3 = 90 mg As kg^{-1} soil

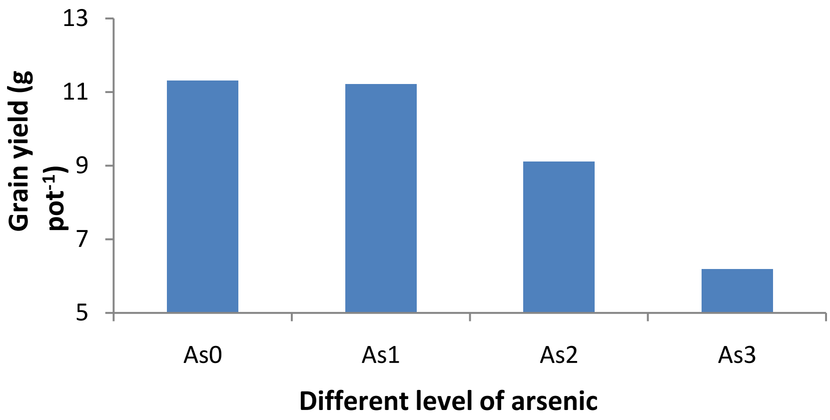
N_0 = 0 mM SNP (control), N_1 = 0.1 mM SNP, N_2 = 0.2 mM SNP

NS = Not significant

4.12 Grain yield (g pot^{-1})

4.12.1 Effect of arsenic

As morphological characters the grain yield (g pot^{-1}) of wheat significantly reduced by different level of As toxicity (Figure 19 and Appendix V). The highest grain yield 11.30 g pot^{-1} was obtained from As_0 or control which was statistically similar with As_1 (11.21 g pot^{-1}) or 30 mg As kg^{-1} soil, whereas the lowest 6.19 g pot^{-1} value from As_3 level or addition of 90 mg As kg^{-1} soil. These results showed the gradual decrease of yield with the increased levels of As toxicity. Grain yield (g pot^{-1}) is consistent with the yield contributing characteristics which are analyzed in this experiment such as number of spikelet spike^{-1} and effective spikelet spike^{-1} (Figure 13), and number of grains plant^{-1} (Figure 15). This information are also dependable on growth measuring parameters of this study such as plant height (Figure 1), number of leaves plant^{-1} (Figure 3) and effective tillers plant^{-1} (Figure 5). In addition, Zhang *et al.* (2009) reported that addition of As (about 50 or 100 mg As/kg soil) significantly ($p<0.05$) reduced root, stem and spike dry weight and yield components, which resulted in the decrease of grain yield per plant. As concentrations in plant tissues were as follows: roots > stems > leaves and rachises > grains > glumes > awns. Similar observations are also mentioned that yield increased due to small additions and decreased with high concentrations of As in wheat, maize and rice (Liu *et al.*, 2009; Liu *et al.*, 2012; Noor *et al.*, 2016). Therefore, altogether these results suggest that As decreases grain yield in higher concentration of wheat as other findings.

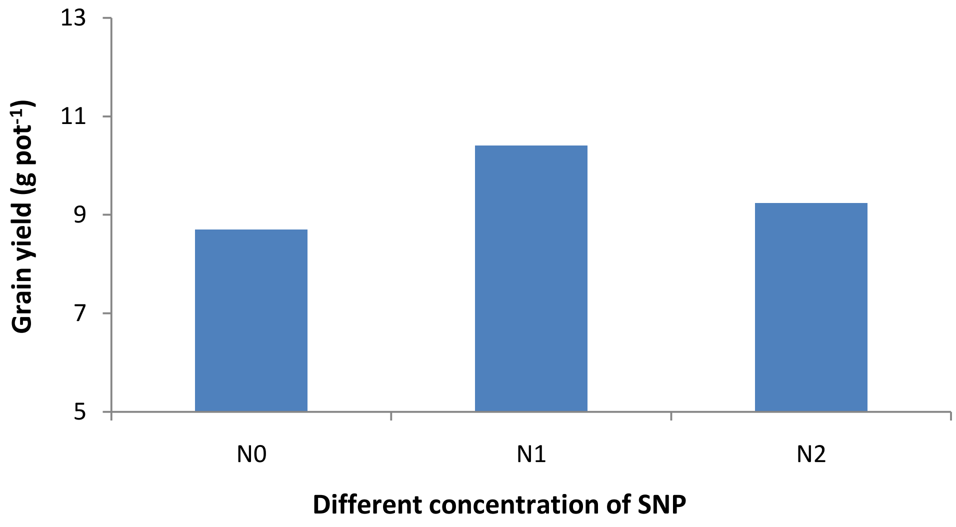


As_0 = Control, As_1 = 30 mg As kg^{-1} soil, As_2 = 60 mg As kg^{-1} soil, As_3 = 90 mg As kg^{-1} soil

Figure 19: Effect of different level of arsenic (As) on grain yield (g pot^{-1}) of wheat ($Lsd_{0.05}$ = 0.29)

4.12.2 Effect of SNP

In this study, the grain yield of wheat (g pot^{-1}) was showed statistically significant variations in the effect of different concentrations of SNP (Figure 20 and Appendix V). The highest grain yield 10.41 g pot^{-1} was observed from N_1 or 0.1 mM SNP whereas the lowest grain yield 8.70 g pot^{-1} was observed from N_0 or zero SNP. These results are consistent with the yield contributing characteristics which are analyzed in this experiment such as number of spikelet $spike^{-1}$ and effective spikelet $spike^{-1}$ (Figure 14), and number of grains $plant^{-1}$ (Figure 16). This information are also dependable on growth measuring parameters of this study such as plant height (Figure 2), number of leaves $plant^{-1}$ (Figure 4) and effective tillers $plant^{-1}$ (Figure 6). Habib *et al.* (2016) reported that seed priming with SNP was found effective in reducing the adverse effects of abiotic stress (salt stress) on grain yield per plant in rice cultivars. Taken together, these results suggest that of all SNP levels, 0.1mM is more effective in mitigating the adverse effects of As stress on grain yield per pot of wheat.



N_0 = 0 mM SNP (control), N_1 = 0.1 mM SNP, N_2 = 0.2 mM SNP

Figure 20: Effect of different concentration of sodium nitroprusside (SNP) on grain yield (g pot^{-1}) of wheat ($Lsd_{0.05}$ = 0.25)

4.12.3 Interaction effect of As and SNP

There was a significant interaction effect of different level of As and SNP concentrations and showed significant variation on the grain yield (g pot^{-1}) of wheat (Table 10 and Appendix V). The maximum grain yield 12.60 g pot^{-1} was observed from As_0N_1 which was statistically similar As_1N_1 (12.35 g pot^{-1}) while the lowest grain yield 5.69 g pot^{-1} was recorded from As_3N_0 treatment which was statistically similar with As_3N_2 (6.00 g pot^{-1}). These results are consistent with the morpho-physiological data as well as yield contributing characters of wheat (Table 1, 2, 3, 4, 7 and 8). Previous reports stated that yield increased due to small additions of As and decreased in high concentrations of As in wheat, maize and rice (Liu *et al.*, 2009; Noor *et al.*, 2016). It is also correlated with the result of Kausar *et al*. (2013) who reported that NO as SNP enhanced the As-stressed condition as well as other stress in case of grain yield per plant (Hasanuzzaman *et al*., 2012; Habib *et al.*, 2016). Taken together, these results indicate that As contamination decreases grain yield and NO increases yield of wheat under with or without As contamination.

Table 4: Interaction effect of different level of arsenic (As) and sodium nitroprusside (SNP) on grain yield (g pot^{-1}) of wheat

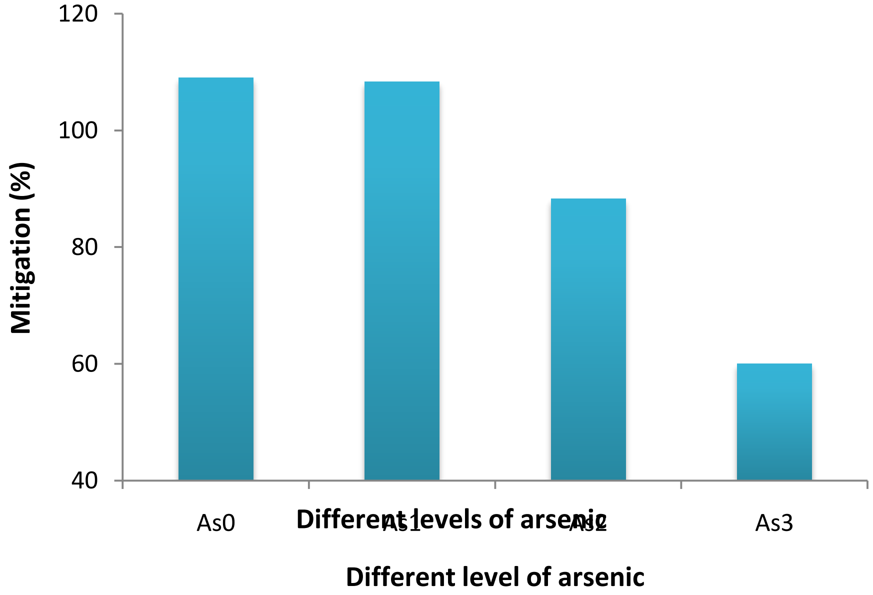
Treatment	Grain yield (g pot^{-1})
As_0N_0	10.31 c
As_0N_1	12.60 a
As_0N_2	11.00 b
As_1N_0	10.30 c
As_1N_1	12.35 a
As_1N_2	10.98 b
As_2N_0	8.50 d
As_2N_1	9.82 c
As_2N_2	8.98 d
As_3N_0	5.69 f
As_3N_1	6.88 e
As_3N_2	6.00 f
$Lsd_{0.05}$	0.49
Significant level	*
CV (%)	7.64

As_0 = Control, As_1 = 30 mg As kg^{-1} soil, As_2 = 60 mg As kg^{-1} soil, As_3 = 90 mg As kg^{-1} soil
N_0 = 0 mM SNP (control), N_1 = 0.1 mM SNP, N_2 = 0.2 mM SNP
* Significant at 5% level of probability

4.13 Mitigation (%)

4.13.1 Effect of arsenic

The percentage of mitigation is the indication of sustain characteristics in stressed condition of crop cultivation. In this study, I analyzed mitigation (%) of As toxicity by conducting the values of grain yield (g pot^{-1}) (Figure 19) which was significantly difference in wheat (Figure 21 and Appendix V). The maximum As mitigation percentage 109.1 was recorded from As_0 treatment or control whereas the minimum mitigation 60.07 % was observed from As_3 level or addition of 90 mg As kg^{-1} soil. Some researchers stated that plant yield was highly reduced by increasing As toxicity as inducing reactive oxygen species (ROS) synthesis which leads to cellular membrane damage and physiological activities (Liu *et al.*, 2012; Kumar *et al.*, 2013, 2014b). So, these results suggest that mitigation percentage decreases chronologically as higher level of As contamination in wheat.

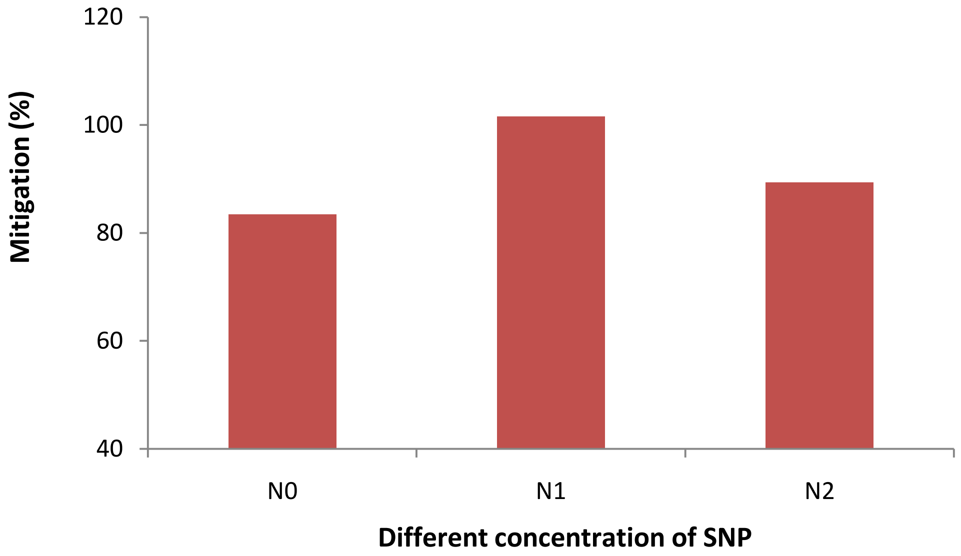


As_0 = Control, As_1 = 30 mg As kg^{-1} soil, As_2 = 60 mg As kg^{-1} soil, As_3 = 90 mg As kg^{-1} soil

Figure 21: Effect of arsenic (As) on mitigation (%) of wheat ($Lsd_{0.05}$ = 2.55)

4.13.2 Effect of SNP

In this experiment, the mitigation (%) of wheat (g pot^{-1}) was showed statistically significant variations in the effect of different concentrations of SNP (Figure 22 and Appendix V). It was measured by conducting the values of grain yield (g pot^{-1}) with SNP (Figure 20). The highest mitigation percentage 101.6 was observed from N_1 or 0.1 mM SNP whereas the lowest percentage 83.4 was observed from N_0 or control. These results showed that high concentration (0.2 mM) decreased the yield of wheat than 0.1 mM SNP. Other report also mentioned by Habib *et al.* (2016) who reported that SNP was found effective in reducing the adverse effects of abiotic stress on grain yield of rice cultivars. Taken together, these results indicate that mitigation percent of the yield of wheat increased with the application of SNP as NO donor.



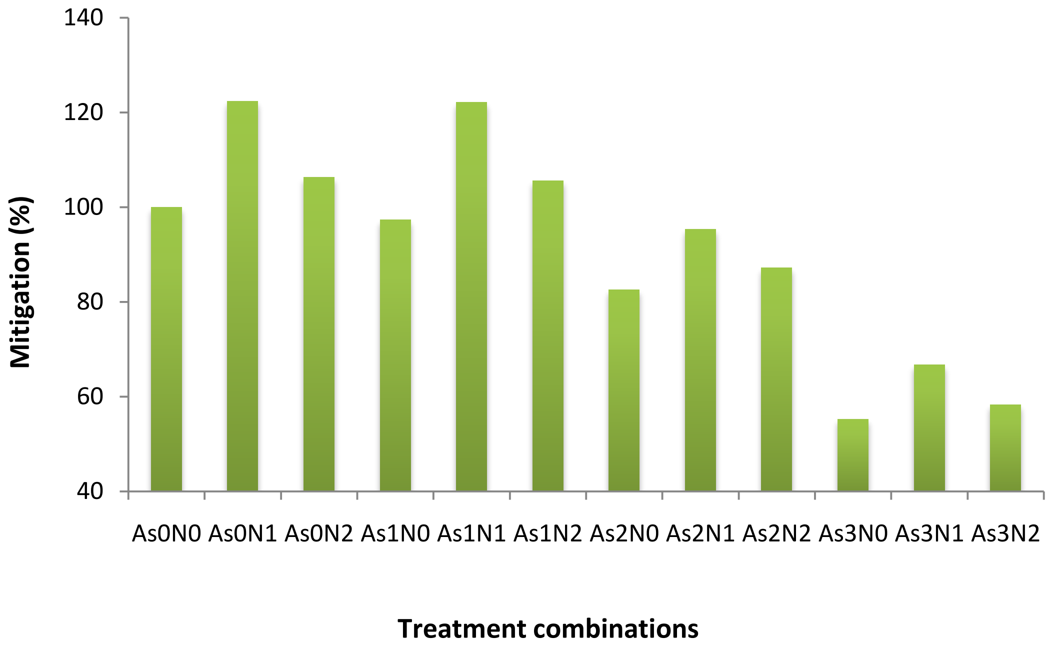
N_0 = 0 mM SNP (control), N_1 = 0.1 mM SNP, N_2 = 0.2 mM SNP

Figure 22: Effect of sodium nitroprusside (SNP) on mitigation (%) of wheat ($Lsd_{0.05}$ = 2.21)

4.13.3 Interaction effect of As and SNP

Interaction effect of As and SNP on mitigation (%) of wheat was showed a significant variation (Figure 23 and Appendix V). In this study, it was measured by conducting the values of grain yield (g pot^{-1}) with combined effects of As and SNP in wheat (Table 10). The highest As mitigation percentage 122.4 was observed from As_0N_1or 0.1 mM SNP in zero As treatment which was statistically similar to As_1N_1 (122.1) or 30 mg As kg^{-1} soil in 0.1 mM SNP, whereas the lowest alleviation percentage 55.23 was recorded from As_3N_0 treatment or 90 mg As kg^{-1} soil in zero SNP which was statistically similar to As_3N_2 (58.42). These results are showed same review as Tan *et al.* (2008) reported that NO has ability to alleviate oxidative damage of wheat seedlings subjected to osmotic stress (Hasanuzzaman *et al.*, 2012).

Considering the above results, it suggests that different concentrations of SNP can successfully mitigate the As-induced stress and increase the yield of wheat.



As_0 = Control, As_1 = 30 mg As kg^{-1} soil, As_2 = 60 mg As kg^{-1} soil, As_3 = 90 mg As kg^{-1} soil

N_0 = 0 mM SNP (control), N_1 = 0.1 mM SNP, N_2 = 0.2 mM SNP

Figure 23: Interaction effect of arsenic (As) and sodium nitroprusside (SNP) on mitigation (%) of wheat (Lsd = 4.42)

CHAPTER FIVE
SUMMARY AND CONCLUSION

CHAPTER 5
SUMMARY AND CONCLUSION

The experiment was conducted in the net house at the field laboratory of Agricultural Botany Department, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka, during the period from November 2015 to March 2016 to find out a method to mitigate the arsenic (As) toxicity in wheat with sodium nitroprusside (SNP). In this experiment, the treatments consisted of four different As levels viz. As_0 = 30 mg As kg^{-1} soil (control), As_1 = 30 mg As kg^{-1} soil, As_2 = 60 mg As kg^{-1} soil, As_3 = 90 mg As kg^{-1} soil and three different levels of sodium SNP viz. N_0 = 0 mM SNP, N_1 = 0.1 mM SNP and N_2 = 0.2 mM SNP. The experiment was laid out in two factors Complete Randomize Design (CRD) with four replications. Data on different growth parameters, physiological parameters and yield with yield contributing characters of wheat were recorded. The collected data were statistically analyzed for evaluation of the treatment effect. A significant variation among the treatments was found while different As levels and SNP concentrations were applied in different combinations.

There are significant differences among the influence of different concentrations of As in case of almost all the parameters. In this experiment, wheat plant was conducted with As contamination by adding As into soil before experiment. Plant grown on normal soil (control treatment) showed the maximum height more or less over the growth period, whereas the lowest height was recorded from 90 mg As kg^{-1} soil treated plants. The longest plant (77.69 cm) was recorded in As_0 or controlled condition, whereas the shortest plant (56.90 cm) in As_3 or 90 mg As kg^{-1} soil. The maximum number of leaves $plant^{-1}$ (19.52), tillers $plant^{-1}$ (4.417), effective tillers $plant^{-1}$ (3.833) and length of spike $plant^{-1}$ (12.50 cm) were found in As_0 or control treatment while the lowest number of leaves $plant^{-1}$ (9.975), tillers $plant^{-1}$ (1.717), effective tillers $plant^{-1}$ (1.208) and length of spike $plant^{-1}$ (9.483 cm) were observed in As_3 or 90 mg As kg^{-1} soil. The SPAD value was not statistically affected by different levels of arsenic. But numerically the maximum SPAD value was recorded from no levels of arsenic (As_0) with minimum from As_3 (90 mg As kg^{-1} of soil). The highest number of spikelet $spike^{-1}$ (20.79), number effective spikelet $spike^{-1}$ (19.62), membrane stability (77.81 %), number of grains $plant^{-1}$ (45.25), 100 grains weight (5.15 g), grain yield (11.30 g pot^{-1}) and mitigation (109.1 %) were found from

As_0treatment or control condition while the lowest number of spikelet spike^{-1} (12.58), number effective spikelet spike^{-1} (11.56), membrane stability (49.08 %), number of grains plant^{-1} (16.58), 100 grains weight (3.97 g), grain yield (6.188 g pot^{-1}) and mitigation (60.07 %)were recorded from As_3 or 90 mg As kg^{-1} soil.

Plant height of wheat showed significant variation in response of foliar application of sodium nitroprusside (SNP). The highest plant height 72.69 cm was observed from N_1 or 0.1 nM SNP, whereas the lowest 66.80 cm observed from N_0 or controlled condition. The maximum number of leaves plant^{-1} (17.87), tillers plant^{-1} (3.844), effective tillers plant-1 (3.184), and length of spike plant^{-1} (11.94 cm) were recorded from N_1 treatment or 0.1 mM SNP whereas minimum number of leaves plant^{-1} (14.26), tillers plant^{-1} (2.819), effective tillers plant^{-1} (2.269) and length of spike plant^{-1} (10.21 cm) were observed from N_0 treatment or control. The SPAD value was not statistically influenced by the application of SNP. But numerically the highest SPAD value (55.51 SPAD units) was observed from N_1 whereas the lowest SPAD value (54.63 SPAD units) was observed from N_0. The highest 100 grains weight (4.83 g), number of spikelet spike^{-1} (18.61), number of effective spikelet spike^{-1} (17.63), number of grains plant^{-1} (37.51), membrane stability (70.40 %), grain yield (10.41 g pot^{-1}) and mitigation (101.6 %) were found from N_1 treatment or 0.1 mM SNP while the lowest 100 grains weight (4.63 g), number of spikelet spike^{-1} (16.22), number of effective spikelet spike^{-1} (15.16), number of grains plant^{-1} (30.71), membrane stability (62.91 %), grain yield (8.699 g pot^{-1}) and mitigation (83.40 %) were recorded from N_0 or control treatment.

The combinations of As and SNP had significant effect on almost all growth and yield contributing parameters. The tallest plant height 80.60 cm was found from As_0N_1 (0.1 mM SNP with zero arsenic) treatment combination, whereas the lowest plant height 55.08 cm was found from As_3N_0 (90 mg As kg^{-1} soil with zero SNP) treatment combination. The maximum number of leaves plant^{-1} (22.19), tillers plant^{-1} (5.250), and effective tillers plant-1 (4.50), were recorded from As_0N_1 treatment or 0.1 mM SNP with zero arsenic. On the other hand, minimum number of leaves plant^{-1} (9.075), tillers plant^{-1} (1.500), and effective tillers plant^{-1} (1.000) were observed from As_3N_0 treatment or 90 mg As kg^{-1} soil with zero SNP. The highest length of spike plant^{-1} (14.38 cm) was recorded in As_0N_1 treatment while the lowest length (9.555 cm) in

As_3N_0 treatment combination. The SPAD value and 100 grains weight were not statistically influenced by the combined application of As and SNP. But numerically the highest SPAD value (56.63 SPAD units) and 100 grains weight (5.3 g) were observed from As_0N_1 whereas the lowest SPAD value (53.55 SPAD units), and 100 grains weight (3.91 g) %) were observed from As_3N_0. The highest number of spikelet spike^{-1} (22.20), number of effective spikelet spike^{-1} (21.05), number of grains plant^{-1} (48.25), membrane stability (80.69 %), grain yield (12.60 g pot^{-1}) and mitigation (122.4 were found from As_0N_1 (0.1 mM SNP with zero arsenic) treatment combination, while the lowest number of spikelet spike^{-1} (12.00), number of effective spikelet spike^{-1} (10.98), number of grains plant^{-1} (30.71), membrane stability (62.91 %), grain yield (8.699 g pot^{-1}) and mitigation (55.23 %) were recorded from As_3N_0 (90 mg As kg^{-1} soil with zero SNP) treatment combination. Considering the above mentioned results, it may be concluded that, the yield of wheat was gradually decreased by the increase of As levels. But 30 mg As kg^{-1} soil conducted a beneficial effect like as control treatment. Among the SNP concentrations, almost 0.1 mM SNP showed the highest result in growth, physiological and yield parameters. Morphological parameters, grain yield and yield contributing parameters of wheat are consistent with As and SNP application. Therefore, the present experimental results suggest that 0.1 mM SNP would be beneficial to mitigate the adverse effect of As and increase the yield of wheat variety BARI Gom-24 than 0.2 mM SNP concentration.

Considering the situation of the present experiment, further studies in the following areas may be suggested:

1. Such study is needed in different agro-ecological zones (AEZ) of Bangladesh for analogy the accuracy of the experiment.
2. It needs to conduct more experiments with arsenic and sodium nitroprusside (SNP) to find out whether these can regulate the morphophygiology, yield and seed quality of wheat var. BARI Gom-24.
3. More experiments may be carried out with other wheat varieties.

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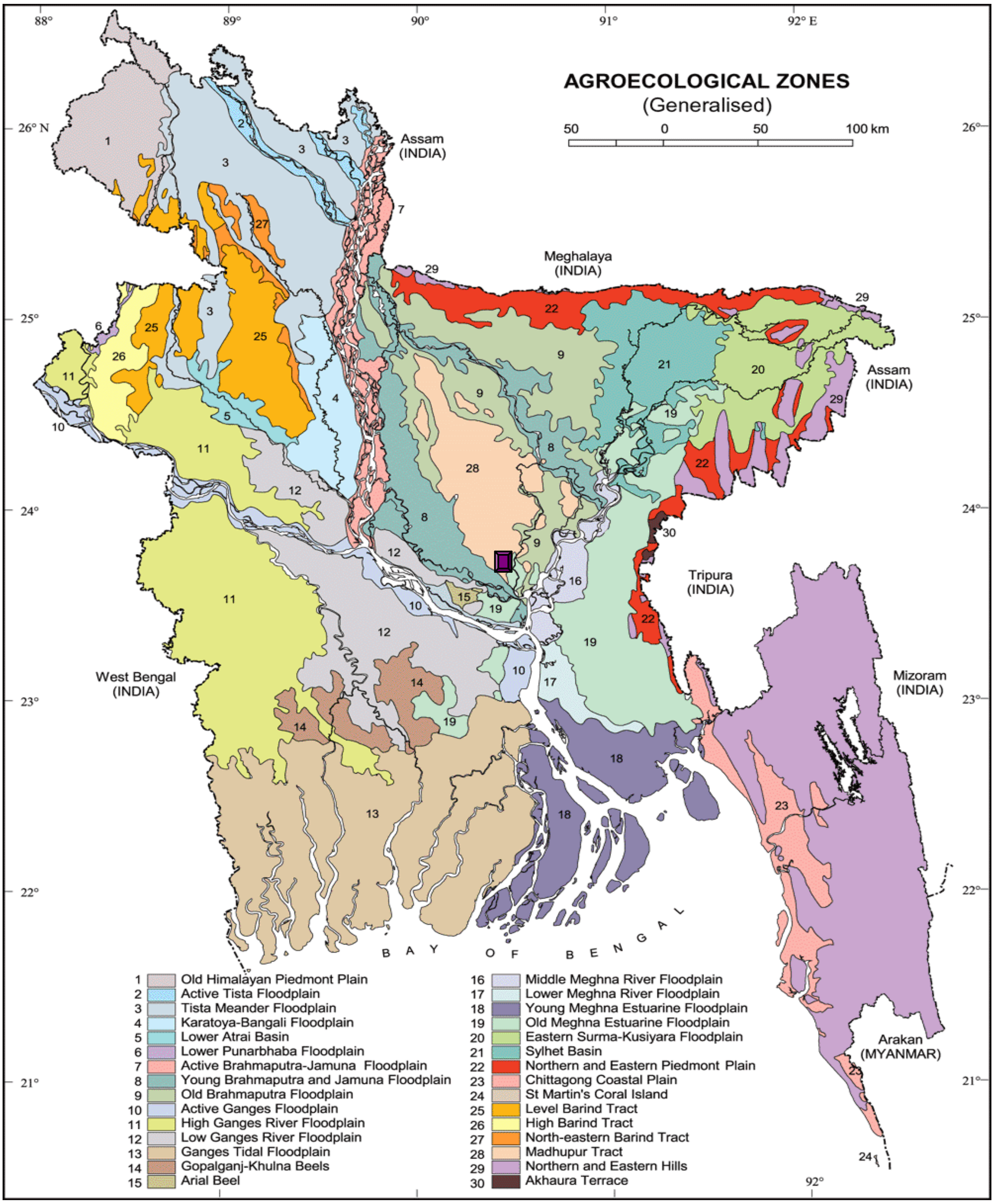
APPENDICES

APPENDICES

Appendix I: Map showing the experimental sites under study



The experimental site under study

Appendix II: Results of mechanical and chemical analysis of soil of the experimental plot

A. Mechanical analysis

Constituents	Percentage (%)
Sand	28.78
Silt	42.12
Clay	29.1

B. Chemical analysis

Soil properties	Amount
Soil pH	5.8
Organic carbon (%)	0.95
Organic matter (%)	0.77
Total nitrogen (%)	0.075
Available P (ppm)	15.07
Exchangeable K (%)	0.32
Available S (ppm)	16.17

Source: Soil Resource Development Institute (SRDI)

Appendix III: Analysis of variance of the data on plant height (cm), number of leaves plant^{-1}, number of tillers plant^{-1} and number of effective tillers plant^{-1} of wheat as influenced by different levels of arsenic along with sodium nitroprusside (SNP)

Source of variation	Degrees of freedom(df)	Meansquare of			
		Plant height (cm)	Number of leaves plant^{-1}	Number of tillers plant^{-1}	Number of effective tillers plant^{-1}
Replication	3	0.338	0.277	0.004	0.057
Factor A (Arsenic)	3	1168.741**	250.188**	21.339*	19.697*
Factor B (SNP)	2	138.686**	54.735**	4.473**	3.359**
Interaction (A X B)	6	3.971**	2.209*	0.215**	0.177**
Error	33	0.567	0.217	0.037	0.015

**: Significant at 1% level of probability

*: Significant at 5% level of probability

Appendix IV: Analysis of variance of the data on length of spike (cm), SPAD value, membrane stability (%) and number of spikelet spike^{-1} of wheat as influenced by different levels of arsenic along with sodium nitroprusside (SNP)

Source of variation	Degrees of freedom (df)	Mean square of			
		Length of spike (cm)	SPAD value	Membrane stability (%)	Number of spikelet spike^{-1}
Replication	3	0.067	24.201	0.027	0.154
Factor A (Arsenic)	3	19.081**	9.67^{NS}	2290.758**	176.579**
Factor B (SNP)	2	12.286**	3.103^{NS}	225.183**	23.016**
Interaction (A X B)	6	1.908**	0.162^{NS}	14.653**	1.085**
Error	33	0.16	87.246	0.293	0.192

**: Significant at 1% level of probability

NS: Not significant

Appendix V: Analysis of variance of the data on number of effective spikelet spike^{-1}, number of grains plant^{-1}, and hundred grains weight (g) of wheat as influenced by different levels of arsenic along with sodium nitroprusside (SNP)

Source of variation	Degrees of freedom (df)	Mean square of		
		Number of effective spikelet spike^{-1}	Number of grains plant^{-1}	hundred grain weight (g)
Replication	3	0.018	1.723	0.0136
Factor A (Arsenic)	3	173.04**	2192.287**	3.6754**
Factor B (SNP)	2	24.543**	172.071**	0.1723**
Interaction (A X B)	6	1.031**	3.335*	0.006^{NS}
Error	33	0.102	1.018	0.0077

**: Significant at 1% level of probability

*: Significant at 5% level of probability

NS: Not significant

Appendix VI: Analysis of variance of the data on grain yield (g pot^{-1}) and mitigation (%) of wheat as influenced by different levels of arsenic along with sodium nitroprusside (SNP)

Source of variation	Degrees of freedom (df)	Mean square of	
		Grain yield (g plot^{-1})	Mitigation (%)
Replication	3	0.097	52.423
Factor A (Arsenic)	3	69.125**	6362.778**
Factor B (SNP)	2	12.252**	1382.709**
Interaction (A X B)	6	0.314*	51.52**
Error	33	0.118	9.432

**: Significant at 1% level of probability

*: Significant at 5% level of probability

Appendix VII: Monthly average temperature, relative humidity and total rainfall of the experimental site during the period from October 2015 to May 2016

Month	Air temperature (^0C)		R. H. (%)	Total rainfall (mm)
	Maximum	Minimum		
October,15	29.18	18.26	81	39
November,15	25.82	16.04	78	0
December,15	22.4	13.5	74	0
January,16	24.5	12.4	68	0
February,16	27.1	16.7	67	3
March,16	31.4	19.6	54	11
April, 16	35.3	22.4	51	15
May, 16	38.2	23.2	62	17

Source: Bangladesh Metrological Department (Climate and weather division) Agargaon, Dhaka

PICTORIAL VIEW

PICTORIAL VIEW

Photo1: Seedlings at 30 DAS

Photo 2: Spraying SNP

Photo 3: Harvesting Stage

Photo 4: Data Collection