MITIGATION OF NICKEL STRESS IN RICE BY EXOGENOUS APPLICATION OF BIOCHAR AND CHITOSAN

MD. RAKIB HOSSAIN RAIHAN

DEPARTMENT OF AGRONOMY

SHER-E-BANGLA AGRICULTURAL UNIVERSITY

DHAKA-1207

JUNE, 2022

MITIGATION OF NICKEL STRESS IN RICE BY EXOGENOUS APPLICATION OF BIOCHAR AND CHITOSAN

BY

MD. RAKIB HOSSAIN RAIHAN

REGISTRATION NO. 14-06185

E-mail: rakib.raihan.75491@gmail.com

Mobile: +8801521210440

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
AGRONOMY

SEMESTER: JANUARY-JUNE, 2022

Approved by:

Dr. Mirza Hasanuzzaman **Professor** **Department of Agronomy** **Supervisor**	**Dr. Anisur Rahman** **Professor** **Department of Agronomy** **Co-supervisor**

Professor Dr. Md. Abdullahil Baque
Chairman
Examination Committee

DEPARTMENT OF AGRONOMY

Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207

CERTIFICATE

*This is to certify that the thesis entitled **"MITIGATION OF NICKEL STRESS IN RICE BY EXOGENOUS APPLICATION OF BIOCHAR AND CHITOSAN"** 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 AGRONOMY**, embodies the result of a piece of bonafide research work carried out by **MD. RAKIB HOSSAIN RAIHAN**, Registration No. **14-06185** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

Dated:
Place: Dhaka, Bangladesh

Professor Dr. Mirza Hasanuzzaman
Supervisor
Department of Agronomy
Sher-e-Bangla Agricultural University

ACKNOWLEDGEMENTS

All praises to the most merciful and omnipotent ***Allah SWT*** for keeping me safe and sound throughout the period of COVID-19. I am also thankful for bestowing His blessings upon me to accomplish my research work successfully for the degree of Master of Science in Agronomy.

I would like to express my heartfelt respect and gratitude to my research supervisor, ***Professor Dr. Mirza Hasanuzzaman***, Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka for his scholastic guidance to walk on an unknown path of research. I really cannot express in words how fortunate I am to have had the opportunity to work under his supervision. I am indebted to him for his endless inspiration and conscientious suggestions throughout the period of the research work and thesis preparation. I am grateful to him for making me realize my capability and patience, and also for his encouragement to achieve my desired aspiration.

I am thankful to my respected co-supervisor, ***Professor Dr. Anisur Rahman***, Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka for his valuable advice and suggestions on the research and thesis writing. I would like to express my profound thanks to ***Dr. Md. Mahabub Alam***, Assistant Professor, Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka for his continuous help and suggestions during research work. I would like to thank ***Abdul Awal Chowdhury Masud***, Assistant Professor, Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka for his invaluable support in any troublesome situation.

I would like to acknowledge the Government of Bangladesh for providing the financial support through the Ministry of Science and Technology by granting the National Science and Technology Fellowship (***NST Fellowship***) to conduct this research work.

I would like to convey my wholehearted appreciation to ***Faria Jannat***, ***Mira Rahman***, ***Khussboo Rahman***, ***Naznin Ahmed***, **and** ***Mst. Mahbuba Khatun*** for their cordial support and help throughout the research work. I would like to thank ***Yobaida Afrin Ara Eti, Md. Shawkat Zaman*** and ***Md. Fajla Rabbi*** for their generous help during the experimental work.

I would like to express my immense thankfulness to my mother who never let me fall down in any adversities. I am also thankful to my father, elder sister and younger brother for their unconditional support and encouragement. Finally, I would like to say "Thank you" to all the people who criticize me, which makes me more energetic and confident.

The Author

MITIGATION OF NICKEL STRESS IN RICE BY EXOGENOUS APPLICATION OF BIOCHAR AND CHITOSAN

ABSTRACT

Nickel (Ni) is considered as an essential micronutrient for plants, but it becomes phytotoxic at supra-optimal level which leads to inhibition of seed germination, growth and yield reduction as a response of physiological and biochemical dysfunction. Therefore, an experiment was conducted at the shed house of the Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh from December 2020 to April 2021 in order to investigate the morphological, physiological, and biochemical responses of rice (*Oryza sativa* L. cv. BRRI dhan96) upon exposure to different levels of Ni and to study the protective role of biochar (BC) and chitosan (CHT) in mitigating Ni stress. Twenty-one days after transplanting (DAT), rice seedlings were exposed to Ni stress by treating with 0.5, 1.0, and 2.0 mM $NiSO_4 \cdot 6H_2O$ for four times. Exogenous supplementation of BC (0.5 g kg^{-1} soil) was done once before seedling transplantation and the CHT (200 mg L^{-1}) was applied at seven-day intervals from 14 DAT to panicle initiation as foliar spray. The experiment was conducted in a completely randomized design with three replications and all the obtained data was subjected to one-way analysis of variance. Exposure to Ni stress exhibited a notable increase in hydrogen peroxide content, lipid peroxidation, and electrolyte leakage indicating Ni-mediated oxidative damages in the rice plants. Consequently, the Ni stressed plants showed a reduction of plant height, tillers $hill^{-1}$, leaf area, root fresh weight, shoot fresh weight, root dry weight, shoot dry weight, soil and plant analysis development value, and yield attributes in a dose dependent manner. The relative water content of the Ni treated plants also reduced with a concomitant increase in the proline accumulation. Supplementation with BC and CHT mitigated the deleterious effects of Ni toxicity in plants as reflected in enhanced growth and physiological attributes under different levels of Ni. Increase of ascorbate and glutathione with the concomitant reduction of dehydroascorbate and glutathione disulfide was observed when BC and CHT was supplemented in the Ni stressed plants. Moreover, the yield attributes such as effective tillers $hill^{-1}$, panicle length, rachis $panicle^{-1}$, filled grains $panicle^{-1}$, and 1000-grain weight were also increased when BC and CHT were applied, thus the grain yield $hill^{-1}$ was increased under Ni stress. These findings indicated a protective role of BC and CHT against Ni-induced damages by enhancing physiological and biochemical processes of rice.

TABLE OF CONTENTS

TABLE OF CONTENTS (Cont'd)

TABLE OF CONTENTS (Cont'd)

TABLE OF CONTENTS (Cont'd)

TABLE OF CONTENTS (Cont'd)

LIST OF FIGURES

LIST OF FIGURES (Cont'd)

LIST OF PLATES

LIST OF APPENDICES

LIST OF APPENDICES (Cont'd)

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
APX	Ascorbate peroxidase
AsA	Ascorbate
BC	Biochar
BRRI	Bangladesh Rice Research Institute
Car	Carotenoid
CAT	Catalase
Cd	Cadmium
Chl	Chlorophyll
CHT	Chitosan
C_i	Intercellular CO_2 concentration
°C	Degree Celsius
cm	Centimeter
Cr	Chromium
CRD	Completely randomized design
CV	Coefficient of variance
cv.	Cultivar
d	Day
DAS	Days after sowing
DAT	Days after transplanting
dH_2O	Distilled water
DHA	Dehydroascorbate
DHAR	Dehydroascorbate reductase
DW	Dry weight
EL	Electrolyte leakage
et al.	*et alibi* (and others)

LIST OF ABBREVIATIONS (Cont'd)

etc.	Etcetera
FAO	Food and Agriculture Organization
FW	Fresh weight
g	Gram
GPX	Glutathione peroxidase
GR	Glutathione reductase
g_s	Stomatal conductance
GSH	Reduced glutathione
GSSG	Glutathione disulfide
GST	Glutathione *S*-transferase
h	Hour
H_2O_2	Hydrogen peroxide
ha	Hectare
i.e.	id est (That is)
K-P buffer	Potassium-Phosphate buffer
LSD	Least significant difference
m	Meter
M	Molar
MDA	Malondialdehyde
MDHAR	Monodehydroascorbate reductase
mg	Milligram
µg	Microgram
ml	Milliliter
mM	Millimolar
N	Nitrogen
Ni	Nickel

LIST OF ABBREVIATIONS (Cont'd)

nm	Nanometer
$O_2^{\bullet-}$	Superoxide radical
$^{\bullet}OH$	Hydroxyl radical
Pb	Lead
P_n	Net photosynthesis
POD	Peroxidase
Pro	Proline
ROS	Reactive oxygen species
RWC	Relative water content
SOD	Superoxide dismutase
SPAD	Soil and plant analysis development
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TCA	Trichloroacetic acid
T_r	Transpiration rate
viz.	Namely
WHC	Water holding capacity
WUE	Water use efficiency

Chapter I

INTRODUCTION

Due to rapid industrial and urban development, the climate is abruptly affected and continuously changing which is accountable for the imbalance of the environmental components posing a great threat. Contamination with heavy metal/metalloids is the most alarming one responsible for the degradation of soil health as well as harmful for the living organisms, and ecological disruptions (Hamsa *et al*., 2017). Among the toxic metal/metalloids, arsenic, aluminium, cadmium (Cd), chromium (Cr), copper, lead (Pb), nickel (Ni), and zinc are the vital pollutants produced through different natural and anthropogenic sources viz., weathering of the parent rocks, metal mining, burning of fossil fuels, smelting, industrial and municipal wastes, vehicle emissions, and excessive application of fertilizers that degrade the physicochemical properties of soil which hampers proliferation of soil microbes, and also threatened the human health (Hassan *et al*., 2019). Though some of these toxic metals are beneficial at a lower concentration required for the successful completion of the life cycle of the plants, but at higher concentrations it becomes phytotoxic and can cause serious damages to the metabolic activities and reduced productivity owing to cause serious threat in the food chain (Hasanuzzaman *et al*., 2019).

Nickel is one of the essential micronutrients of plants particularly needed at the rate of 0.05-10 mg kg^{-1} (dry weight basis) as it is a vital component of metalloenzymes such as urease and hydrogenase where it plays a role of coordinator to join the *S*- and *O*-ligands with the enzymes (Mosa *et al*., 2016). Activity of urease enzymes greatly deteriorates upon Ni deficiency, thus hampering nitrogen (N) metabolisms, and the over accumulation of urea in the shoot of plants resulted in the necrosis and chlorosis of leaf tips. Substantial reduction of hydrogenase enzymes could occur in case of Ni deficiency and is liable for the destruction of the amino acid metabolism, ureide catabolism and intermediary components of ornithine cycles (Hassan *et al*., 2019). Thus, Ni shows a key role in promoting plant growth and development through enhancing the enzymes and metabolic activities in the presence of lower concentrations.

Besides this, the occurrence of Ni at a higher rate could be detrimental for deteriorating the soil health, fertility status, enzymatic activities i.e., acid phosphatase, β-glucosidase, catalase, etc. and ultimately negatively affecting the plants as well as humans and animals (Tanzeem-ul-Haq *et al*., 2021). Excessive Ni interrupts in the cellular components and leads to over accumulation of reactive oxygen species (ROS) such as superoxide radical, $O_2^{\bullet -}$; hydrogen peroxide, H_2O_2; and singlet oxygen, $^1O_2$, etc. as a by-product of these metabolic pathways. Overaccumulated ROS interact with the primary and secondary metabolites and disrupt physiological and biochemical processes leading to oxidative stress in plants (Hasanuzzaman and Fujita, 2012). Nickel-induced oxidative stress causes lipid peroxidation, photosynthetic pigment breakdown, hampers water uptake, membrane damages and in many cases can cause death of the plants (Hasanuzzaman *et al*., 2019).

To combat the Ni-induced damages, plants evolve an array of tolerance or avoidance mechanisms to protect the cellular organelles. Among them osmolytes accumulation, vacuolar sequestration, ion efflux, metal chelation, metal binding proteins and the reduction of metal transportation are the key ways to inhibit the deleterious effects in plants (Rizwan *et al*., 2017). Activation of enzymatic and non-enzymatic antioxidant defense mechanisms in another course of action to counteract the ROS-induced oxidative damages in plants. The enzymatic antioxidants such as ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR), peroxidase (POD), glutathione peroxidase (GPX), glutathione *S*-transferase (GST), superoxide dismutase (SOD) are work in coordination with the non-enzymatic components like as ascorbate (AsA), glutathione (GSH), anthocyanins, tocopherols, flavonoids, etc. to scavenge the ROS in plant cells, thus aids in reducing the ROS-induced oxidative damages (Hasanuzzaman *et al*., 2020).

Researchers adopt different remediation techniques such as leaching, soil flooding, chemical flushing, phytoremediation, immobilizing with the application of organic or inorganic elicitors, etc. to get rid of heavy metal toxicity from the contaminated sites

(Turan *et al*., 2018a). Among the strategies use of organic amendments (i.e., biochar, chitosan, vermicompost, bentonite, humic acid, etc.) are attracted a lot for their sustainability and the ecofriendly nature (Ozfidan-Konakci *et al*., 2018; Tanzeem-ul-Haq *et al*., 2021).

Biochar (BC) is produced through pyrolysis of organic feedstock from agricultural and forest wastes. It is a highly porous charcoal-like substance, rich in organic carbons which is broken thermally in an oxygen deficit condition, and contains some essential nutrients (calcium, magnesium, etc.) of plants (Leng *et al*., 2019). It has capacity to increase the cation exchange sites, surface area, water holding capacity (WHC), porosity, and pH of the soil, thereby enhancing the Ni absorption capacity of soil in the Ni-spiked soils (Turan *et al*., 2018b).

Chitosan (CHT) is a natural biopolymer comprising 2-glucosamine and *N*-acetyl-2-glucosamine processed from the crustacean's exoskeletons. It contained many functional groups of hydroxyl and amino groups providing a cationic polyelectrical surface for Ni chelation in both soil and water (Katiyar *et al*., 2015; Turan *et al*., 2018b). Beside this, the supplementation of CHT enhances soil enzyme activities, soil WHC, and also regulates plant antioxidants activities in the Ni stressed plants (Shaheen *et al*., 2015).

Climate change enhanced the vulnerability of the plants to various abiotic stressors such as water-deficit, waterlogging, salinity, nutrient imbalance, heavy metals, and xenobiotic stress, which accounts for 50% productivity losses worldwide (Saini *et al*., 2018). Among the cereals, rice (*Oryza sativa* L.) is considered as the major grain crop due to its highest calorie consumption by humans, around 85% (IRRI, 2022). It is notable to mention that its growth and productivity greatly hampered under the diverse climatic conditions. Moreover, the yield and quality of crops also adversely affected with heavy metal toxicity, including Ni (Rizwan *et al*., 2017; Tanzeem-ul-Haq *et al*., 2021).

Furthermore, very little literature has enlightened the adversity of Ni-induced oxidative stress and still has no convincing information regarding the use of BC and CHT in mitigating Ni toxicity in rice. Considering the above-mentioned phenomenon, the following objectives has been taken:

i. To access the morphological, physiological, and biochemical responses of rice to nickel stress
ii. To explore the effects of biochar and chitosan on the growth and physiobiochemical attributes of rice under nickel stress
iii. To evaluate the potential of biochar and chitosan to enhance the yield and yield attributes of rice under nickel stress

Chapter II

REVIEW OF LITERATURE

2.1 Rice and its importance

Global population is increasing rapidly and is expected to reach around 9 billion by 2050, thus ensuring food security will be a big challenge. Rice is one of the major crops among the cereals, providing around 20% of the caloric supply to half of the world's population (IRRI, 2022). Rice cultivation occupies around 12% of the world's croplands and 90% of its total production comes from Asian countries (FAOSTAT, 2021; FAO, 2022).

Bangladesh ranks third on the production basis among the rice growing countries, thereby it has a significant contribution to the country's global domestic production (FAO, 2022). For cultivating rice, 75% of the total cropped area and around 80% of the total irrigated land are being used, reported by Imrul *et al.* (2016).

Besides caloric supplies, rice is a major source of some important minerals (i.e., magnesium, iron, phosphorus) and vitamins (i.e., niacin, thiamin, folic acid). It also contains bioactive compounds like anthocyanins, flavonoids, and polyphenols. Rice bran also contains plenty of these bioactive compounds such as amino acids, tocopherols, dietary fibers, and γ-oryzanol (Ravichanthiran *et al*., 2018).

2.2 Crop responses to abiotic stress

Abiotic factors are considered as the most devastating environmental components which adversely affect the distribution and productivity of plants. Due to rapid climate change, alteration of different environmental services has been observed, thus plants become more susceptible to the abiotic stresses during ontogenesis (Bulgari *et al*., 2019; Sachdev *et al*., 2021). In nature, most common abiotic factors are salinity, drought, waterlogging, drought, heat shock, chilling/freezing, nutrient deficiencies heavy metals, and xenobiotic, etc. are constantly faced by plants and limit the crop production up to 50% (Saini *et al*., 2018). These environmental factors abruptly affect the growth and development of the plants through reducing the plants cellular, physiological, and metabolic activities due to the combined effect of one or more of these factors (He *et al*., 2018).

Plant growth is adversely affected upon exposure to salt stress. To explore the extent of salt-induced damages in the *Phaseolus vulgaris*, ElSayed *et al.* (2021) exposed the plants to 200 mM NaCl for 3 hours (h) at 14 days after sowing (DAS) and found the highest increment of the malondialdehyde (MDA) and H_2O_2 content by 39 and 50%, respectively. The growth rate of the plants in terms of fresh weight (FW) and dry weight (DW) of the salt affected plants were declined under salt stress (ElSayed *et al*., 2021). Besides this, Derbali *et al*. (2020) screened four cultivars of quinoa (*Chenopodium quinoa* cvs. Tumeko, Red Faro, Kcoito and UDEC-5) at different concentrations of NaCl, viz., 0, 100, 300 and 500 mM for 2 weeks and reported that Tumeko and Kcoito exhibited a lower content of Chlorophyll (Chl) *a*, Chl *b* and Chl (*a*+*b*) in the salt affected plants, whereas the contents were remained similar in the Red Faro and UDEC-5 varieties. In cv. UDEC-5, the highest proline (Pro) accumulation was found, but surprisingly the least accumulation of MDA and H_2O_2 were observed in the 300 mM NaCl treated plants. Finally concluded that the Red Faro and UDEC-5 were the most tolerant cultivars under saline conditions.

The root length, shoot length, root FW, root DW, shoot FW, and shoot DW of the maize (*Zea mays* cvs. Pearl and Malka) plants were significantly retarded under drought stress (60% field capacity). Moreover, the drought stress notably declined photosynthetic pigments (viz., Chl *a*, Chl *b*, and carotenoid, Car) and increased the osmolyte accumulation (i.e., Pro, glycine betaine, and soluble sugars) in the maize cultivars (Parveen *et al*., 2019).

Drought stress at different growth stages of wheat (*Triticum aestivum* cv. Galaxy 2013), viz., tillering, flowering, and grain filling stages retarded the growth, physiology, and yield attributes. The plant height by 52, 43, and 28%, number of fertile tillers by 22, 26, and 32%, spike length by 40, 38, and 30%, number of spikelets spike^{-1} by 28, 31, and 41%, number of grains spike^{-1} by 19, 18, and 30%

were declined, respectively at the tillering, flowering, and grain filling stages of the plants when exposed to the drought conditions (Zulfiqar *et al*., 2022).

Excessive water at the root zone caused an alteration of the growth and development of the plants due to hypoxia (O_2 insufficiency) or anoxia (lack of O_2) leading to some important physiochemical changes in soil properties such as pH, redox potential, and oxygen access (Pan *et al*., 2021). Waterlogging to the wheat plants for 96 h and 168 h increased the MDA accumulation by 20 and 70%, respectively over the control, whereas the H_2O_2 content increased by 30 and 60%, respectively. The non-enzymatic antioxidants, such as phenolics and thiols levels were increased at the 96 h and 168 h waterlogging affected plants (Katerova *et al*., 2021). Hasanuzzaman *et al*. (2022) exposed soybean plants (*Glycine max* cv. Sohag) under different durations of waterlogging, viz., 3, 6, and 9 days (d) and reported that the growth, physiological, and oxidative stress indicators were affected in the plants in a dose dependent manner.

High temperature accelerated leaf chlorosis, necrosis, membrane injury, denatured proteins, oxidative damage, and dehydration in plants (Kaushal *et al*., 2016). Bhardwaj *et al*. (2021) reported that upon exposure to the heat stress (32/20 °C, day/night; 12 h), it notably reduced the pollen germination, pollen viability, stigma receptivity, and ovule viability of the heat sensitive genotypes of lentil (*Lens culinaris*) compared to the control.

2.3 Nickel stress

Nickel is one of the 23 most toxic metal pollutants which adversely disrupts the ecosystem and human health through incorporating with soil and water bodies (Duda-Chodak and Baszczyk, 2008). Both natural and anthropogenic activities are responsible for the Ni contamination and it is ubiquitously found in the soil and surface water in lower than 100 and 0.005 mg kg^{-1}, respectively (McIlveen and Negusantil, 1994; McGrath, 1995).

Nickel is an essential micronutrient of the plants required at the rate of 0.05–10 mg kg^{-1} dry weight, but induces a phytotoxic effect on plants at a higher concentration (Ragsdale, 1998). Owing to its essentiality in plant growth and development, Ni is involved in different biological factors. Thus, deficiency of Ni is responsible for declining the growth, occurs senescence and necrosis of young leaves, perturbs N assimilation and hampers iron uptake in plants (Bai *et al*., 2006; Wood and Reilly, 2007). Besides these, Ni is a vital component of many enzymes such as urease, and reduction in its activity disturbs N assimilation, and metabolic reactions such as hydrogen metabolism, acetogenesis, and methanogenesis taking place by bacterial activity (Ragsdale, 1998; Kupper and Kroneck, 2007). Moreover, synthesis of phytoalexin and confronting different stress inducing agents in plants is also a key role of Ni (Wood and Reilly, 2007).

2.4 Crop responses to nickel stress

The extent of the Ni-induced damages to the plants depends on the concentrations of Ni, soil and crop type, genotypic variation, and also on the growth stages of the plants.

2.4.1 Effect on germination and seedling establishment

The vetch seedlings were exposed to different concentrations of Ni, i.e., 1, 5, 10, 50, 100, 500, and 1000 μM Ni, Ivanishchev and Abramova (2015) reported that the sustainability index to the seedlings were dropped from gradually from 5 μM to 1000 μM at a rate of three times lower than the initial concentrations (1 μM).

The germination percentage of soybean (*Glycine max* L. cv. SL-525) were declined upon exposure to 2 mM Ni to the media reported by Sirhindi *et al*. (2015).

Gupta *et al.* (2017) exposed ten pearl millet varieties (RIB 494, RIB 57, RIB 3135, RIB 192, RIB 20K86, RHB 90, RHB 121, RHB 58, RHB 173, and RHB 177), five finger millet varieties (PR 202, PES 110, GPU 28, GPU 20, and GPU 66), and three oat varieties (Quandel jai, H-Javi 08, and NDO 1) at different concentrations of Ni (0, 10, 15, 20, 25, 30, and 40 ppm), and reported that highest inhibition of germination was found in oats at 30 ppm by 23%, whereas no seeds were germinated at 40 ppm.

2.4.2 Effect on growth and development

Growth of mungbean (*Vigna radiata* L. var. AZRI-2006mun) was adversely affected with the increase of Ni concentrations. Upon incorporation of Ni to the soil at the rate of 20, 40, and 60 mg kg^{-1}, declined the root length (21, 21, and 64%), shoot length (27, 36, and 66%), root FW (27, 33, and 71%), shoot FW (36, 47, and 74%), root DW (29, 41, and 74%), and shoot DW (36, 46, and 75%) of the plants (Ali *et al*., 2015).

Upon exposure to 2 mM Ni caused a reduction in the root length, shoot length, total Chl, Chl *a*, Chl *b*, and Chl *a*/*b* of the soybean plants compared to the controls (Sirhindi *et al*., 2015).

Khan *et al*. (2016) exposed two different varieties of mustard (*Brassica juncea* L. cvs. Varuna and RH30) to Ni stress, and found the leaf area and DW were reduced in both varieties. Moreover, the total Chl content also declined in both varieties under Ni stress, and more prominent declination was seen in cv. RH30 compared to the cv. Varuna.

Toxic effects of Ni were clearly noticeable in dose dependent manner on the growth of seedlings. In comparison to the shoots, the roots were more injured by the toxic effect of Ni in all the varieties of finger millet, pearl millet, and oats. However, in the finger millet and pearl millet the magnitude of the root length was least, in contrast at 20 ppm, there was negligible root growth found in oats. Besides this, the color of the roots and stem portion of the shoots become brownish under Ni stress and the shoot length was also declined (Gupta *et al*., 2017).

Upon exposure to 4 mM Ni, stunted growth of soybean (*G. max* L. cv.SL-525) plants has been reported by Mir *et al*. (2018). Plants which were exposed to Ni stress showed a significant reduction in the shoot length by 20 and 30%, and root length by 33 and 46% at days 20 and 24, respectively. A drastic declination of total Chl content was also found by 34 and 44% at days 20 and 24, respectively in the Ni treated soybean plants (Mir *et al*., 2018).

The shoot length, shoot FW, and leaf area of Indian mustard (*B. juncea* L. cv. Type 59) were decreased in the Ni affected plants. Induction of 150 μM Ni to the plants caused a rapid reduction of the shoot length by 41%, shoot FW by 36%, and leaf area by 48% over the controls (Zaid *et al*., 2019).

Helaoui *et al.* (2020) reported that when the alfalfa (*Medicago sativa*) plants were treated with 50, 150, 250, and 500 mg kg^{-1} Ni for 60 days, the root length, shoot length, root FW, shoot FW, root DW, and shoot DW reduced notably in a concentration-dependent manner. The Chl *a*, Chl *b*, and Chl (*a*+*b*) were declined with the increase of Ni concentration, whereas the ratio of Chl *a*/*b* was increased in the Ni affected plants.

Two varieties of mungbean (*V. radiata* cv. M-93 and M-1) were exposed to Ni stress by Aqeel *et al.* (2021) and reported that the growth of the plants was severely affected with the toxicity of Ni. Upon increase of Ni concentrations, the plant growth of both varieties in terms of number of leaves, plant height, leaf area, and leaf chlorosis were increased significantly, whereas the shoot FW and root DW showed non-significant differences in the Ni treated plants.

Naheed *et al*. (2022) imposed Ni stress to the quinoa (*C. quinoa*) at 100, 200, and 400 μM doses and revealed that the root and shoot length were slightly affected at 100 μM, however, a profound declination of these attributes was observed at 400 μM Ni stressed plants.

2.4.3 Effect of physiology and metabolism

Nickel toxicity adversely affects the physiology and metabolic activity of the plants. Upon exposure to Ni stress to mustard (*B. juncea* L. cvs. Varuna and RH30) severely affected the gas exchange parameters, i.e., net photosynthesis (P_n), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) of the plants. The P_n, T_r, and C_i were declined more prominently by 39, 28, and 28%, respectively in cv. Varuna, while these attributes reduced by 49, 40, and 42%, respectively in RH30 under Ni stress (Khan *et al*., 2016).

Plants accumulate a higher level of Pro under Ni stress. Gupta *et al*. (2017) reported that the free Pro accumulation was accelerated with the increase of Ni concentrations. The Pro accumulation was increased by 4.3-, 4-, and 2.8-fold in the shoots of finger millet, pearl millet, and oats, respectively, whereas in roots, it was increased by 5.7-, 5.4-, and 3.5-fold, respectively at 40 ppm.

Mir *et al*. (2018) reported that Ni stress to the soybean plants lead to osmotic shock indicated by declined relative water content (RWC) of the plants. However, to mitigate this osmotic imbalance, plants accumulate a higher level of Pro in the Ni treated plants by 101% at day 20 and by 129% at day 24 over the controls.

Induction of Ni stress led to decrease of P_n, g_s, and C_i by 44, 46, and 38% in the mustard plants. Moreover, the water use efficiency (WUE) of the plants was also reduced by 39% upon exposure to Ni stress (Zaid *et al*., 2019).

Imposition of Ni stress to mustard plants showed a reduction of water potential by 1.8- and 2.1-times at 30 and 60 DAS, respectively over the control. Moreover, the photosynthetic attributes such as P_n, g_s, and C_i were also declined in the Ni treated plants (Khan *et al*., 2020a).

The gas exchange parameters such as P_n, g_s, transpiration rate (T_r), and C_i exhibited perturbations under metal stress. Both the varieties of mungbean showed a noticeable declination of P_n, g_s, T_r, and C_i when exposed to the Ni stress to the plants reported by Aqeel *et al*. (2021).

2.4.4 Effect on yield and yield attributes

The number of seeds plant^{-1} and seeds weight plant^{-1} were significantly declined in the Ni stressed mungbean plants. Ali *et al.* (2015) reported that upon exposure of Ni stress to the plants, the seed weight plant^{-1} was decreased by 22, 31, and 51%, respectively at 20, 40, and 60 mg kg^{-1} soil Ni contamination.

Aqeel *et al.* (2021) reported that yield attributes such as seed yield plant^{-1} and 100-seed weight were affected by Ni stress in both varieties of mustard (*B. Juncea* cv. M-93 and M-1). The highest reduction in the seed yield plant^{-1} by 70% was recorded in the M-1 variety.

2.5 Nickel-induced oxidative stress and antioxidant defense system

Due to the sessile nature of the plants, they undergo adverse environmental events. These environmental stresses are occurring due to both climate change and anthropogenic activities which are contributing to induce oxidative stress through excessive generation of ROS. The primary sites of ROS generation are chloroplasts, mitochondria, peroxisomes, apoplast, and plasma membranes, but the chloroplast is considered as the leading ROS producing site (Singh *et al*., 2019). These stresses also hinder CO_2 availability and carbon fixation, this disturbs photosynthetic processes and yields ROS (Gill and Tuteja, 2010).

2.5.1 Oxidative stress under nickel stress

Lipid peroxidation of the roots and shoots of finger millet, pearl millet, and oats as indicated by MDA content was increased significantly with the increase of Ni doses. However, the lowest MDA content was found in oats at 30 ppm Ni (Gupta *et al*., 2017).

Oxidative stress indicators such as H_2O_2, MDA, and electrolyte leakage (EL) were increased enormously in the Ni stressed plants. Upon exposure to Ni stress, the contents of H_2O_2 by 45 and 111%, MDA by 158 and 179%, and EL by 543 and 555%, respectively at days 20 and 24 in soybean plants over the control (Mir *et al.*, 2018). Zaid *et al.* (2019) also reported that Ni stress increased the EL and H_2O_2 contents with the increase of Ni concentrations. However, a highest level of H_2O_2 by 39% was observed at the 150 µM Ni stressed mustard plants (Zaid *et al*., 2019). Similarly, Khan *et al*. (2020a) also found that mustard plants treated with Ni accelerated the thiobarbituric acid reactive substances (TBARS) and EL by 86% and 2.9-fold compared to the control at 60 DAS.

Naheed *et al*. (2022) reported that plants treated with Ni showed a significant increase of the H_2O_2 and MDA content in a dose dependent manner. Upon exposure of 100, 200, and 300 μM Ni to the quinoa plants, the H_2O_2 content was increased by 2-, 5-, and 9- folds, respectively over the control. Similarly, the MDA content was also augmented by 2.5-, 3.75-, and 5-fold, respectively under 100, 200, and 300 μM Ni stressed quinoa plants.

2.5.2 Antioxidant defense system under nickel stress

Plants can overcome the metal-mediated oxidative damages with their inherently equipped antioxidant defense system which comprises enzymatic and non-enzymatic antioxidants. The enzymatic antioxidants such as CAT, SOD, GPX, APX, MDHAR, DHAR, DHAR, GR, as well as non-enzymatic antioxidants such as GSH, AsA, tocopherols, flavonoids, etc. which are worked together in order to detoxify ROS in plants (Gill and Tuteja, 2010).

Mir *et al.* (2018) found the content of AsA declined at both days 20 and 24, but a highest reduction was observed at days 24 by 32%, whereas the dehydroascorbate (DHA) content increased under Ni stress. Moreover, the GSH content showed a variable activity which decreased by 12% at day 20, but increased at day 24 by 5%. In contrast, the glutathione disulfide (GSSG) content increased noticeably by 25 and 30 at days 20 and 24, respectively. Other antioxidants such as APX, MDHAR, DHAR, GR, GPX, GST, and CAT activities were decreased upon exposure of Ni stress to the soybean plants.

A notable increase of SOD, APX, GR, and GPX activities were reported by Khan *et al*. (2020a) in the Ni affected mustard plants. At 30 DAS, the activities of SOD, APX, GR, and GPX were accelerated by 39, 58, 56 and 20%, respectively, whereas the acceleration of their activities were more at 60 DAS by 46, 63, 68 and 61%, respectively compared to the controls.

Saroy and Garg (2021) reported that the Ni stressed *Cajanus cajan* plants, the AsA, DHA, GSH and GSSG were increased in a concentration dependent manner. However, a significant enhancement of the activities of APX, MDHAR, DHAR, GR, CAT, SOD, and POD were also found in the Ni treated plants.

Moreover, plants treated with Ni showed a noticeable increase of APX, SOD, and CAT activities in the quinoa plants. Upon exposure of 400 μM Ni, 3-, 4-, and 8-fold increase in the activities of SOD, APX and CAT was found in the Ni affected quinoa plants (Naheed *et al*., 2022).

2.6 Responses of rice to nickel stress

Two rice varieties (*O. sativa* cvs. KSK-282 and Basmati-385) were exposed to Ni stress at the rate of 100, 250, 500, and 1000 ppm and found that at 1000 ppm, the germination rate and germination percentage declined noticeably in both varieties. However, maximum inhibition of seed germination was found in the cv. KSK-282 compared to the cv. B-385. Besides, the Chl *a* and Chl *b* contents were decreased in the Ni stressed plants, and highest reduction was observed in the cv. KSK-282 than cv. B-385 (Jamil *et al*., 2014)

Aziz *et al*. (2015) reported that upon exposure to 20 and 40 mg kg^{-1} soil Ni significantly decreased the root and shoot DW of rice (cv. Super Basmati) in a dose dependent manner. Moreover, the Ni toxicity leads to the declination of total Chl, P_n, g_s, and T_r of the Ni stressed rice plants.

A sensitive cultivar of rice (cv. Malviya-36) was exposed to 200 μM Ni, a notable reduction in the height, biomass production, photosynthetic pigment accumulation, as well as increased the level of Ni in the plants were observed. Moreover, Ni-induced oxidative stress indicated by increased level of ROS such as $O_2^{\bullet-}$, H_2O_2, and hydroxyl radicals ($^{\bullet}OH$), and accelerated lipid peroxidation in the rice plants. However, the antioxidant enzymes such as SOD, CAT, and GPX were increased under Ni stress in order to mitigate the ROS-induced damages (Rajpoot *et al*., 2016).

Exposure of Ni stress to the rice plants revealed that the root and shoot length, FW, and DW were declined with a concomitant reduction of the contents of photosynthetic pigments. Nickel stress also induced a deleterious effect on plants through generating higher amounts of H_2O_2 and MDA contents in the plants in a dose dependent manner. The H_2O_2 content was increased by 2.49-fold in roots and 3.62-fold in shoots at 200 μM Ni. However, a similar trend of increase of MDA content in the Ni stressed plants by 3.62- and 2.49-fold, respectively in shoots and roots was found at 200 μM over control. The enzymatic and non-enzymatic antioxidants such as AsA, GSH, POD, and CAT were increased and the SOD was decreased in the Ni stressed rice plants (Rizwan *et al*., 2017).

Hasanuzzaman *et al*. (2019) reported that induction of Ni stress at 0.25 and 0.5 mM doses lead to a declination of growth attributes of rice seedlings such as shoot length (20 and 24%), root length (32 and 36%), FW (27 and 43%), and DW (15 and 27%), respectively. The photosynthetic pigment accumulation, i.e., Chl *a* (15 and 31%) and Chl *b* (22 and 31%) were also decreased in the Ni stressed plants. Moreover, Ni stress at 0.25 and 0.5 mM doses accelerated the oxidative stress indicated by increased H_2O_2 by 28 and 35%, and TBARS by 172 and 199%, respectively. The antioxidants such as AsA, CAT and GST were declined whereas the DHA, GSH, GSSG, APX, MDHAR, DHAR, GR, GPX, and SOD were increased in the Ni affected plants.

Shoot length and FW were adversely affected when the plants were treated with Ni, whereas the effect of Ni on the root length was not significant. Moreover, the Ni toxicity triggers the excessive accumulation of ROS (viz., $O_2^{\bullet-}$, H_2O_2, and $^{\bullet}OH$) which ultimately caused a notable increment of the MDA levels in the rice seedlings. The GR and vitamin E were only increased in the Ni stressed plants (Khan *et al*., 2020b).

2.7 Strategies to mitigate nickel stress in crops

Plants adopt different types of physical barriers in order to defend the toxic effect of metals. When plants undergo heavy metal toxicity, plants change their morphological structures including thick cuticles, trichomes, and cell walls (Harada *et al*., 2010). Plants can synthesize secondary metabolites such as GSH, Pro, histidine, and spermidine which accelerate the antioxidant activities and thereby reduce the adverse effect of heavy metals stress in plants (Hauser, 2014). Moreover, the roots of plants also excrete different organic complexes that reduce the mobility and bioavailability of metals in the soils that reduce the entry of metal ions in the plant parts (Hassan *et al*., 2019).

Remediation of Ni from soil is the prime concern as the concentration of Ni in the soil is increasing day by day due to rapid industrialization and anthropogenic activities. Thus, use of different types of remediation techniques such as use of hyperaccumulator plants, metal chelators, metal accumulating microbes (i.e., *Aspergillus* and arbuscular mycorrhiza), plant growth promoting rhizobacteria, addition of organic and inorganic elicitors, and cultivation of genetically modified plants, etc. have been adopt to reclaim the soil from Ni toxicity (Audet and Charest, 2008; Namgay *et al*., 2010; Hassan *et al*., 2019).

To alleviate the Ni-induced damages, phytohormones are exogenously applied to the plants that can regulate growth and retard the inhibitory effect of toxic metals. Ali *et al*. (2015) supplemented Ni stressed mungbean plants with gibberellic acid, and found that the accumulation of Ni in the roots and shoots were declined, therefore improved the growth and yield attributes of the plants. Sirhindi *et al.* (2015) also applied jasmonic acid to the Ni stressed soybean plants and reported that the oxidative stress indicators such as MDA and H_2O_2 were declined and improved the antioxidant activities. Moreover, similar results were also reported on application of salicylic acid (Zaid *et al*., 2019) and ethophan (Khan *et al*., 2020a) to different crop species. Other signaling molecules, such as nitric oxide (Rizwan *et al*., 2018a), silicon (Abd_Allah *et al*., 2019, Hasanuzzaman *et al*., 2019), and sodium hydrosulfide (Valivand and Amooaghaie, 2021) were also used in different crops to mitigate the Ni-induced damages in plants.

2.8 Role of biochar on plant growth and stress mitigation

Incorporation of BC is an alternative and sustainable way for retaining the physicochemical properties of soil. Improvement of soil physicochemical properties have an immediate effect on the growth and development of plants (Turunen *et al*., 2020). When the soil is supplemented with BC, it improves water holding capacity, pH, cation exchange capacity, nutrient availability, fertility status, microbial activities, carbon sequestration, and also absorbs toxic metals. Therefore, the BC incorporation increases organic matter content, improves soil fertility status, and thereby increases the productivity of crops (Sofy *et al*., 2022; Hasanuzzaman *et al*., 2021). Besides these, BC has a higher water holding capability which enhances water and nutrient availability in the soil by protecting them from leaching losses, and also increases fertilizer use efficiency of the plants (Borges *et al*., 2020). Moreover, the water retention properties and saturated hydraulic conductivity have also been elevated as a result of BC application which ultimately reduce the irrigation requirements and promotes crop cultivation under water limited conditions (Nikolaou *et al*., 2020; Abideen *et al*., 2020). In Figure 1, the role of BC on soil and plant level have been depicted.

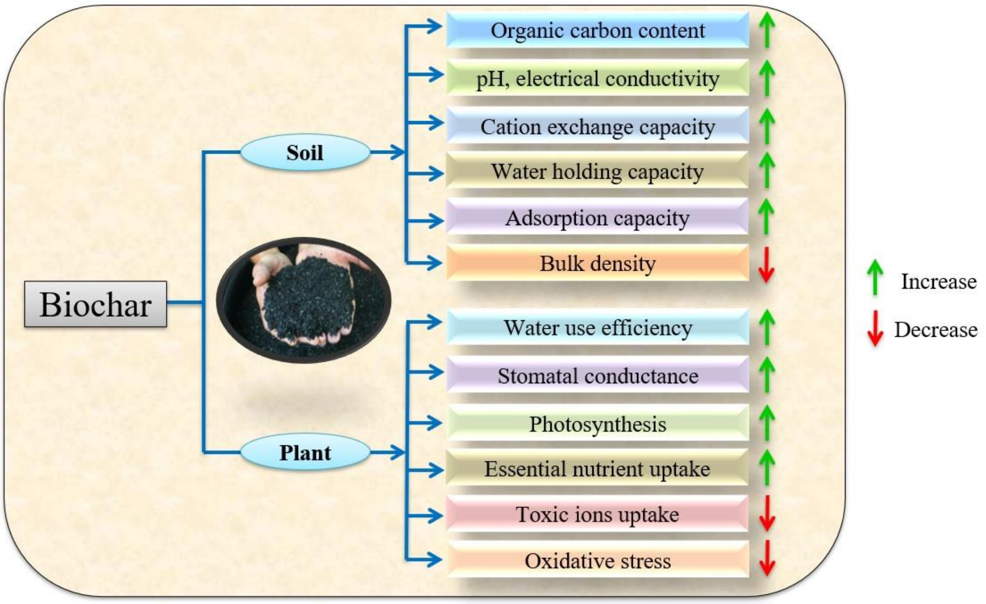


Figure 1. Possible effects of biochar at both soil and plant level

Upon exposure to 25 and 50 mM NaCl, the photosynthetic attributes such as P_n, g_s, and T_r were decreased in the plants. But when BC was supplemented at the rate of 5% of the total soil mass, increased P_n, g_s, T_r and also antioxidant enzyme activities, i.e., CAT, POD, and SOD in the salt affected plants. Thus, the improvement of physiological responses of plants ultimately increased the yield of the plants. (Akhtar *et al*., 2015).

Under drought conditions, seed vigor and germination percentage of the soybean were greatly hampered. Hafeez *at el*. (2017) demonstrated under water deficit conditions seed vigor and germination percentage are declined significantly compared to the well-watered conditions. But when BC was supplemented at 5 t ha^{-1} and 10 t ha^{-1}, these parameters were enhanced under drought.

Kanwal *et al*. (2018) demonstrated the leaf water potential and osmotic potential has been reduced in the salt stressed wheat plants. Under stressful conditions, incorporation of BC at 1 and 2% increased the water potential by 14 and 16%, respectively at 150 mM NaCl. A higher value of leaf water potential was found in the case of NARC 2009 as compared to NARC 2011. Moreover, leaf osmotic potential was also increased by 4, 6 and 6% at 1% BC, while 10, 7 and 8% at 2% BC application under 50, 100, and 150 mM NaCl, respectively. Higher values of osmotic potential were recorded in the case of NARC 2009.

Ali *et al.* (2018) reported that the BC supplementation under Cr-contaminated soil enhanced the plants growth and development through enhancing Chl *a*, Chl *b*, total Chl and Car content of mustard plants. Similarly, in the Pb stressed pea plants, BC application increased the contents of Chl *a* and Chl *b*, thus resulting in better growth and development (Haider *et al*., 2019).

Supplementation of BC to the 10 dS m^{-1} saline soils increased the P_n, leaf area index, and enhanced K^+ uptake in wheat, thus decreasing Na^+/K^+ ratio. Amendment with BC improved the grain formation and salt endurance capacity of plants (Huang *et al*., 2019). Similarly, Parkash and Singh (2020) also reported that g_s and P_n were increased, whereas decreased leaf temperature and EL in the *Solanum melongena* plants when exposed to salt stress at 2 and 4 dS m^{-1} NaCl but supplemented with 5% BC.

Tanzeem-ul-Haq *et al*. (2021) also reported that application of BC in the soil affected by Ni significantly mitigated the generation of $O_2^{\bullet-}$, H_2O_2, and MDA, whereas, upgraded the activities of APX, CAT, and SOD in the leaves of lentil over control.

Zaheer *et al*. (2021) demonstrated that growth and yield attributes of wheat such as plant height, spike length, number of spikelets spike^{-1}, number of grains spike^{-1}, 1000-grain weight, and grain yield plant^{-1} were adversely affected when drought stress was imposed at tillering and grain filling stage. But supplementation with BC at different rates (28 and 38 g kg^{-1}) improved these parameters, thereby increasing the grain yield plant^{-1} of wheat.

Exposure to salt stress significantly and dose-dependently increased the contents of MDA, H_2O_2, and EL in jute leaves. The MDA content was increased by 24, 36, and 49%, and H_2O_2 content increased by 51, 68, and 76% in plants upon exposure to 50, 100, and 150 mM NaCl, respectively over the control. Moreover, the increase of EL in jute leaves is an indication of salt-induced membrane damage which was increased by 25, 34, and 39%, respectively, following exposure of plants to 50, 100, and 150 mM, respectively. The oxidative stress indicators such as MDA, H_2O_2, and EL were declined with the BC amendment with the upregulation of antioxidant defense system of the plant (Hasanuzzaman *et al*., 2021).

2.9 Role of chitosan on plant growth and stress mitigation

Chitosan (CHT) is an unbranched polymer of β-1,4-ᴅ glucosamine. This biopolymer is obtained from chitin which is a co-polymer of *N*-acetyl-ᴅ-glucosamine and ᴅ-glucosamine. These co-polymers are the main constituent of arthropods exoskeleton, and the chitin is found in various organisms such as fungi, diatoms, mollusks, and fresh and marine water sponges. The shell of shrimps and crabs is the largest and convenient source of this natural polymer. Chitin is considered as the second largest renewable carbon source with the production of around 10^{11} t year^{-1} after cellulose. Therefore, utilization of these products has gained immense interest throughout the world (Kurita, 2006). Moreover, the CHT induces resistance against abiotic stresses such as drought salinity, heat stress, and heavy metal toxicity through augmenting various physiological and biochemical pathways of plants (Malerba and Cerana, 2015). Role of CHT under stressful conditions has been depicted in Figure 2.

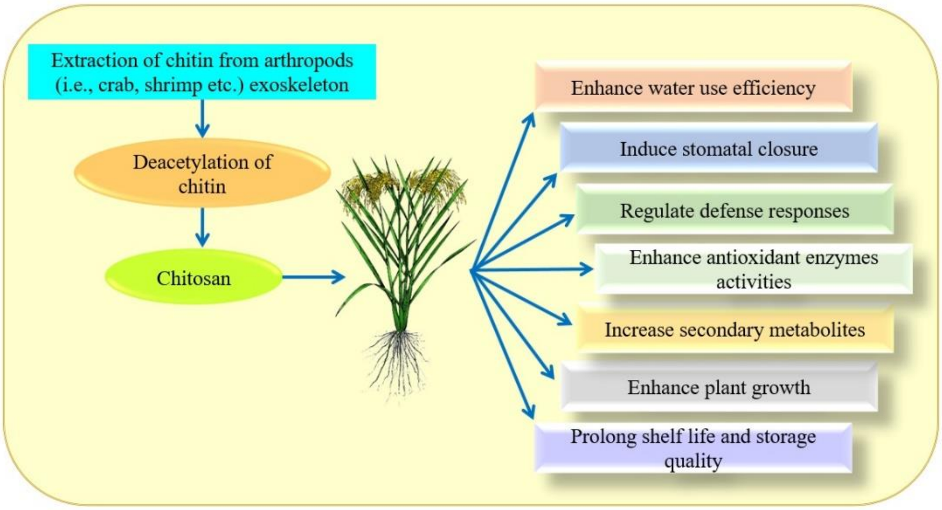


Figure 2. Role of chitosan in plants

Zong *et al*. (2017) reported that foliar application CHT enhanced the plant growth and Chl content, and decreased MDA levels in the mustard plants under Cd stress. Upon application of CHT, the MDA content decreased by 40, 47, and 60%, respectively in comparison to the Cd stressed plants only. Moreover, the C_i, T_r, g_s, and P_n were also increased by 6, 52, 99, and 61%, respectively when the Cr affected plants were supplemented with CHT.

Application of CHT nanoparticles increased the leaf area, soil and plant analysis development (SPAD) value, number of grain spike^{-1}, grain yield, and harvest index of the drought affected barley plants. Moreover, the use of CHT nanoparticle in the plants notably increased the RWC, 1000-grain weight, grain protein, Pro content, CAT and SOD activity under drought stress (Behboudi *et al*., 2018).

Upon exposure to salt stress to the rosemary plants caused a decline of the plant growth, photosynthetic pigments, minerals contents, and essential oil percentage, whereas increased the sodium (Na^+) and chloride (Cl^-) ions accumulation in the plants. But when the salt affected plants was supplemented with CHT suppressed the negative effect of salt stress and increased the plant growth, pigment accumulation, essential oil content and also minerals. Moreover, toxic ion contents such as Na^+ and Cl^- were also reduced when CHT was applied to the plant (Helaly *et al*., 2018).

Salt stress significantly retard the root length and plant height, whereas the foliar supplementation of CHT ameliorated the salt-induced adverse effect in maize seedling. Moreover, the CHT application in the salt affected maize plants increased the activities of SOD, CAT, and APX enzymes by 35, 45, and 120%, in contrast, decreased the GPX activity by 20% compared to the salt stressed maize seedlings (Turk *et al*., 2019).

Water stress in barley negatively affected the morphological, physiological, and yield traits, whereas the EL, MDA, soluble sugars, sucrose, and starch contents were increased in the plants. However, a profound increase of plant height, leaf numbers, Chl contents, and RWC of the drought stressed plants were found with the CHT application, in contrast CHT treatment led to decrease of EL and MDA content of the barley. Furthermore, yield attributes such as 1000-grain weight, grain yield, and biological yield were also enhanced with CHT under drought stress (Hafez *et al*., 2020).

Liu *et al*. (2021) demonstrated that the foliar spraying of CHT improved seedling growth, decreased MDA content and ROS accumulation in the leaves of wheat, whereas decreased the Cd deposition in the roots and shoots of the plants under Cd stress. The Cd stress was alleviated by CHT application with the increase of SOD, POD, and CAT by 7-13%, 17-33%, and 20-26%, respectively in the plants. Moreover, the exogenous supplementation of CHT increased the soluble protein and soluble sugar content in the Cd affected wheat plants.

Chapter III

MATERIALS AND METHODS

A brief description of the experimental location, climate of the experimental site, planting materials, treatments, experimental design and layout, soil preparation, fertilization techniques, seed sowing, seedling transplanting, different intercultural operations, data collection procedures and statistical analysis are being described in this chapter.

3.1 Location

The experiment was conducted at the experimental shed house of the Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh which is located at 90º 77´ E longitude and 23º 77´ N latitude with an altitude of 8.6 meter above the sea level. This site belongs to the Agro-ecological zone of "Madhupur Tract', AEZ 28. The geographical location of the experimental site has been depicted in the Appnendix I.

3.2 Climate and weather conditions of the experimental site

The climate of the experimental site is sub-tropical. High temperature, high relative humidity and heavy rainfall are the salient features of this zone. Gusty winds in *Kharif* and scattered rainfall in *Rabi* with low temperature are also observed in this zone. The field experiment was conducted from December 2020 to April 2021, and the monthly maximum and minimum temperature, relative humidity, and rainfall during this period have been collected from the Bangladesh Meteorological Department, Dhaka, Bangladesh and shown in Appendix II.

3.3 Plant materials

A rice variety of BRRI dhan96 was selected as the plant material for conducting the study. This is a substitute of the widely cultivated variety BRRI dhan28 for *Boro* season of Bangladesh which was released by Bangladesh Rice Research Institute (BRRI) in 2020.

3.3.1 Salient features of BRRI dhan96

The salient features of BRRI dhan96 has given below-

- The variety was developed through a hybridization method between BR(Bio 9787-BC2-63-2-2) and *O. rufipogon* (IRGC103404).
- Average height of the variety is 87 cm.
- Flag leaf is upright.
- Grain size is medium short and golden colored.
- 1000-grain weight is 18.4 g.
- The average grain yield is about 7 t ha^{-1}.
- Amylose content is 28% and protein content is 10.4%.

3.4 Collection of seed

Seeds of BRRI dhan96 were collected from Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh.

3.5 Sprouting of seed

Seeds were washed with running water to remove the dust and soaked in water for 24 h. After then the seeds were taken out from the water and kept in a gunny bag for sprouting. After 48 h, the seeds started sprouting and it will be ready for sowing in the seedbed in 72 h.

3.6 Preparation of seedbed

A raised seedbed was prepared by puddling with repeated ploughing and laddering to grow the seedlings. Weeds were removed from the seedbed and no fertilizers were applied in the seedbed.

3.7 Seed sowing to the seedbed

Sprouted seeds were sown uniformly on the seedbed on November 16, 2020 for raising seedling for transplantation. Gentle irrigation was provided when necessary.

3.8 Preparation of pot soil

To prepare the soil for transplanting the seedlings, 15 kg of sun-dried and crushed soil was filled in the plastic pots (18-inch depth and 14-inch diameter). The recommended basal dose of the fertilizers was incorporated to the soil and puddling was done to prepare the soil for transplantation.

3.9 Application of fertilizers

Recommended dose of fertilizer for BRRI dhan96 (BRRI, 2020) as follows-

Fertilizers	**Dose (kg ha^{-1})**
Urea	160
Triple superphosphate (TSP)	40
Muriate of potash (MoP)	60
Gypsum	40
Zinc sulphate	4

During final soil preparation, a full dose of TSP, gypsum and zinc sulphate, and half of MoP were incorporated into the pots. Urea was top-dressed in three installments, viz., after seedling recovery, during the vegetative stage and before panicle initiation. Rest of the MoP was applied with the third installment of urea.

3.10 Transplanting of seedlings

Fourty-days-old seedlings were carefully uprooted from the seedbed and kept in shade. For uprooting the seedlings, an adequate amount of water should be irritated to the seedbed to soften the soil which minimize injuries and root damages. Four seedlings were transplanted in each pot maintaining 15 cm × 15 cm spacing. Each pot contains four hills.

3.11 Treatments

The experiment consists of the following 12 treatments as follows:

i. Control
ii. BC (0.5 g biochar kg^{-1} soil)
iii. CHT (200 mg chitosan-100 L^{-1})
iv. Ni_1 (0.5 mM $NiSO_4{\bullet}6H_2O$)
v. Ni_1+BC
vi. Ni_1+CHT
vii. Ni_2 (1.0 mM $NiSO_4{\bullet}6H_2O$)
viii. Ni_2+BC
ix. Ni_2+CHT
x. Ni_3 (2.0 mM $NiSO_4{\bullet}6H_2O$)
xi. Ni_3+BC
xii. Ni_3+CHT

Biochar (Merck Pvt. Ltd, India) at 0.5 g kg^{-1} soil was supplemented to the sets of pots and thoroughly mixed with the soil during final soil preparation. After seedling establishment at 21 days after transplanting (DAT), sets of plants were irrigated with Ni solutions, viz., 0.5, 1.0, and 2.0 mM $NiSO_4{\bullet}6H_2O$ concentrations, whereas only water was irrigated to the controls. Nickel solutions were applied at 21, 35, 49, and 56 DAT. Foliar spraying of CHT (Chitosan-100; Wako, Japan) was done at 200 mg L^{-1} and the solution was prepared after dissolving in 1% acetic acid by maintaining a neutral pH 7.0. Spraying was done at 7 d interval from 14 DAT to panicle initiation.

3.12 Experimental layout and design

The experiment was conducted maintaining a completely randomized design (CRD) with three replications. There were two sets of pots to conduct the experiment, where one set used for growth, physiological and biochemical parameters and another set was for measuring the yield and yield contributing parameters.

3.13 Intercultural operations

3.13.1 Gap filling

Gap filling was done to maintain a uniform plant population in each pot.

3.13.2 Weeding

Weeds were uprooted manually from the pots whenever observed.

3.13.3 Application of irrigation water

Irrigation water was applied to the pots whenever required. Frequency of irrigation was reduced during panicle initiation and grain filling stage and stopped 7 d prior to harvesting.

3.13.4 Plant protection measures

To protect the plants from the infestation of rice stem borer (*Scirphophaga incertolus*) and leafhopper (*Nephotettix nigropictus*), Actara @ 0.12 g L^{-1} of water was sprayed for two times during vegetative stage and grain filling stage.

3.13.5 Harvesting

Harvesting was done at 105 DAT, when the 80% of the grains became matured and golden colored, then the hills were harvested with serrated edged sickle manually.

3.13.6 Threshing

Pedal thresher was used for threshing to separate the grains from the hills. Then the grains were cleaned and dried up to 12% moisture content. Straw was also dried properly to determine the straw yield.

3.14 Collection of data

Growth, physiological, biochemical, and phenotypic attributes were observed after completion of the treatments at 63 DAT. The yield and yield contributing parameters were recorded after harvesting.

3.14.1 Crop growth attributes

- Plant height
- Tillers hill^{-1}
- Leaf area
- Fresh weight of root and shoot hill^{-1}
- Dry weight of root and shoot hill^{-1}

3.14.2 Physiological attributes

- Soil and plant analysis development (SPAD) value
- Relative water content
- Proline content

3.14.3 Oxidative stress indicators

- Malondialdehyde content
- Hydrogen peroxide content
- Electrolyte leakage

3.14.4 Ascorbate and glutathione content

3.14.5 Yield and yield contributing attributes

- Effective tillers hill^{-1}
- Ineffective tillers hill^{-1}
- Panicle length
- Rachis panicle^{-1}
- Filled grains panicle^{-1}
- Unfilled grains panicle^{-1}
- Total number of grains panicle^{-1}
- 1000-grain weight
- Grain yield hill^{-1}
- Straw yield hill^{-1}

3.14.6 Phenotypic observations

3.15 Procedure of measuring crop growth attributes

3.15.1 Plant height

Plant height was measured from the base to the uppermost portion of the plant using a measuring scale. Height of four plants from each treatment was measured at 35, 49, 63, 77 DAT and harvest maintaining 14 days interval. The data was presented as the average value of four plants and expressed as centimeters (cm).

3.15.2 Tillers hill^{-1}

Number of tillers were counted from four hills of each treatment at 49, 63, 77 DAT and harvest. The tillers of four hills were then averaged and expressed as number hill^{-1}.

3.15.3 Leaf area

Leaf area was calculated based on the technique of leaf length-width measurement at 49, 63 and 77 DAT. For that, length (cm) of five randomly selected leaf blades was measured using a measuring scale and the width (cm) of leaf blades were taken from three different points, viz., base, middle, and top. Then, the leaf area was calculated from the average value of length and width of five leaves following the equation of Yoshida (1981) using a correction factor (K) of 0.75 and expressed as square centimeters (cm^2). The equation is as follows:

$$\text{Leaf area } (cm^2) = K \times \text{Length (cm)} \times \text{Width (cm)}$$

3.15.4 Fresh weight of root and shoot hill^{-1}

Three hills from each treatment were uprooted and washed with running tap water to clean the adhering soils. After then, roots and shoots were separated from each other and weighed in a digital balance to estimate the FW of roots and shoots. The weight of roots and shoots were divided by three and expressed as g hill^{-1}.

3.15.5 Dry weight of root and shoot hill^{-1}

For determining the DW, the root and shoot of three hills were separated, sun-dried for 72 h to reduce the initial moisture content and followed by oven-dried at 80 °C for 72 h and weighed for DW (g). Finally, the weight of roots and shoots were divided by three and expressed as g hill^{-1}.

3.16 Procedure for estimating physiological attributes

3.16.1 Soil and plant analysis development (SPAD) value

A portable SPAD meter (FT Green LLC, Wilmington, DE, USA) was used for estimating the SPAD value. Five fully matured leaves were randomly selected from each treatment and reading was taken at 35, 49, 63, and 77 DAT. Values of five leaves were then averaged for the SPAD value.

3.16.2 Relative water content

Three leaf blades from each treatment were plucked and weighted for the FW. After that, leaf blades were dipped into distilled water (dH_2O) in the Petri dish by covering it with two layers of filter paper. Dipping of leaf blades was done for 24 h by keeping them in a dark place. Later, adhesive surface water was removed and weighted for the turgid weight (TW). Oven drying of the leaf blades were done for 72 h in an electric oven and the temperature was maintained at 80 °C. Then the DW of the leaf blades were taken. Finally, the RWC was calculated following the formula of Barrs and Weatherly (1962) using the values of FW, TW, and DW. The formula is as follows:

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

3.16.3 Proline content

Leaf sample of 0.5 g was taken and homogenized in an ice-cooled mortar pestle by using a 5 ml extraction buffer of 3% aqueous sulfosalicylic acid. The homogenate was centrifuged for 15 min at 11,500 ×g and a clear supernatant was obtained. Then 1 ml of supernatant was mixed with 1 ml of acid ninhydrin containing 6 M phosphoric acid and 1 ml of glacial acetic acid. The mixture was then incubated in a water bath for 60 min at 100 °C. After that, the mixture was cooled down at room temperature and 4 ml of toluene was added to it followed by vortexed for removing the free Pro from the mixture. Then the colored upper chromophore was subjected to the spectrophotometer to observe the optical density at 520 nm. Finally, the value of Pro was determined by plotting the absorbance reading of each treatment against a standard curve and expressed as μmol g^{-1} FW. The Pro content was estimated following the method of Bates *et al*. (1973).

3.17 Procedure for estimating oxidative stress indicators

3.17.1 Malondialdehyde content

Procedure of Heath and Packer (1968) was followed to determine the level of lipid peroxidation as MDA content using thiobarbituric acid (TBA) reagent. Leaf sample (0.5 g) was homogenized in 3 ml of 5% trichloroacetic acid (TCA) solution. Then the homogenate was centrifuged for 12 min at 11,500 ×g at 4 °C. After that 1 ml of aliquot was diluted with 4 ml of TBA reagent containing 20% TCA and 0.5% TBA. The composition of aliquot and TBA reagent heated for 30 min at 95 °C in a water bath. Immediately after cooling in an ice bath and centrifuged again at 11,500 ×g for 10 min. The absorbance of colored chromophore was recorded at 532 nm and the non-specific absorbance was taken at 600 nm with a spectrophotometer. Then the values were corrected by subtracting the non-specific values and the calculation was done by using an extinction coefficient of 155 mM^{-1} cm^{-1}. The content of MDA was expressed as nmol g^{-1} FW.

3.17.2 Hydrogen peroxide content

From the procedure of Yu *et al*. (2003), 0.5 g leaf sample was homogenized in 3 ml of 5% TCA and followed by centrifugation for 12 min at 11,500 ×g at 4 °C. Then 1 ml of the supernatant was mixed with 1 ml of 10 mM potassium-phosphate (K-P) buffer pH 7 and 1 ml of 1 mM potassium iodide. Then the mixture was vortexed and incubated for 1 h. Then the optical absorbance of the mixture was taken at 390 nm with a spectrophotometer. Finally, the H_2O_2 content was calculated and expressed as nmol g^{-1} FW.

3.17.3 Electrolyte leakage

Following the method of Dionisio-Sese and Tobita (1998), 0.5 g of plant samples from each treatment was chopped into small pieces and put in the Falcon tubes. After adding 15 ml of dH_2O in the tube, these were incubated in the water bath at 40 °C for 60 minutes. Then the electrical conductivity (EC_1) was measured with an electrical conductivity meter (HI-993310, Hanna, USA) when the solution came to the room temperature. After that the solution in the Falcon tube was again heated at 121 °C for 30 min in an autoclave. Final conductivity (EC_2) of the solution was measured after cooling within the ice bath. Then, the EL of root and leaf was determined following the equation as follows:

$$\mathrm{EL}\ (\%) = \frac{\mathrm{EC_1}}{\mathrm{EC_2}} \times 100$$

3.18 Procedure of quantifying ascorbate and glutathione content

Homogenization of 0.5 g of leaf sample was done with 5% metaphosphoric acid and 1 mM ethylenediaminetetraacetic acid. Homogenate was centrifuged for 15 min at 11,500 ×g at 4 °C and the clear aliquot was collected for the determination of AsA and GSH.

Procedure of Huang *et al*. (2005) was followed for the determination of reduced and total AsA. The supernatant was neutralized with 0.5 M K-P buffer pH 7 and dH_2O for determining the reduced AsA. Whereas 0.1 M dithiothreitol instead of dH_2O was used for the estimation of total AsA. Then the absorbance of reduced and total AsA were assayed spectrophotometrically at 265 nm with 100 mM K-P buffer pH 6.5 and 0.5 unit of ascorbate oxidase. For quantification of AsA and total AsA, a specific standard curve was used, and the DHA content was determined by deducting the value of reduced AsA from the total AsA. Units of AsA and DHA were expressed as nmol g^{-1} FW.

Glutathione content was determined by the method of Hasanuzzaman *et al*. (2018). For determining total GSH neutralization of the supernatant was done with 0.5 M K-P buffer pH 7 and dH_2O, whereas 2–vinylpyridine was added for the GSSG along with the K-P buffer. Then the absorbance was taken after oxidizing with 5,5-dithio-bis(2-nitrobenzoic acid) and again reduced by nicotinamide adenine dinucleotide phosphate in the presence of GR at 412 nm with a spectrophotometer. A standard curve was used for the determination of total GSH and GSSG, and the content of GSH was determined after subtracting the values of GSSG from total GSH. Units of GSH and GSSG were expressed as nmol g^{-1} FW.

3.19 Procedure of determining yield and yield contributing attributes

3.19.1 Effective tillers hill^{-1}

Tillers that bear panicles were considered effective tillers. The number of effective tillers were counted from the four hills of each treatment. Then the average value from the four hills was expressed as the number of effective tillers hill^{-1}.

3.19.2 Ineffective tillers hill^{-1}

No panicle bearing tillers were considered as ineffective tillers. The number of ineffective tillers were counted from the four hills of each treatment. Then the average value from the four hills was expressed as the number of ineffective tillers hill^{-1}.

3.19.3 Panicle length

Panicle length was determined from 10 randomly selected panicles from each treatment. The length of panicles was measured from the base to the apex of each panicle. Then the averaged value was expressed as cm.

3.19.4 Rachis panicle^{-1}

Primary branches from 10 panicles were counted for the estimation for rachis number from each treatment. Average value was expressed as the number of rachis panicle^{-1}.

3.19.5 Filled grains panicle^{-1}

Fully filled kernel was considered as filled grain. The number of filled grains were counted from the 10 panicles of each treatment and the average value was expressed as the number of filled grains panicle^{-1}.

3.19.6 Unfilled grains panicle^{-1}

Partially filled or unfilled kernels were regarded as unfilled grain. The number of unfilled grains were counted from the 10 panicles from each treatment and the average value was expressed as the number of unfilled grains panicle^{-1}.

3.19.7 Total number of grains panicle^{-1}

Summation of the number of filled and unfilled grains panicle^{-1} was considered as the number of total grains panicle^{-1}.

3.19.8 1000-grain weight

Dried and cleaned 1000 grains was counted using a seed counter from each treatment and weighted, and expressed as g.

3.19.9 Grain yield hill^{-1}

After threshing and winnowing, the grains were sun-dried upto 12% moisture content and the weight of the grains was recorded. The weight of the grains of four hills was averaged and expressed as g hill^{-1}.

3.19.10 Straw yield hill^{-1}

Straw yield was estimated from the weight of four hills of each treatment after separating the grains and dried until a constant weight was obtained. Then the value was averaged and expressed as g hill^{-1}.

3.20 Phenotypic observations

Phenotypic features of the plants were observed and pictures were taken with a digital camera.

3.21 Statistical analysis

CoStat v.6.400 computer-based software (CoHort Software, Monterey, CA, USA) was used for the statistical analysis. The mean values were computed from three replications and compared among them after the application of Fisher's LSD test. The treatments were statistically significant when the differences among them was at $p \leq 0.05$ (CoStat, 2008).

Chapter IV

RESULTS AND DISCUSSION

4.1 Crop growth attributes

4.1.1 Plant height

The plant height was reduced by 21, 24, and 29% in plants treated with 0.5, 1.0, and 2.0 Ni, respectively compared to the control at 35 DAT. The application of BC improved the plant height under 0.5 and 1.0 mM Ni stress by 28 and 26%, respectively over the corresponding Ni stressed plants only. On the contrary, foliar spray of CHT increased the plant height by 18% in 0.5 mM and 23% in 1.0 mM Ni stressed plants compared to the Ni stressed plant only (Figure 3A).

A notable reduction of the plant height at 49 DAT was observed by 16, 20, and 25% under 0.5, 1.0, and 2.0 mM Ni stress, respectively compared to the control. The highest improvement of the plant height was observed under 0.5 mM Ni stress with BC application by 20% compared to the Ni stressed plants only. Under 1.0 and 2.0 mM Ni stress, the BC application improved the plant height by 9 and 17%, respectively compared to the corresponding Ni treated plants only. Similarly, the CHT application enhanced the plant height by 10, 12, and 15% in plants treated with 0.5, 1.0, and 2.0 mM Ni, respectively compared to the corresponding Ni stressed plants only (Figure 3B).

At 63 DAT, the plant height was declined by 12% in 0.5 mM, 18% in 1.0 mM, and 22% in 2.0 mM Ni stressed plants compared to the control. Supplementation with BC increased the plant height by 18, 18, and 15% in plants exposed to 0.5, 1.0, and 2.0 mM Ni, respectively, while the plant height was increased by 12, 17 and 15% with CHT spray, respectively (Figure 3C).

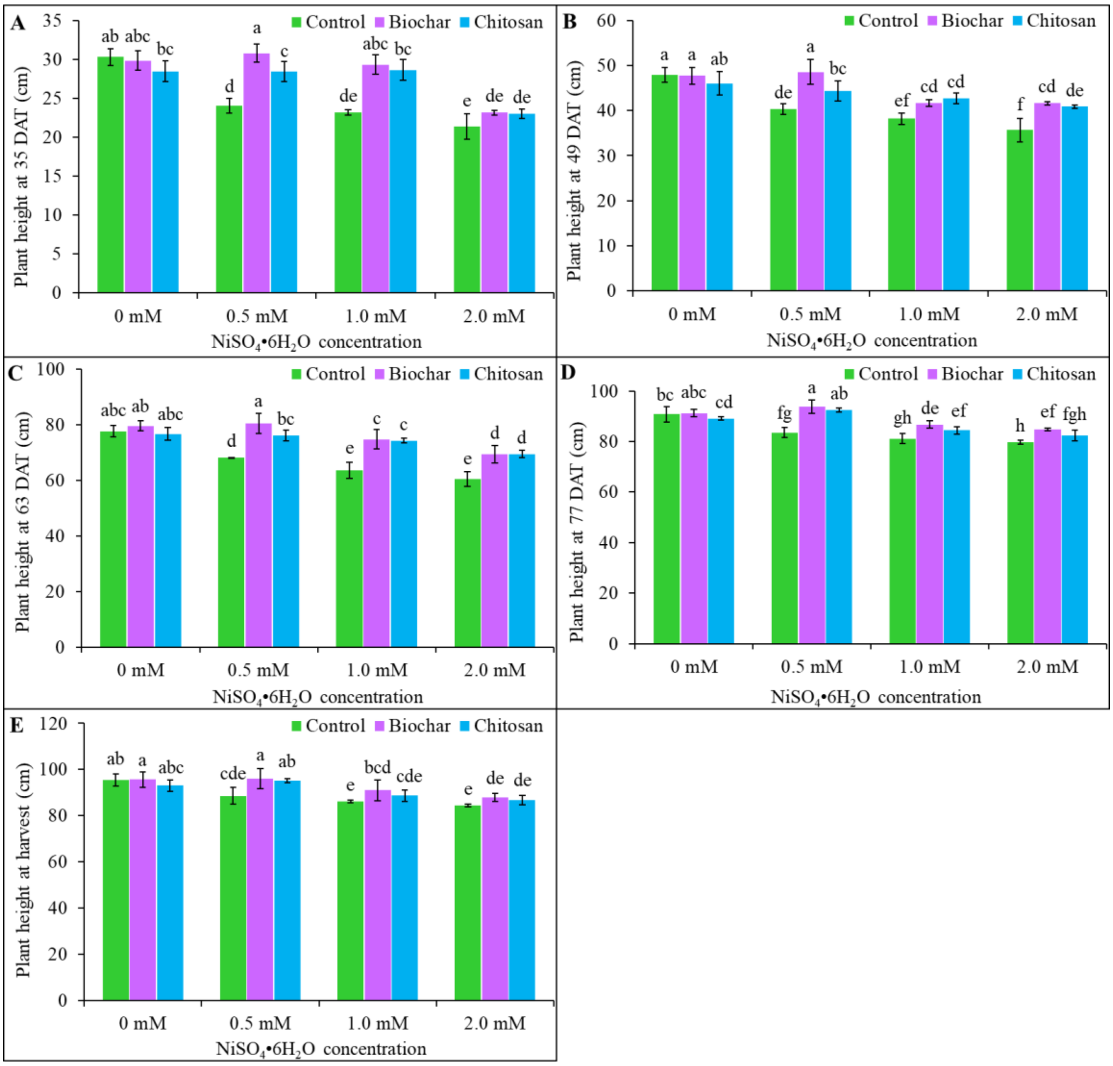


Figure 3. Effect of exogenous application of biochar (0.5 g kg^{-1} soil) and chitosan (200 mg L^{-1}) on the plant height of rice plants at 35 (A), 49 (B), 63 (C), 77 (D) DAT and harvest (E) upon exposure to different concentrations of nickel. Mean (± SD) was computed from three replications (n=3). Different letters in each column indicate a significant difference between the treatments at $p \leq 0.05$ after applying Fisher's LSD test

The reduction of plant height was observed at 77 DAT by 8, 11, and 12% upon exposure to 0.5, 1.0, and 2.0 mM Ni, respectively compared to the control. Moreover, the BC application increased the plant height by 12% in 0.5 mM, 7% in 1.0 mM, and 6% in 2.0 mM Ni over the Ni affected plants only. Whereas the CHT application only improved the height under 0.5 and 1.0 mM Ni stress by 11 and 4%, respectively (Figure 3D).

The plant height was declined by 7, 10, and 11% under 0.5, 1.0, and 2.0 mM Ni stress, respectively compared to the control at harvest. However, the plant height was increased by 8 and 7% under 0.5 mM Ni with BC and CHT supplementation, respectively over the respective Ni affected plants only. Under 1.0 mM Ni, the BC application only showed better results which increased by 6% compared to the Ni stressed only. No improvement was seen with BC or CHT application under 2.0 mM Ni stress (Figure 3E).

Plants' response to metal toxicity could be identified as growth retardation. The plant's stress endurance capability is assessed on the basis of root and shoot growth inhibition caused by the metal ions (Wang and Zhou, 2005). Unlike other metals, Ni also inhibit the plant growth by reducing water uptake and distorting pigments involved in photosynthesis, thereby causing wilting, chlorosis, necrosis, and also hampers photoassimilate production (Vinterhalter and Vinterhalter, 2005; Baligarx 2012; Awasthi and Sinha, 2013). In the present study, growth in terms of plant height was retarded upon exposure to Ni stress (Figure 3A-E). Similar growth reduction due to Ni toxicity has been reported in rice (Hasanuzzaman *et al*., 2019), mungbean (Ali *et al*., 2015), soybean (Sirhindi *et al*., 2015; Mir *et al*., 2018), wheat (Parlak, 2016), mustard (Khan *et al*., 2016; Khan *et al*., 2020a), oats (Gupta *et al*., 2017), and maize (Azeem, 2018; Amjad *et al*., 2019) plants. Interference in nutrient uptake also occurs in presence of excess amounts of Ni (Gajewska *et al*., 2012). However, the supplementation of BC and CHT enhanced the plant height under Ni toxicity. Biochar has the ability to bind the toxic metals to the soils and solubilize the essential nutrients to the plants (Alharby *et al*., 2020). Moreover, it can lessen the nutrient losses, enhance fertility status, accelerate soil acidification, and improve soil biogeochemical properties of the soil, thus improving the growth and development of the plants (Imran *et al*., 2022). Soliman *et al*. (2022) reported the BC application to the wheat under salt stress decreased the accumulation of toxic ions and improved the plant height. Previous reports on maize (Rehman *et al*., 2016), wheat (Shahbaz *et al*., 2019), and jute (Hasanuzzaman *et al*., 2021) also demonstrated that the plant height was increased under different stress when BC was applied exogenously. Moreover, the CHT can improve photosynthetic activity of the plants through retrieving pigment acquisition, thereby enhancing the plant growth. In *Calendula tripterocarpa* plants, when CHT was applied to the Ni stressed plants, a profound increase of the plant height was observed (Heidari *et al*., 2020). However, the CHT has a role in regulating signaling cascades, and phytohormones accumulation, thus accelerating the growth of the plants under stress (Rabêlo *et al*., 2019; Hasanuzzaman *et al*., 2021). Reports on soybean (Sadeghipour, 2021), durum wheat (Quitadamo *et al*., 2021), and maize (Rabêlo *et al*., 2019) plants also suggested that the CHT application improved the growth of plants. Application of BC and CHT showed a positive response to increase the plant height in the present study corroborated the previous reports.

4.1.2 Tillers hill^{-1}

The number of tillers hill^{-1} was significantly dropped by 17, 22, and 38% in plants upon exposure to 0.5, 1.0, and 2.0 mM Ni stress, respectively compared to the control at 49 DAT. When compared to the Ni stressed plants only, the BC application showed an improvement of the number of tillers hill^{-1} by 19, 22, and 18% in plants treated with 0.5, 1.0, and 2.0 mM Ni, respectively. Moreover, the CHT supplementation also increased the number of tillers hill^{-1} by 9, 27, and 13% under 0.5, 1.0, and 2.0 mM Ni, respectively in comparison to the corresponding Ni stressed plants only (Figure 4A).

The reduction of the number of tillers hill^{-1} was observed at 63 DAT by 9% in 0.5 mM, 12% in 1.0 mM, and 14% in 2.0 mM Ni stress compared to the control. Highest increase of the number of tillers hill^{-1} was found with BC supplementation by 12% in plants under 0.5 mM Ni stress over the Ni stressed plants only (Figure 4B).

The highest declination of the number of tillers hill^{-1} was observed at the highest dose of Ni (2.0 mM) by 12% at 77 DAT and 13% at harvest compared to the control. Supplementation with BC further improved the number of tillers hill^{-1} under 0.5 mM Ni treated plants by 8% at harvest in comparison to the corresponding Ni stressed plants only. While in 1.0 and 2.0 mM Ni stress the improvement of the number of tillers hill^{-1} was statistically similar either with BC and CHT supplementation compared to the Ni stressed plants only at both 77 DAT and harvest (Figure 4C, D).

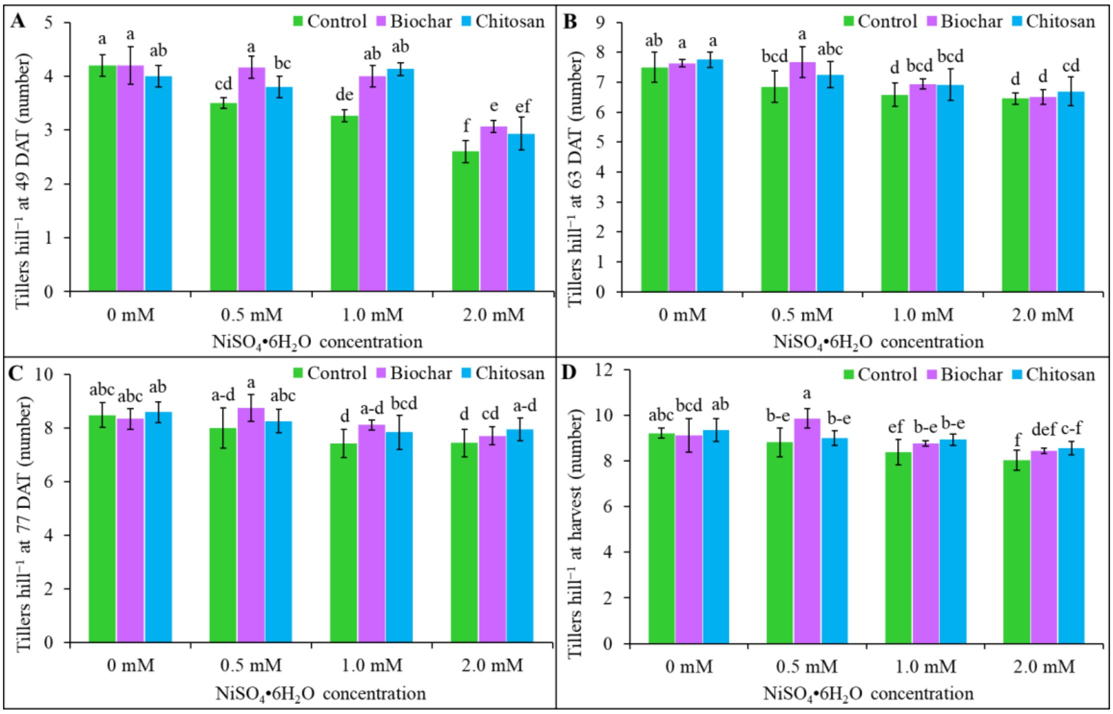


Figure 4. Effect of exogenous application of biochar (0.5 g kg^{-1} soil) and chitosan (200 mg L^{-1}) on the number of tillers $hill^{-1}$ of rice plants at 49 (A), 63 (B), 77 (C) DAT and harvest (D) upon exposure to different concentrations of nickel. Mean (± SD) was computed from three replications (n=3). Different letters in each column indicate a significant difference between the treatments at $p \leq 0.05$ after applying Fisher's LSD test

In monocots, tillering is considered as the determinant factor of plant developmental process (Wang and Jiao, 2018). Moreover, tillering is considered as an imperative annual growth of rice, originated through two developmental processes. At the beginning, the dormant axillary meristem in the leaf axil turned into axillary bud, and subsequent growth of the axillary bud ultimately gave rise to new tiller (Wai and An, 2017; Zhuang *et al*., 2019). The outgrowth of this axillary bud is genetically controlled through endogenous regulatory factors, such as hormones (Domagalska and Leyser, 2011). Under Ni stress, the number of tillers $hill^{-1}$ of rice declined notably in the current study (Figure 4A-D). Niu *et al*. (2021) reported that the heavy metal toxicity inhibits the axillary bud formation and further suppresses the outgrowth of the buds. Moreover, Ni toxicity hampers nutrient availability, and also reduces the N assimilation rate in plants (Shin *et al*., 2005; Mishra and Dubey, 2011; Khan *et al*., 2020b). Nitrogen assimilation rate inhibited due to the reduction of nitrate reductase activity in the rice seedlings under Ni stress. Therefore, a higher level of Ni hampers the availability and transportation of NO_3^- to the plant, thus the roots growth is reduced and inhibits plant growth (Dubey and Pessarakli, 2002). The number of tillers hill^{-1} was declined when Ni stress, however, with BC and CHT supplementation further increased the tillers numbers of the rice (Figure 4A-D). This type of enhancement of plant growth occurred due to the mitigation of toxic effects of metals. Moreover, BC has the capacity to enrich nutrient availability to accelerate the growth of plants, whereas CHT is involved in the synthesis of growth accelerating phytohormones under stress (Hidangmayum *et al*., 2019; Imran *et al*. 2022). Further report of Rizwan *et al*. (2018b) demonstrated that the number of tillers hill^{-1} of rice was increased with BC application at both 3 and 5% levels under Cd stress. Whereas, Behboudi *et al*. (2018) also found an improvement of the number of tillers plant^{-1} in barley with CHT application. Such findings are in agreement with the current study with BC and CHT application where the number of tillers hill^{-1} was increased under Ni stress.

4.1.3 Leaf area

Leaf area at 49 DAT was decreased by 14, 26, and 29% in plants treated with 0.5, 1.0, and 2.0 mM Ni, respectively compared to the control. The highest increase of leaf area was observed under 1.0 mM Ni stress with BC application by 16% compared to the Ni stressed plants only. The enhancement of the leaf area was statistically similar at both 0.5 and 2.0 mM Ni stress with BC or CHT supplementation (Figure 5A).

A notable reduction of leaf area was observed at 63 DAT. Upon exposure to 0.5, 1.0, and 2.0 mM Ni, the leaf area was declined by 13, 15, and 24%, respectively compared to the control. The BC application under 0.5, 1.0, and 2.0 mM Ni stress further increased the leaf area by 22, 10, and 12%, respectively compared to the Ni affected plants only, while the CHT application improved it by 17, 15, and 15%, respectively (Figure 5B).

Leaf area at 77 DAT was reduced by 16, 18, and 18% under 0.5, 1.0 and 2.0 mM Ni treated plants compared to the control. Moreover, it was improved with BC application by 22, 10, and 12% in 0.5, 1.0, and 2.0 mM Ni treated plants, respectively compared to the Ni stressed plants only. Whereas the foliar spray of CHT increased the leaf area by 17, 15, and 15% under 0.5, 1.0, and 2.0 mM Ni stress, respectively compared to the Ni treated plants only (Figure 5C).

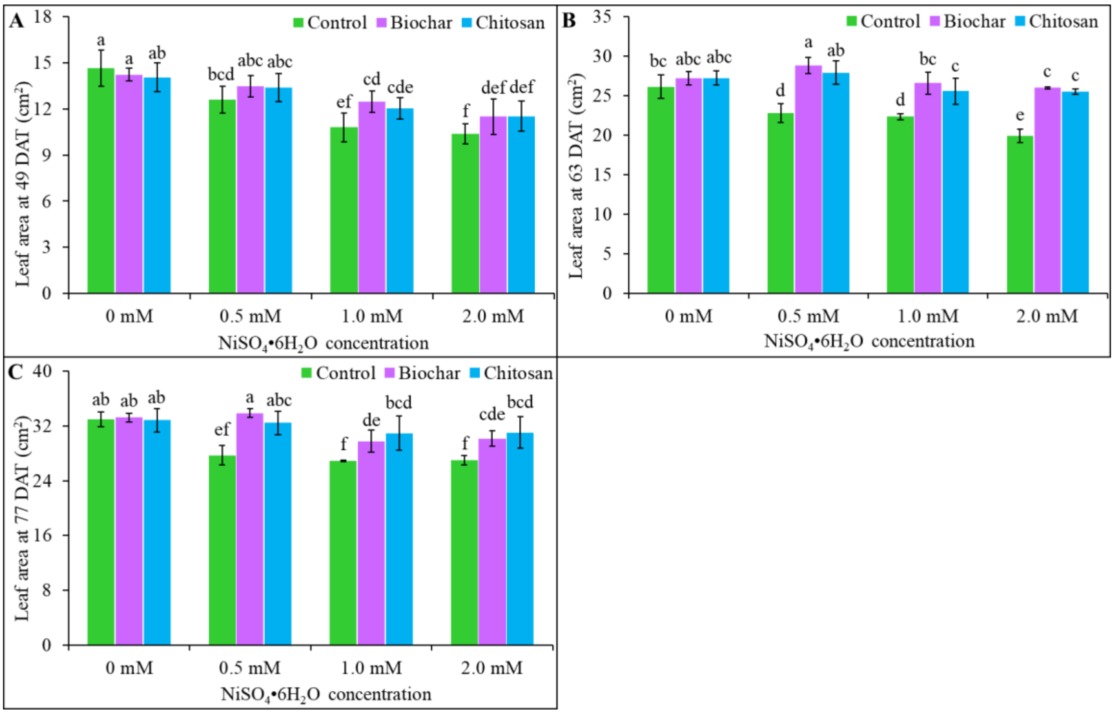


Figure 5. Effect of exogenous application of biochar (0.5 g kg^{-1} soil) and chitosan (200 mg L^{-1}) on the leaf area of rice plants at 49 (A), 63 (B), and 77 (C) DAT upon exposure to different concentrations of nickel. Mean value (± SD) was computed from three replications (n=3) from each treatment. Different letters in each column indicate significant differences between the treatments at $p \leq 0.05$ after applying Fisher's LSD test

Leaf area is also affected like plant growth when exposed to metal stress. In this study, plants exhibited declination of the leaf area under Ni stress in a dose dependent manner (Figure 5A-C). The plant's response to the retardation of leaf growth is considered an immediate effect of Ni toxicity (Hassan *et al*., 2019). Metal ions induce ionic and osmotic stress in plants and consequently lead to the declination of the growth and development of different plant parts (Bhalerao *et al*., 2015). Besides this, induction of Ni stress to the plants inhibits cell growth through suppressing the cell division and proliferation. Cell wall lignification also hampers cell development and ultimately restricts the growth of plants (Bhalerao *et al*., 2015). Moreover, the metal toxicity induces physiological disorders and decreases the plasticity of cell walls due to excessive pectin formation also reported by Anjum *et al*. (2016). Khair *et al.* (2020) reported that imposition of Ni stress caused a reduction of the leaf area along with number of leaves plant^{-1} and leaf weight of the plants The finding of the Khair *et al.* (2020) corroborated with the present study. Moreover, in the Ni stressed wheats, the number of leaves declined in a dose dependent manner as reported by Azeem (2018). However, the leaf area was improved when they were supplemented with BC and CHT under Ni stress in the current study. Both of them are capable of improving the physiological properties of the plants through increasing nutrient and water uptake, restricting toxic ion accumulation, and also improving metabolic processes (Hidangmayum *et al.*, 2019). Hence, increasing the cell division and development which ultimately increase the leaf area as well as growth of the plants (Imran *et al.*, 2022). The BC application enhanced the leaf area of the drought affected wheat (Kanwal *et al.*, 2018) and Cr stressed mustard (Ali *et al.*, 2018) plants, whereas the CHT application increased the leaf area in barley (Behboudi *et al.*, 2018) and maize (Qu *et al.*, 2019), respectively under drought and Cd stress. These reports are in agreement with the current finding where the application of BC and CHT under Ni stress is an indication of improvement of the physiological processes of plants.

4.1.4 Fresh weight of root and shoot hill^{-1}

A sharp declination of the FW of root and shoot was observed upon exposure to Ni stress. The root FW was decreased by 13, 23, and 41% in plants treated with 0.5, 1.0, and 2.0 mM Ni, respectively compared to the control, while the shoot FW was reduced by 23, 29, and 32%, respectively. The BC application showed an increase in the root FW by 17, 16, and 33%, and the shoot FW by 26, 31, and 24% in plants under 0.5, 1.0, and 2.0 mM Ni stress, respectively over Ni affected plants only. Moreover, the CHT application also improved the root FW (by 7, 12, and 13%) and shoot FW (by 13, 28, and 22%) in 0.5, 1.0, and 2.0 mM Ni treated plants in comparison to the respective Ni stressed plants only (Figure 6A, B).

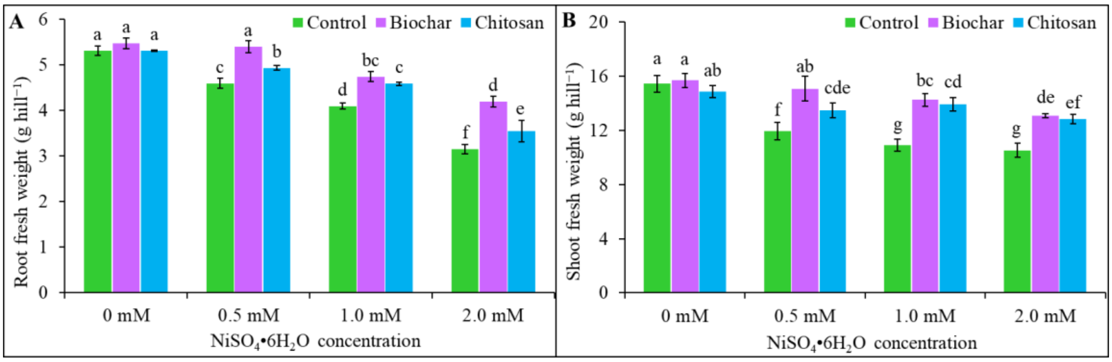


Figure 6. Effect of exogenous application of biochar (0.5 g kg^{-1} soil) and chitosan (200 mg L^{-1}) on the fresh weight of root (A) and shoot (B) of rice plants upon exposure to different concentrations of nickel. Mean (± SD) was computed from three replications (n=3). Different letters in each column indicate a significant difference between the treatments at $p \leq 0.05$ after applying Fisher's LSD test

Photosynthetic activity of the plants was abruptly affected under Ni stress due to the inhibition of Chl biosynthesis. Excessive accumulation of Ni suppresses magnesium acquisition from the soil and replaces the Mg^{2+} ion in the tetrapyrrole ring of Chl, which is considered as the prime light harvesting apparatus of plants (Pandey and Sharma, 2002). Thus, inhibition of photoassimilate production and reduction of the biomass accumulation in plants which ultimately resulted in stunted plant growth (Tränkner *et al*., 2018). The root FW and shoot FW of rice were declined with the increasing Ni concentrations here in the present study (Figure 6A, B). Several findings also demonstrated the FW of various plants have been declined under Ni stress (Yusuf *et al*., 2011; Ali *et al.*, 2015) Amjad *et al.* (2019) reported that the shoot FW and root FW were decreased with increasing Ni concentrations in both varieties of maize. Similarly, under Ni stress, the FW of the mungbean (Ali *et al.*, 2015) and rice (Hasanuzzaman *et al.*, 2019) were declined compared to the control. Moreover, the BC further improved the FW of the Ni stressed rice in the present study. Previous report of Hasanuzzaman *et al*. (2021) stated that the BC as well as CHT application increased the FW of jute under salt stress. Similarly, fresh mass accumulation was also increased in wheat (Abbas *et al*., 2017) and rice (Rizwan *et al*., 2018b) plants upon incorporation of BC. Conversely, the CHT application showed an increase of the shoot FW and root FW in the mustard under Cd stress reported by Zong *et al*., (2017). In the Ni stressed *C. tripterocarpa* (Heidari *et al*., 2020) and Cd stressed maize (Qu *et al*., 2019), the CHT application also improved the FW of the plants. Furthermore, Mehmood *et al*. (2020) modified CHT and BC in a formulated product and applied it to the salt affected quinoa plants. This formulated CHT and BC also improved the root FW and shoot FW in quinoa under salinity (Mehmood *et al*., 2020). These findings are in congruence with the present study which also demonstrated that on application of BC and CHT, the biomass production of the rice also improved under Ni stress.

4.1.5 Dry weight of root and shoot hill^{-1}

When compared to control, the reduction of the root DW by 31, 38, and 42%, and the shoot DW by 32, 41, and 49% was observed under 0.5, 1.0 and 2.0 mM Ni stress, respectively. Further improvement of the root DW by 35, 44, and 17%, and shoot DW by 48, 59, and 51% were found in plants treated with 0.5, 1.0, and 2.0 mM Ni, respectively in comparison to the corresponding Ni affected plants only, while the CHT spray also enhanced the root DW by 22, 28, and 10%, and shoot DW by 33, 52, and 37%, respectively (Figure 7A, B).

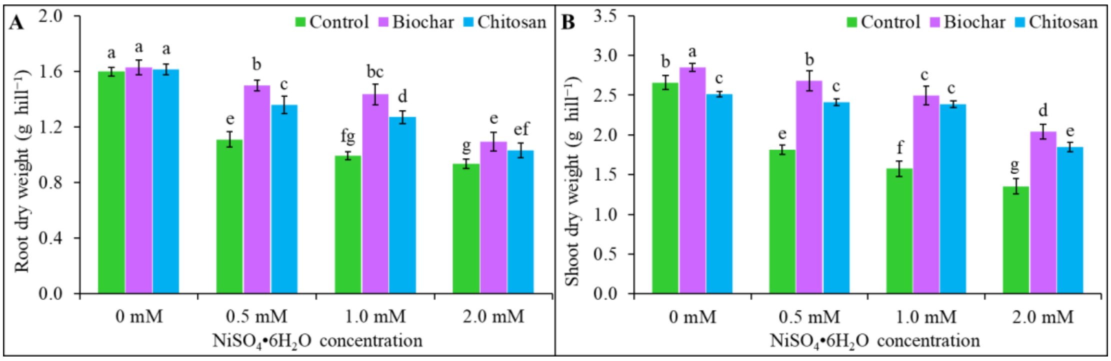


Figure 7. Effect of exogenous application of biochar (0.5 g kg^{-1} soil) and chitosan (200 mg L^{-1}) on the dry weight of root (A) and shoot (B) of rice plants upon exposure to different concentrations of nickel. Mean (± SD) was computed from three replications (n=3). Different letters in each column indicate a significant difference between the treatments at p ≤ 0.05 after applying Fisher's LSD test

The DW of the plants is closely associated with the FW of the plants. However, a sharp declination of the DW was seen in rice under Ni stress here in the present study (Figure 7A, B). Ali *et al*. (2015) reported that the root DW and shoot DW of mungbean were declined under Ni stress. As earlier mentioned, Ni inhibits photosynthesis through distorting the photosynthetic apparatus and reducing biomass products in plants. In a study, Khan *et al*. (2016) reported that the dry mass accumulation of mustard was more in cv. Varuna which has high photosynthetic capacity in comparison to a low photosynthetic capacity variety (cv. RH30) under Ni stress. Similar findings of DW reduction were also reported in several studies (Mir *et al*., 2018; Abd_Allah *et al*., 2019; Hasanuzzaman *et al*., 2019; Aqeel *et al*., 2021; Fiala *et al*., 2021). However, the BC and CHT supplemented plants showed an improvement in the DW of roots and shoots of rice under Ni toxicity (Figure 7A, B). The root DW and shoot DW in maize under Cd stress (Rizwan *et al*. 2019), quinoa under salt stress (Naveed *et al*., 2020), and wheat under drought conditions (Bashir *et al*. 2020) were also increased when BC was applied to the plants. On the contrary, Heidari *et al*. (2020) reported that the CHT application to the Ni affected *C. tripterocarpa* has improved the DW of the plants. Whereas, the DW of the roots and shoots was also increased with supplementing with formulation of CHT and BC as reported by Mehmood *et al*. (2020). Furthermore, Hasanuzzaman *et al*. (2021) reported that the root DW and shoot DW of jute were increased in the plants with BC application under salinity. Thus, such improvement in the DW of the plants with BC and CHT corroborated the present study and indicate ameliorative effect of Ni stress.

4.2 Physiological attributes

4.2.1 Soil and plant analysis development (SPAD) value

In comparison to the control, the SPAD value of 0.5, 1.0 and 2.0 mM Ni stressed plants decreased by 9, 13, and 15% at 35 DAT, respectively. The supplementation of BC increased the SPAD value by 8, 6, and 4%, respectively under 0.5, 1.0, and 2.0 mM Ni stress compared to the corresponding Ni affected only, whereas the CHT application increased it by 5, 5, and 6%, respectively (Figure 8A).

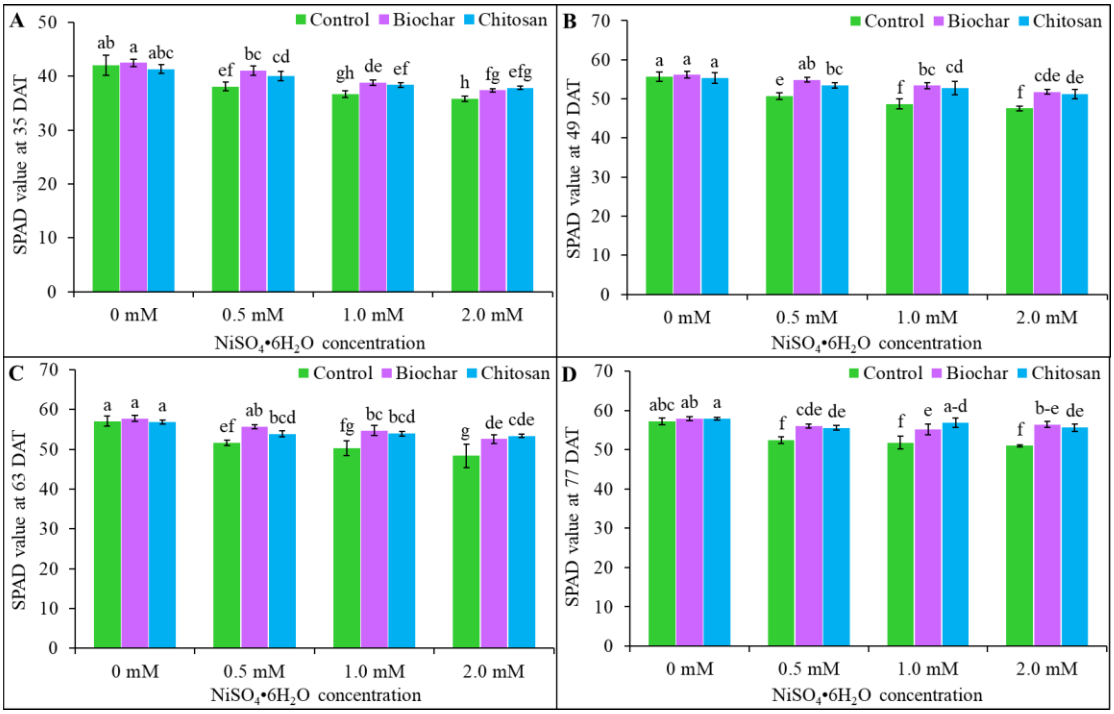


Figure 8. Effect of exogenous application of biochar (0.5 g kg^{-1} soil) and chitosan (200 mg L^{-1}) on the SPAD value of rice plants at 35 (A), 49 (B), 63 (C), and 77 (D) DAT upon exposure to different concentrations of nickel. Mean (± SD) was computed from three replications (n=3). Different letters in each column indicate a significant difference between the treatments at $p \leq 0.05$ after applying Fisher's LSD test

The SPAD value of the 0.5, 1.0, and 2.0 mM Ni treated plants declined by 9, 14, and 16% at 49 DAT, respectively compared to the control. However, the BC amendment increased the SPAD value by 8% in 0.5 mM, 10% in 1.0 mM, and 9% in 2.0 mM Ni compared to the Ni stressed only. Whereas the CHT application also increased it by 5, 8, and 8% under 0.5, 1.0, and 2.0 mM Ni stress, respectively compared to the Ni stressed plants only (Figure 8B).

At 63 DAT, the SPAD value was reduced by 10, 12, and 15% under 0.5, 1.0, and 2.0 mM stress, respectively compared to the control. The BC amendment improved the SPAD value by 8, 9, and 9%, whereas it was enhanced by 4, 7, and 10% with CHT application under 0.5, 1.0, and 2.0 mM Ni stress, respectively over the Ni stressed plants only (Figure 8C).

The reduction of SPAD value was 8, 9, and 11% in plants under 0.5, 1.0, and 2.0 mM Ni stress, respectively compared to the control at 77 DAT. However, compared to the Ni stressed plants only, the BC amendment enhanced the SPAD value by 7, 7, and 11% in plants treated with 0.5, 1.0, and 2.0 mM Ni, respectively, while it was increased by 6, 10, and 9% with CHT application (Figure 8D).

Nickel stress is responsible for the reduction of photosynthetic apparatus of the plants through disrupting the molecular structure of Chl (Hassan *et al*., 2019). In Chl, Mg^{2+} occupied in the center of the tetrapyrrole ring, however upon accumulation of higher amounts of Ni^{2+} replaced the Mg^{2+} and distort the Chl structure. Thus, disruption of Chl *a* and Chl *b* structures hamper photosynthesis which in turn causes chlorosis, necrosis, and leaf senescence of the plants (Tränkner *et al*., 2018). Under Ni stress, the SPAD value of the rice was declined which is an indication of breakdown of photosynthetic pigments. However, Hasanuzzaman *et al*. (2019) reported that the induction of Ni stress to the rice seedling disrupted the photosynthetic pigments such as Chl *a*, Chl *b*, and total Chl contents. Similar declination of the total Chl content (Khan *et al*., 2016) and Car content (Abd_Allah *et al*., 2019) were reported in mustard plants under Ni stress. In the current experiment, the application of BC and CHT showed an improvement in the SPAD value of rice under Ni stress (Figure 8A-E). Previous findings also supported that the BC (Abbas *et al*., 2017; Kanwal *et al*., 2018) and CHT (Zong *et al*., 2017; Behboudi *et al*., 2018) application improved the photosynthesis in various crops. Moreover, Naveed *et al*. (2020) reported that salt stress is responsible for the declination of the SPAD value of quinoa, but with BC application, it was improved in plants. Incorporation of BC under other stresses, viz., under drought also showed an improvement in the contents of Chl *a* and Chl *b* in wheat reported by Bashir *et al*. (2020). Under Cd stress, Rizwan *et al*. (2018b) observed that the Chl *a*, Chl *b*, and gas exchange parameters such as P_n, g_s, and T_r of rice were increased with BC supplementation. Other reports of Rizwan *et al*. (2019) in the Cd stressed maize and Hasanuzzaman *et al*. (2021) in the salt stressed jute also found an improvement of the photosynthetic activities with BC. In contrast, CHT can also accelerate the pigment accumulation such as Chl *a*, Chl *b*, and Car in mustard under Cd stress reported by Zong *et al*. (2017). Ali *et al*. (2018) also found an increase of the Chl *a*, Chl *b*, total Chl, and Car in the Cr stressed mustard. Moreover, Behboudi *et al*. (2018) and Qu *et al*. (2019) reported that the CHT application enhanced the Chl *a* and Chl *b* accumulation in the drought affected barley and Cd stress maize, respectively. Under Ni stress, the application of CHT also retrieved the contents of Chl *a* and Chl *b* in lentils reported by Tanzeem-ul-Haq *et al*. (2021). Furthermore, Heidari *et al*. (2020) found an improvement of the contents of Chl *a*, Chl *b*, and Car in the Ni stressed *C. tripterocarpa*. Thus, these findings are in congruence with the present study where the BC and CHT application increased the SPAD value under Ni stress.

4.2.2 Relative water content

The leaf RWC was reduced by 13, 21, and 26% under 0.5, 1.0, and 2.0 mM Ni stress, respectively compared to the control. However, the BC application increased the RWC by 6, 10 and 10% in plants treated 0.5, 1.0, and 2.0 mM Ni, respectively. Moreover, the CHT application also improved the RWC by 4, 9, and 9% in plants under 0.5, 1.0, and 2.0 mM Ni stress, respectively over the Ni stressed plants alone (Figure 9).

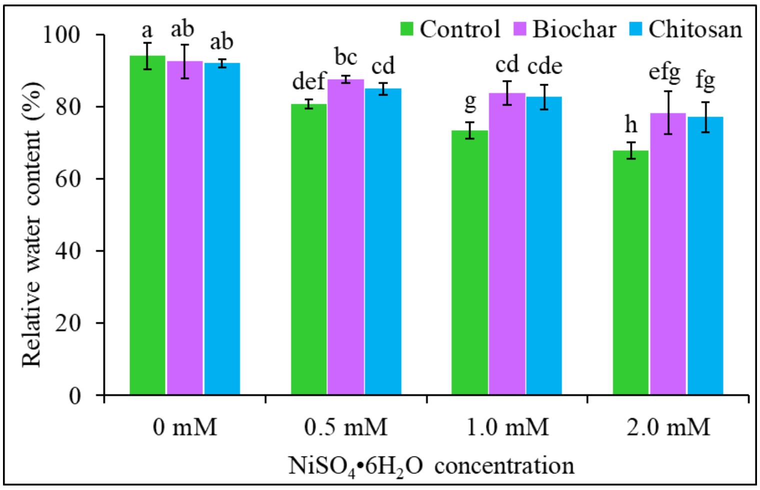


Figure 9. Effect of exogenous application of biochar (0.5 g kg^{-1} soil) and chitosan (200 mg L^{-1}) on the relative water content of rice plants upon exposure to different concentrations of nickel. Mean (± SD) was computed from three replications (n=3). Different letters in each column indicate a significant difference between the treatments at $p \leq 0.05$ after applying Fisher's LSD test

Metal toxicity restricts the root growth and accelerates root damage, therefore inhibiting water uptake. Moreover, excess amounts of metal ions also alter soil water potential and result in osmotic stress due to hindrance in water uptake (Fahr *et al*., 2013). In response to Ni toxicity, the RWC of the rice was decreased with the increase of Ni concentrations here in the present study (Figure 9). This result is in agreement with the previous findings (Mir *et al*., 2018; Abd_Allah *et al*., 2019; Hasanuzzaman *et al*., 2019). Under Ni stress, the RWC of the soybean was decreased (Mir *et al*., 2018), moreover, similar reduction was also reported in mustard (Abd_Allah *et al*., 2019) and rice (Hasanuzzaman *et al*., 2019) plants. However, the application of BC and CHT enhanced the RWC of the Ni affected rice plants. Such improvement of RWC was also reported by Hafez *et al*. (2020) in the water stressed barley plants which increased with BC supplementation. Naveed *et al*. (2020) also reported that salt stress caused a reduction of the RWC of quinoa, however the BC incorporated plants showed an improvement in the RWC of salt stressed plants. On the contrary, the CHT foliar spray under drought notably increased the RWC of the barley plants (Behboudi *et al*., 2018). Similarly, when CHT was applied to the Ni affected lentils, the RWC of the plants was increased reported by Tanzeem-ul-Haq *et al*. (2021). Further report of Hasanuzzaman *et al*. (2021) also demonstrated that the RWC of jute was enhanced under salt stress with BC and CHT application. These results support the findings of the current study that BC and CHT can ameliorate the osmotic stress in plants and enhance water uptake.

4.2.3 Proline content

A notable increase of the Pro accumulation was observed with the increase of Ni concentrations. Upon exposure to 0.5, 1.0, and 2.0 mM Ni, the Pro content was increased by 64, 82, and 111%, respectively compared to the control. Application of BC reduced the Pro content by 23, 19, and 15% in plants treated with 0.5, 1.0 and 2.0 mM Ni, respectively compared to the corresponding Ni stressed plants only, whereas it was decreased by 8, 10, and 10%, respectively with CHT application (Figure 10).

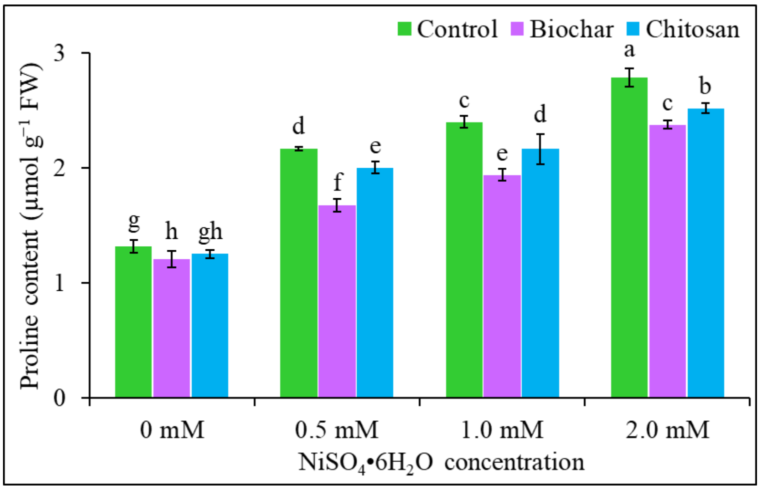


Figure 10. Effect of exogenous application of biochar (0.5 g kg^{-1} soil) and chitosan (200 mg L^{-1}) on the proline content of rice plants upon exposure to different concentrations of nickel. Mean (± SD) was computed from three replications (n=3). Different letters in each column indicate a significant difference between the treatments at $p \leq 0.05$ after applying Fisher's LSD test

To alleviate the osmotic shock, plants accumulate osmolytes in order to mitigate the physiological damages. Upon exposure to Ni stress, the Pro accumulation was increased in the soybean plants (Mir *et al.*, 2018). A notable increase of Pro and glycine betaine found in the Ni affected mustard plants reported by Abd_Allah *et al.* (2019). Moreover, Ni toxicity caused an increase of the Pro levels in rice in a dose dependent manner in the current study, however, the BC and CHT application resulted in the improvement of the RWC in plants, thereby retaining the osmotic balance and reduced Pro accumulation (Figure 10). Similarly, Kanwal *et al.* (2018) reported that the Pro accumulation increased under salt stress, but the BC application reduced the Pro content in both varieties of wheat. Whereas, the Pro accumulation of the salt affected soybean plants also reduced when the plants were supplemented with a modified formulation of CHT and BC in the soils (Mehmood *et al.*, 2020). Further report of Zong *et al.* (2017) showed that the Pro accumulation in the Cd affected mustard was decreased with the CHT application. Similarly, in the drought affected barley, the Pro accumulation was reduced with CHT supplementation reported by Behboudi *et al.* (2018). Whereas, Hasanuzzaman *et al.* (2021) reported that BC as well as CHT application to the jute under salt stress reduced the accumulation of Pro in the plants. These findings supported the present report on the reduction of Pro content with the BC and CHT application under Ni stress.

4.3 Oxidative stress indicators

4.3.1 Malondialdehyde content

A prominent increase of the MDA content was found in a dose dependent manner under Ni stress. Upon exposure to 0.5, 1.0, and 2.0 mM Ni, the MDA content was accelerated by 46, 85, and 119%, respectively compared to the control. The BC supplementation lowered the MDA generation by 29, 23, and 13%, whereas CHT reduced it by 17, 12, and 8% in plants treated with 0.5, 1.0, and 2.0 mM Ni compared to the corresponding Ni stressed plants only (Figure 11).

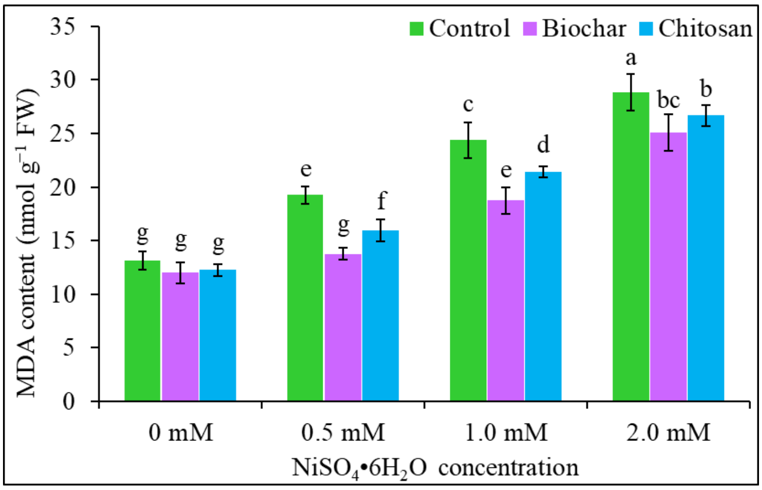


Figure 11. Effect of exogenous application of biochar (0.5 g kg^{-1} soil) and chitosan (200 mg L^{-1}) on the malondialdehyde (MDA) content of rice plants upon exposure to different concentrations of nickel. Mean (± SD) was computed from three replications (n=3). Different letters in each column indicate a significant difference between the treatments at $p \leq 0.05$ after applying Fisher's LSD test

Heavy metals negatively affect the physiological and metabolic processes of plants through inducing ionic toxicity, nutrient deficiency, and water scarcity. Therefore, it accelerates the ROS generation and increases the lipid peroxidation in plants under stress (Hasanuzzaman *et al*., 2021). Induction of Ni stress leads to an increase of lipid peroxidation indicated by MDA in the rice plants here in the current study (Figure 11). Previous reports on mustard (Khan *et al*., 2016), soybean (Mir *et al*., 2018) and rice (Hasanuzzaman *et al*., 2019) showed an increase of the MDA content in the plants under Ni stress. Moreover, here in the present study, the BC and CHT application to the Ni affected rice plants showed a notable reduction of the MDA levels (Figure 11). The MDA accumulation of quinoa under salinity (Naveed *et al*., 2020), barley under drought (Hafez *et al*., 2020), and mustard under Cr toxicity (Ali *et al*., 2018) were also reduced with the application of BC. Conversely, the CHT application to the Cd stressed mustard (Zong *et al*., 2017) and maize (Qu *et al*., 2019) decreased the MDA levels in plants. Moreover, Anee *et al*. (2022) reported that the BC and CHT supplementation decreased the MDA of wheat under drought conditions. Furthermore, the BC and CHT application to the salt stressed jute, the MDA content was lowered notably as reported by Hasanuzzaman *et al*. (2021). In rice, the accumulation MDA was also decreased when BC was exogenously applied to the Cd treated plants (Rizwan *et al*., 2018b). Another report of Rizwan *et al*. (2019) further demonstrated that the BC application to the maize inhibits the MDA accumulation in the roots and shoots under Cd stress. Whereas, the CHT application to the Ni affected lentils suppressed the MDA production as reported by Tanzeem-ul-Haq *et al*. (2021). Moreover, the content of MDA was reduced with the combined application of CHT and BC in the salt stressed soybean plants (Mehmood *et al*., 2020). Another report of Heidari *et al*. (2020) also showed a declination of the MDA levels in the roots and leaves of *C. tripterocarpa* under Ni stress with CHT application. These results support the present reports on rice where the BC and CHT mitigated the MDA contents and protects the plants from Ni-induced oxidative damages.

4.3.2 Hydrogen peroxide content

Upon exposure to 0.5, 1.0, and 2.0 mM Ni, the H_2O_2 content was accelerated by 48, 80, and 99%, respectively compared to the control. However, the supplementation with BC reduced the H_2O_2 content by 19, 18, and 7% in plants treated with 0.5, 1.0, and 2.0 mM Ni, respectively, while the CHT application reduced it by 11, 15, and 4%, respectively, in comparison to the Ni stressed plants only (Figure 12).

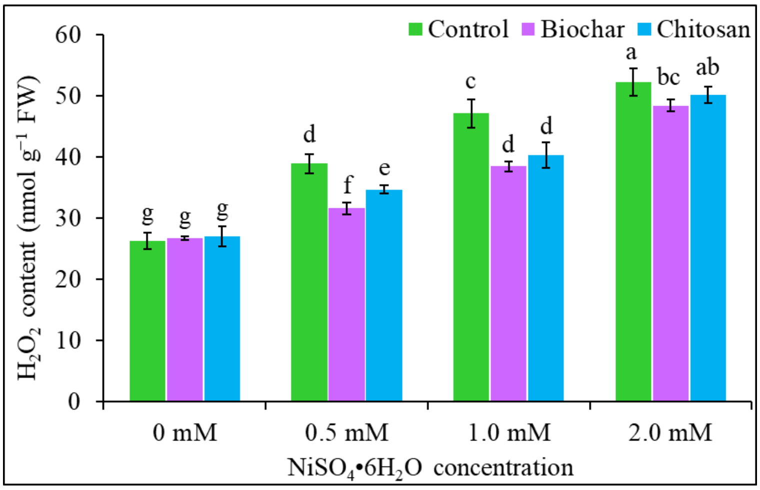


Figure 12. Effect of exogenous application of biochar (0.5 g kg^{-1} soil) and chitosan (200 mg L^{-1}) on the hydrogen peroxide (H_2O_2) content of rice plants upon exposure to different concentrations of nickel. Mean (± SD) was computed from three replications (n=3). Different letters in each column indicate a significant difference between the treatments at $p \leq 0.05$ after applying Fisher's LSD test

Metal toxicity leads to an increase in the ROS generation in plants. Though ROS acts as a signaling molecule in order to activate the defensive mechanisms, excessive accumulation led to ROS-mediated oxidative damages in plants through disrupting the cellular organelles such as proteins, lipids, DNA, etc. (Hasanuzzaman *et al*., 2020; Sachdev *et al*., 2021). The Ni toxicity disrupts electron flow and photosynthetic apparatus, thus accelerating the H_2O_2 generation in rice under Ni stress here in the present study (Figure 12). Several studies reported that the Ni toxicity increased the H_2O_2 in soybean (Mir *et al*., 2018), mustard (Abd_Allah *et al*., 2019), and rice (Hasanuzzaman *et al*. 2019) plants. However, Ali *et al*. (2018) reported that the generation of H_2O_2 contents in the roots and shoots of mustard were decreased with BC application under Cr stress. Similarly, in rice, the H_2O_2 content was reduced when they were supplemented with BC under Cd stress (Rizwan *et al*., 2018b). Moreover, the H_2O_2 production was also restricted in the Cd stressed maize plant when supplemented with BC (Rizwan *et al*., 2019). Furthermore, the H_2O_2 accumulation in the drought stressed barley was also declined with BC supplementation as reported by Hafez *et al*. (2020). On the contrary, Qu *et al*. (2019) reported that the exogenous application of CHT to the Cd stressed maize suppressed the toxic accumulation of $O_2^{\bullet-}$ and H_2O_2 in plants. On application of a modified formulation of CHT with BC inhibited the H_2O_2 generation in soybean under salinity (Mehmood *et al*., 2020).

Hasanuzzaman *et al*. (2021) also reported the H_2O_2 generation was decreased in the plants with BC as well as CHT application to salt stressed jute plants. Furthermore, Tanzeem-ul-Haq *et al*. (2021) also reported the CHT supplementation mitigated the H_2O_2 in lentil plants under Ni toxicity. Such findings are in congruence with the present study, where the BC and CHT mitigated the H_2O_2 generation and protects cellular organelles from ROS-induced damages in plants.

4.3.3 Electrolyte leakage

Electrolyte leakage was increased with the increasing concentrations of Ni in both roots and leaves of rice. The root EL was increased by 11, 21, and 29%, whereas the leaf EL was increased by 6, 17 and 24% in plants under 0.5, 1.0, and 2.0 mM Ni stress, respectively compared to the control. The reduction of root EL was observed with BC supplementation by 4, 5, and 6%, and by 3, 3, and 6% with CHT in plants under 0.5, 1.0, and 2.0 mM Ni stress, respectively in comparison to the corresponding Ni affected plants alone. Similarly, the leaf EL also decreased with the BC application by 4, 8, and 10%, whereas the CHT spray reduced it by 4, 4, and 10% in plants under 0.5, 1.0, and 2.0 mM Ni stress, respectively (Figure 13A, B).

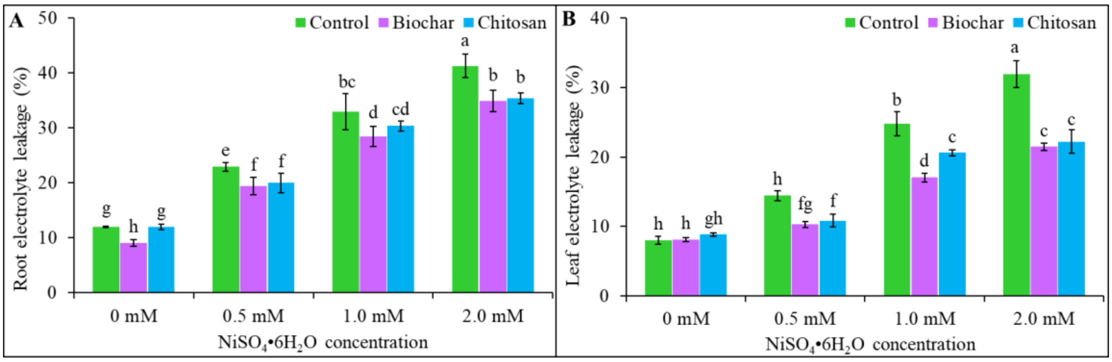


Figure 13. Effect of exogenous application of biochar (0.5 g kg^{-1} soil) and chitosan (200 mg L^{-1}) on the electrolyte leakage of root (A) and leaf (B) of rice plants upon exposure to different concentrations of nickel. Mean (± SD) was computed from three replications (n=3). Different letters in each column indicate a significant difference between the treatments at $p \leq 0.05$ after applying Fisher's LSD test

Acceleration of oxidative stress leads to an increase in the disruption of cellular organelles, thereby increasing electron leakage in plants (Sachdev *et al*., 2021). Here in the current study, the MDA and H_2O_2 content were increased under Ni stress, thereby increasing the EL of rice, in contrast, the BC and CHT supplemented plants showed a reduction of the EL under Ni toxicity (Figure 13). Mir *et al*. (2018) also reported that in soybeans, the EL was increased under Ni stress. The EL of the Cd affected rice plants decreased with the BC application and the highest reduction of the EL was found at 3% BC supplemented plants as reported by Rizwan *et al*. (2018b). In jute, the BC as well as CHT showed a declination of the EL in plants under salt stress (Hasanuzzaman *et al*., 2021). Such reduction of EL occurred due to the mitigation of ROS-induced damages with BC and CHT application in plants under stress which supports the current study.

4.4 Ascorbate and glutathione content

Upon exposure to 0.5, 1.0, and 2.0 mM Ni stress, the AsA content was reduced by 20, 27, and 39%, respectively, while the DHA content was increased by 20, 39, and 59%, respectively compared to the control. Thus, the ratio of AsA/DHA dropped in the Ni stressed plants compared to the control. The BC amendment increased the AsA content by 21, 19, and 24%, while decreased the DHA content by 16, 21, and 14% in plants under 0.5, 1.0, and 2.0 Ni, respectively compared to the Ni stressed plants only. Similarly, the foliar application of CHT increased the AsA content by 15, 12, and 18%, and decreased the DHA content by 11, 12, and 9% in plants treated with 0.5, 1.0, and 2.0 Ni, respectively. Thus, the ratio of AsA/DHA was retrieved with the supplementation of BC and CHT in the Ni stressed plants (Figure 14A-C).

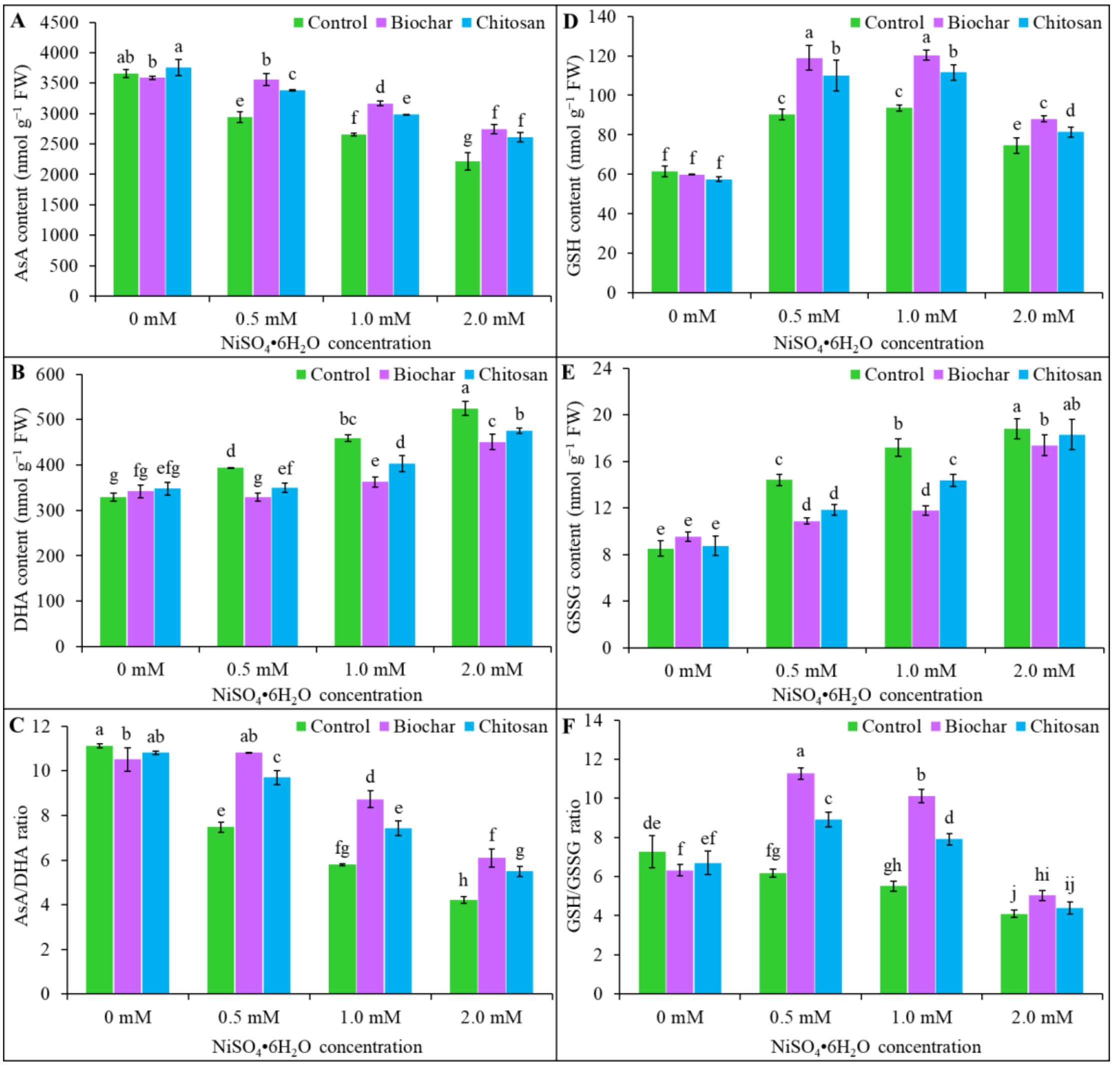


Figure 14. Effect of exogenous application of biochar (0.5 g kg^{-1} soil) and chitosan (200 mg L^{-1}) on the AsA content (A), DHA content (B), AsA/DHA ratio (C), GSH content (D), GSSG content (E), and GSH/GSSG ratio (F) of rice plants upon exposure to different concentrations of nickel. Mean (± SD) was computed from three replications (n=3). Different letters in each column indicate a significant difference between the treatments at $p \leq 0.05$ after applying Fisher's LSD test

Compared to the control, the GSH content was increased by 47, 52, and 21%, while the GSSG content was increased by 69, 101, and 121%, respectively under 0.5, 1.0, and 2.0 mM Ni stress. Thus, the ratio of GSH/GSSG was reduced in the Ni stressed plants compared to the control. Moreover, the GSH content was increased with BC application by 32, 29, and 18%, whereas it was increased by 22, 19, and 9% with CHT in plants under 0.5, 1.0, and 2.0 Ni stress, respectively over the Ni stressed plants only. On the contrary, the GSSG content was decreased by 24, 31, and 8% with BC application, while the reduction was by 18, 16, and 3% with CHT in plants treated with 0.5, 1.0, and 2.0 Ni, respectively. Furthermore, the ratio of GSH/GSSG was uplifted with BC and CHT supplementation under Ni stress (Figure 14D-F).

The AsA and GSH plays a pivotal role in ROS scavenging through the AsA-GSH cycle. In order to maintain a redox balance and to protect the cellular organelles and biomolecules from the oxidative damages, the AsA-GSH cycle plays a crucial role (Hasanuzzaman *et al*., 2020). Upon exposure to Ni stress in the current study, the AsA content was declined, whereas the contents of DHA, GSH, and GSSG were increased in a dose dependent manner, thus the ratios of AsA/DHA and GSH/GSSG were dropped notably in rice. Mir *et al*. (2018) reported that the contents of AsA and GSH decreased with the increase of DHA and GSSG content, thus the AsA/DHA and GSH/GSSG were also reduced notably in soybean under Ni stress. Reports of Khan *et al*. (2016) also demonstrated the reduction of the GSH content in mustard in a dose dependent manner. Abd_Allah *et al*. (2019) further reported that the AsA content decreased whereas the GSH and GSSG contents were increased in the mustard plants under Ni stress. The ratio of AsA/DHA and GSH/GSSG were declined in the Ni affected rice also reported by Hasanuzzaman *et al*. (2019). Moreover, the AsA and GSH content were increased with the reduction of DHA and GSSG levels, thus improving the ratios of AsA/DHA and GSH/GSSG in Ni affected rice in the present study (Figure 14A-F). The supplementation of BC as well as CHT improved the AsA and GSH contents with the reduction of DHA and GSSG contents, thus retrieved the AsA/DHA and GSH/GSSG ratios of the salt stressed jute plants (Hasanuzzaman *et al*., 2021). The CHT application also increased the AsA content in the Cd affected mustard (Zong *et al*., 2017). The report of Qu *et al*. (2019) also demonstrated that the AsA and GSH contents were reduced in maize under Cd stress, however their contents were enhanced when the plants were additionally treated with CHT. Thus, the findings indicated a protective role of BC and CHT in regulating AsA-GSH cycle to mitigate the ROS-induced damages in different plants which is in congruence of the present findings.

4.5 Yield and yield attributes

4.5.1 Effective tillers hill^{-1}, ineffective tillers hill^{-1}, panicle length, and rachis panicle^{-1}

Upon exposure to 0.5, 1.0, and 2.0 mM Ni, the number of effective tillers hill^{-1} was decreased by 19, 32, and 39%, respectively compared to the control. However, the BC application increased the number of effective tillers hill^{-1} by 28, 26, and 17% in plants treated with 0.5, 1.0, and 2.0 mM Ni, respectively compared to the corresponding Ni affected plants only. Conversely, the CHT application also enhanced the number of effective tillers hill^{-1} by 13, 23, and 17% in plants under 0.5, 1.0, and 2.0 mM Ni stress, respectively (Figure 15A).

The number of ineffective tillers hill^{-1} was increased by 102, 161, and 182% upon exposure to 0.5, 1.0, and 2.0 mM Ni stress, respectively compared to the control. However, the supplementation of BC reduced the number of ineffective tillers hill^{-1} by 35, 36, and 13% in plants treated with 0.5, 1.0, and 2.0 mM Ni, respectively compared to the Ni affected plants only, whereas it was also decreased by 29, 24, and 9%, respectively with CHT application (Figure 15B).

The panicle length was decreased with the increase of Ni concentrations. The highest reduction of panicle length was found in 2.0 mM Ni stress by 18%, then at 1.0 mM Ni by 14%, and at 0.5 mM Ni by 9% compared to the control. However, the supplementation of with BC increased the panicle length by 10, 9, and 11% in plants treated with 0.5, 1.0, and 2.0 mM Ni, respectively compared to the Ni stressed plants only, whereas it was increased by 8 and 9% in plants under 0.5 and 2.0 mM Ni stress with CHT application. No significant difference was found under 1.0 mM Ni stressed plants with CHT supplementation (Figure 15C).

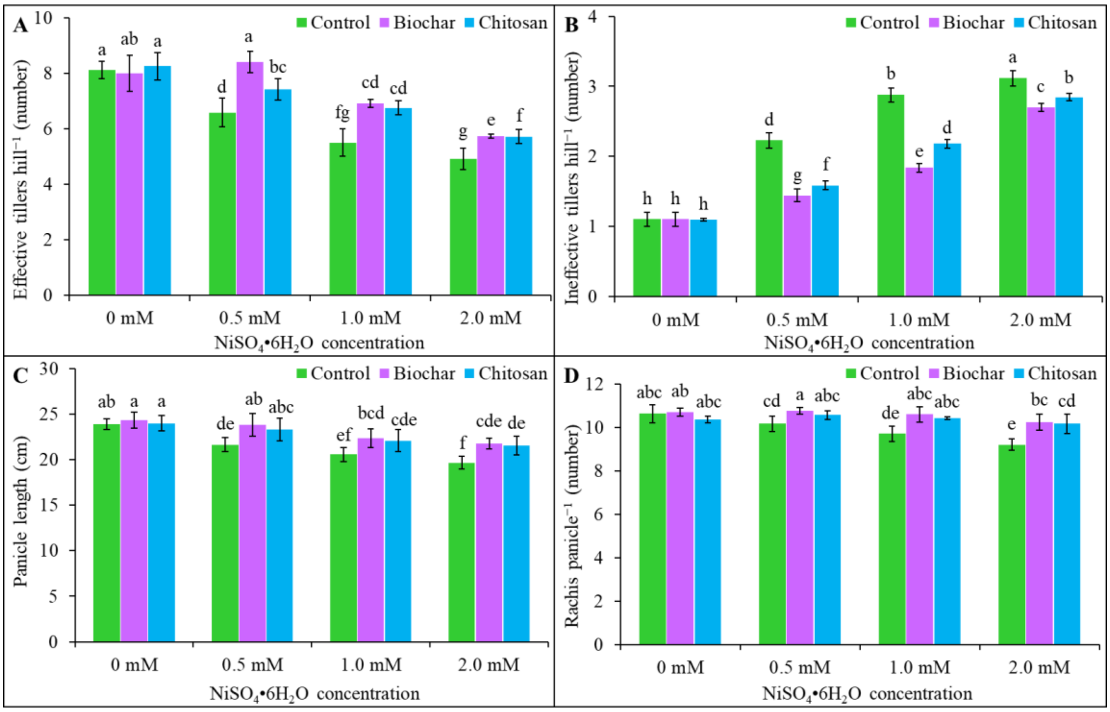


Figure 15. Effect of exogenous application of biochar (0.5 g kg^{-1} soil) and chitosan (200 mg L^{-1}) on the effective tillers $hill^{-1}$ (A), ineffective tillers $hill^{-1}$ (B), panicle length (C), and rachis $panicle^{-1}$ (D) of rice plants upon exposure to different concentrations of nickel. Mean (± SD) was computed from three replications (n=3). Different letters in each column indicate a significant difference between the treatments at $p \leq 0.05$ after applying Fisher's LSD test

When compared to the control, the number of rachis $panicle^{-1}$ was reduced by 4, 9, and 13% in plants upon exposure to 0.5, 1.0, and 2.0 mM Ni stress, respectively. Moreover, the rachis number $panicle^{-1}$ was increased with BC supplementation by 6, 9, and 11% in plants under 0.5, 1.0, and 2.0 mM Ni stress, respectively in comparison to the corresponding Ni stressed plants only. Whereas, the CHT application also increased the number of rachis $panicle^{-1}$ by 8 and 11% at 1.0 and 2.0 mM Ni stress, respectively in comparison to the corresponding Ni stressed plants only (Figure 15D).

Heavy metal stress adversely affects the cell division and ultimately retards the growth of plants (Hassan *et al*., 2019). However, the ionic toxicity due to metals ions inhibit axillary bud formation thus hampers the tiller formation in monocots (Domagalska *et al*., 2011; Zhuang *et al*., 2019). Moreover, the Ni toxicity also reduced the plant growth, FW, DW, and photosynthesis, thereby hampering the yield attributes of the plants (Kanwal *et al*., 2018; Hassan *et al*., 2019; Abd_Allah *et al*., 2019; Hasanuzzaman *et al*., 2019; Aqeel *et al*., 2021). Here, in the Ni stressed rice, the number of effective and ineffective tillers hill^{-1}, panicle length and number of rachis panicle^{-1} were decreased with the increase of Ni concentrations. However, when BC and CHT were applied to the Ni affected rice these parameters were improved notably (Figure 15A-D). Under stress, BC reduce the metal ion accumulation by absorbing it with soil particles, whereas CHT has a role in enhancing phytohormone accumulation, thus ameliorate the plant growth and development (Hidangmayum *et al*., 2019; Imran *et al*., 2020). Moreover, Anee *et al*. (2022) reported that the spike length and number of spikelets spike^{-1} in wheat were enhanced under drought with the supplementation of the BC and CHT. Under Cd toxicity, the spike length was also improved upon incorporation with BC reported by Abbas *et al*. (2017). Similarly, Bashir *et al*. (2020) reported that the spike length of the drought affected wheats was enhanced on the application of BC. Furthermore, Younas *et al*. (2021) reported that the CHT application to the maize plants enhanced the cob length under salt stress. These findings corroborated the present study and indicate that BC and CHT can improve the plant growth and also ameliorate the yield attributes.

4.5.2 Filled grains panicle^{-1}, unfilled grains panicle^{-1}, total number of grains panicle^{-1}, and 1000-grain weight

The number of filled grains panicle^{-1} was decreased by 5, 12, and 17% in plants treated with 0.5, 1.0, and 2.0 mM Ni, respectively compared to the control. Moreover, the number of filled grains was increased with BC application by 10 and 13% under 0.5 and 1.0 mM Ni stress, respectively compared to the Ni affected plants only, whereas the CHT application improved it by 8 and 10%, respectively (Figure 16A).

Upon exposure to 0.5, 1.0, and 2.0 mM Ni to the plants, the number of unfilled grains panicle^{-1} was increased by 20, 44, and 46%, respectively over the control. However, the BC application to the 0.5, 1.0, and 2.0 mM Ni stressed plants decreased the number of unfilled grains panicle^{-1} by 20, 14, and 14%, respectively in comparison to the Ni affected plants only. In contrast, the CHT application also reduced the number of unfilled grains panicle^{-1} by 14, 17, and 13% in plants treated with 0.5, 1.0, and 2.0 mM Ni, respectively (Figure 16B).

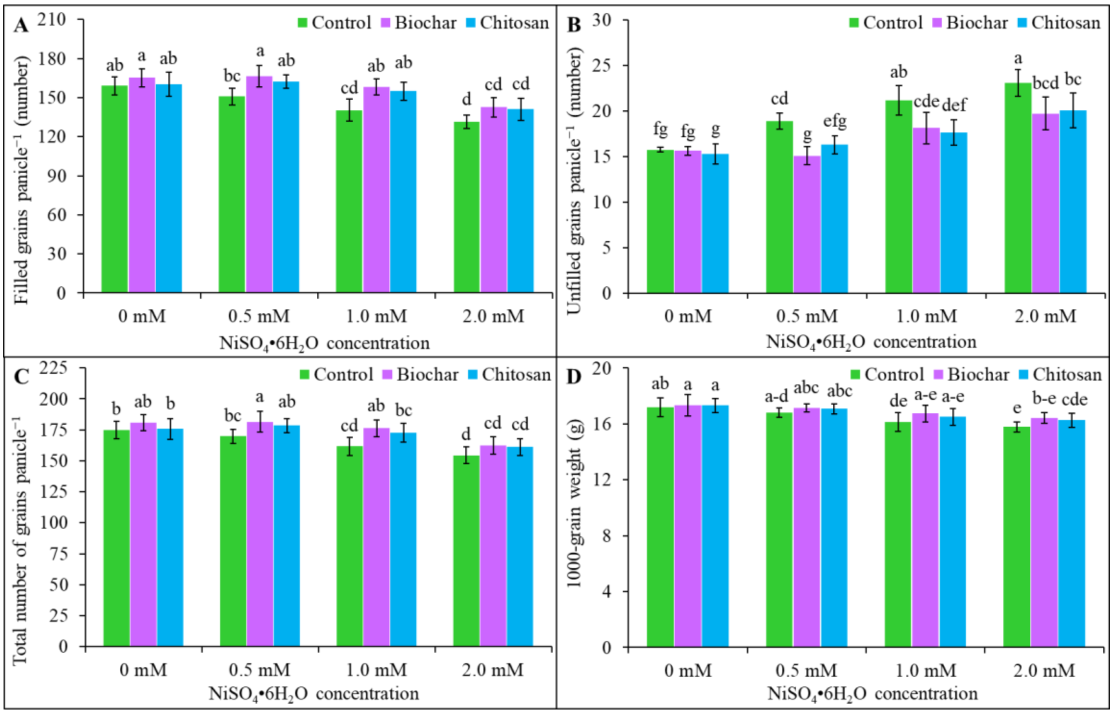


Figure 16. Effect of exogenous application of biochar (0.5 g kg^{-1} soil) and chitosan (200 mg L^{-1}) on the filled grains $panicle^{-1}$ (A), unfilled grains $panicle^{-1}$ (B), total number of grains $panicle^{-1}$ (C), and 1000-grain weight (D) of rice plants upon exposure to different concentrations of nickel. Mean (± SD) was computed from three replications (n=3). Different letters in each column indicate a significant difference between the treatments at $p \leq 0.05$ after applying Fisher's LSD test

A notable reduction of the total number of grains $panicle^{-1}$ was found by 8 and 12% in plants, respectively under 1.0 and 2.0 mM Ni stress compared to the control. However, the BC application increased the total grain numbers $panicle^{-1}$ by 7 and 9% in plants treated with 0.5 and 1.0 mM Ni, respectively over the Ni affected plants only. Whereas, no significant improvement was seen under Ni stress with the CHT supplementation (Figure 16C).

The weight of 1000 grains was decreased under 1.0 and 2.0 mM Ni stress by 6 and 8%, respectively compared to the control. However, the application of BC and CHT did not show any significant improvement of the weight of 1000-grain in the Ni affected plants (Figure 16D).

Physiological mechanisms of the plants alter upon exposure to Ni stress. The disrupted Chl structure, water balance and ion toxicity effects on the developmental processes of the plants (Hassan *et al*., 2019). Moreover, Ni-induced oxidative stress accelerates the cell damage and also hampers metabolic activities, thus reducing the yield traits. Here in the study, Ni stress led to decrease the filled grains panicle^{-1}, total grains panicle^{-1}, and 1000-grain weight of rice, whereas increased the unfilled grains panicle^{-1} in a dose dependent manner (Figure 16A-D). Ali *et al*. (2015) reported that the number of seed plant^{-1} was decreased with the increase of Ni concentration in mungbean plants. However, the BC and CHT application further increased the filled grains panicle^{-1}, total grains panicle^{-1}, and 1000-grain weight with the decrease of the unfilled grains panicle^{-1} under Ni stress in the present study. Moreover, Anee *et al*. (2022) reported that the BC and CHT application to the drought affected wheat enhanced the filled grains spike^{-1} and decreased the unfilled grains spike^{-1} which ultimately improved yield. Whereas, the number of grains spike^{-1} was increased with CHT application in barley reported by Behboudi *et al*. (2018). Similarly, the number of grain cob^{-1} was also increased with the CHT application in maize under salinity (Younas *et al*., 2021). Grain weight has a direct effect on the yield of crops. The 1000-grain weight of barley was increased with BC under drought (Hafez *et al*., 2020). Similarly, the 100-grain weight of wheats was also enhanced under drought stress with the BC and CHT as reported by Anee *et al*. (2022). The CHT application also enhanced the weight of 1000 grains of barley (Behboudi *et al*., 2018). These findings indicated a protective role of BC and CHT to ameliorate the ROS damages and improved the crop yields.

4.5.3 Grain yield hill^{-1} and straw yield hill^{-1}

Upon exposure to 0.5, 1.0, and 2.0 mM Ni to the plants caused a notable reduction of the grain yield hill^{-1} by 23, 38, and 48%, respectively compared to the control. However, the BC supplementation retrieved the adverse effect of Ni stress and increased the grain yield hill^{-1} by 40, 36, and 21% under 0.5, 1.0, and 2.0 mM Ni stress, respectively compared to the Ni affected plants only. Conversely, the CHT application also showed an improvement of the grain yield hill^{-1} by 21, 29, and 19% at the 0.5, 1.0, and 2.0 mM Ni stressed plants, respectively over the Ni treated plants only (Figure 17A).

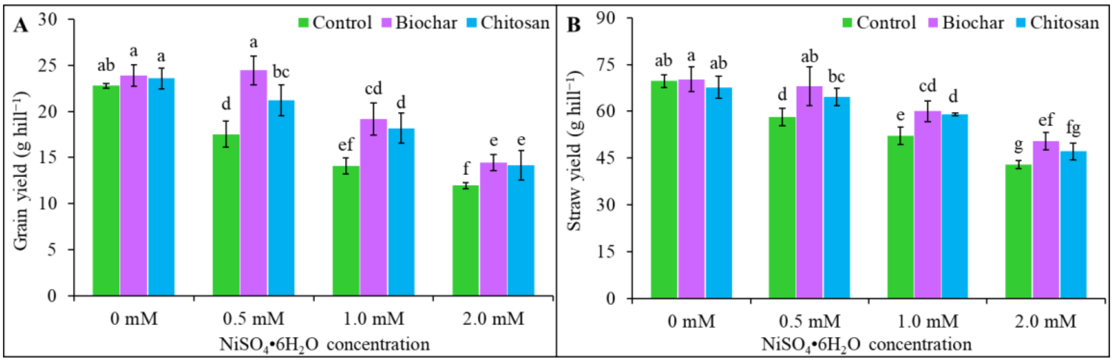


Figure 17. Effect of exogenous application of biochar (0.5 g kg^{-1} soil) and chitosan (200 mg L^{-1}) on the grain yield hill^{-1} (A) and straw yield hill^{-1} (B) of rice plants upon exposure to different concentrations of nickel. Mean (± SD) was computed from three replications (n=3). Different letters in each column indicate a significant difference between the treatments at $p \leq 0.05$ after applying Fisher's LSD test

The straw yield hill^{-1} was also reduced in plants under Ni stress in a dose dependent manner. Upon exposure of 0.5, 1.0, and 2.0 mM Ni, the straw yield hill^{-1} was decreased by 17, 25, and 39% in plants, respectively compared to the control. Moreover, the application of BC under 0.5, 1.0, and 2.0 mM Ni stress, increased the straw yield hill^{-1} by 17, 15, and 18%, respectively over the Ni stressed plants only, whereas the CHT application only showed a notable increase of the straw yield hill^{-1} at 0.5 mM (11%) and 1.0 mM (13%) Ni stressed plants (Figure 17B).

Heavy metal toxicity leads to a decrease of the photosynthetic activity, induce osmotic stress, and oxidative damages of the plants. Therefore, inhibit the photoassimilate production and reduce the yield. The reduction of the filled grains hill^{-1} and 1000-grain weight with the increase of unfilled grains hill^{-1} ultimately decreased the yield of the rice under Ni stress (Figure 17A). Ali *et al*. (2015) reported that the seed yield plant^{-1} was reduced with the increase of Ni concentration in mungbean. However, when the plants were supplemented with BC and CHT further improved the grain yield hill^{-1} under Ni stress (Figure 17A). In the Cd stressed wheat, the grain yield was increased with BC application reported by Abbas *et al*. (2017). Naveed *et al*. (2020) also reported that the seed yield plant^{-1} was enhanced when the plants were treated with BC under salt stress in quinoa. Grain yield of the barley was also increased in plants with BC application under water stress (Hafez *et al*., 2020). On the contrary, the grain yield plant^{-1} was increased in the maize when treated with

CHT under water-deficit conditions (Rabêlo *et al*., 2019). As Ni stress leads to the reduction of photosynthetic activity, FW, and water availability of the plants, thus the straw yield was also declined in a dose dependent manner. However, the BC and CHT supplemented plants ameliorate the growth and development of the plants, thereby enhancing the straw yield hill^{-1} of rice (Figure 17B). Therefore, it could be stated that BC and CHT has a role in promoting growth of plants through enhancing several physiological and biochemical processes, thus ultimately improving the yield of rice.

4.6 Phenotypic observations

The phenotypic appearance of rice treated with different concentrations of Ni (0.5, 1.0, and 2.0 mM) has been depicted in Figure 18A, compared to the control. It is clearly noticeable that the plant growth has been retarded in a dose dependent manner with the increase of Ni concentrations (Figure 18A). Nonetheless, the supplementation of BC and CHT under control (Figure 18B) as well as 0.5, 1.0, and 2.0 mM Ni stress enhanced the growth of the plants in comparison to the Ni stressed plants alone (Figure 18C-E).

Figure 18. Phenotypic appearance of rice plants with different treatment combinations. Here, Ni_1= 0.5 mM $NiSO_4 \cdot 6H_2O$, Ni_2= 1.0 mM $NiSO_4 \cdot 6H_2O$, Ni_3= 2.0 mM $NiSO_4 \cdot 6H_2O$, BC= 0.5 g biochar kg^{-1} soil and CHT= 200 mg chitosan-100 L^{-1}

Metal stress induces ionic toxicity, osmotic stress, and accelerates oxidative damages in plants. Thus, the growth in terms of height, leaf area, FW and DW of the plants were declined due to the disruption of photosynthesis and photoassimilate production (Ali *et al*., 2015; Parlak, 2016; Azeem, 2018; Amjad *et al*., 2019; Hasanuzzaman *et* *al*., 2019; Khan *et al*., 2020a). However, application of BC and CHT ameliorates the damages caused by Ni in plants and improves the developmental processes. The BC has a role in mitigating ionic stress, enhance water uptake, and also inhibit the damages caused by oxidative stress (Rehman *et al*., 2016; Shahbaz *et al*., 2019; Hasanuzzaman *et al*., 2021), thus improve the growth of plants. On the contrary, the CHT acts as a signaling molecules and enhance the phytohormone activity in plants under different environmental conditions, thus improves the metabolic pathways and increased stress endurance capacity of the plants (Rabêlo *et al*., 2019; Heidari *et al*., 2020; Sadeghipour, 2021). Here in the present study the growth of plants was retarded due to the Ni toxicity, but when BC and CHT were applied to the Ni affected plants the growth of plants was enhanced, thus ultimately improving the yield of rice.

Chapter V

SUMMARY AND CONCLUSION

The experiment was conducted at the experimental shed house of the Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh, to investigate the effectiveness of BC and CHT in alleviating the detrimental effects of Ni in rice (*O. sativa* L. cv. BRRI dhan96). The experiment was conducted in a completely randomized design with three replications. Fourty-days-old seedlings were transplanted in the plastic pots and at 21 DAT, sets of plants were imposed to Ni stress at the concentrations of 0.5, 1.0, and 2.0 mM $NiSO_4 \bullet 6H_2O$, whereas only water was irrigated to the control. During pot soil preparation, BC was incorporated at 0.5 g kg^{-1} soil and the foliar spray of CHT was done at 200 mg L^{-1} from 14 DAT to panicle initiation at 7 d interval.

Different parameters on crop growth (plant height, tillers $hill^{-1}$, leaf area, fresh weight of root and shoot $hill^{-1}$ and dry weight of root and shoot $hill^{-1}$), physiology (SPAD value, RWC, and Pro content), and oxidative stress indicators (MDA content, H_2O_2 content, EL, AsA content, DHA content, AsA/DHA ratio, GSH content, GSSG content, GSH/GSSG ratio), and the yield attributes (effective tillers $hill^{-1}$, ineffective tillers $hill^{-1}$, panicle length, rachis $panicle^{-1}$, filled grains $panicle^{-1}$, unfilled grains $panicle^{-1}$, total number of grains $panicle^{-1}$, 1000-grain weight, grain yield $hill^{-1}$, and straw yield $hill^{-1}$) were estimated.

A sharp reduction of the plant height was observed at 35, 49, 63, 77 DAT, and harvest when plants were treated with 0.5, 1.0, and 2.0 Ni, respectively, compared to the control. The lowest plant height was observed at 2.0 mM Ni at 35, 49, 63, 77 DAT and harvest. However, supplementation of BC and CHT showed a better improvement of the plant height under Ni stress at every stage.

The number of tillers $hill^{-1}$ at 49, 63, 77 DAT and harvest was dropped significantly upon exposure to 0.5, 1.0, and 2.0 mM Ni stress in plants, respectively, in comparison to the control. Highest reduction of the tillers $hill^{-1}$ was found at 49 DAT at 2.0 mM Ni stress. However, the BC supplementation increased the number of tillers $hill^{-1}$ notably at 0.5 mM Ni stressed plants at 49, 63, 77 DAT and harvest, whereas both BC and CHT increased the number of tillers $hill^{-1}$ at 1.0 mM Ni stressed plants.

Upon exposure to 0.5, 1.0, and 2.0 mM Ni stress, the leaf area was decreased at 49, 63, and 77 DAT. The reduction of the leaf area was more prominent at 2.0 mM Ni stressed plants. Moreover, the BC and CHT application improved the leaf area under Ni stress. Highest increase of leaf area was observed at 63 DAT with BC (22%) and CHT (17%) application under 0.5 mM Ni stress.

A notable reduction of the root FW by 13, 23, and 41%, and shoot FW by 23, 29, and 32% were observed in plants treated with 0.5, 1.0, and 2.0 mM Ni, respectively, compared to the control. Supplementation with BC and CHT further increased the root FW and shoot FW under Ni stress.

In comparison to the control, the root DW by 31, 38, and 42%, and the shoot DW by 32, 41, and 49% were decreased under 0.5, 1.0 and 2.0 mM Ni stressed plants, respectively. Improvement of the root DW and shoot DW in plants were found when they were supplemented with BC and CHT.

The SPAD value at 35, 49, 63, and 77 DAT was reduced upon exposure to 0.5, 1.0 and 2.0 mM Ni compared to the control. However, both BC and CHT applications improved the SPAD value.

The RWC of leaf was reduced by 13, 21, and 26% under 0.5, 1.0, and 2.0 mM Ni stress, respectively compared to the control. However, the RWC was enhanced when supplemented with BC and CHT in the Ni stressed plants.

Upon exposure to 0.5, 1.0, and 2.0 mM Ni, the Pro content was increased by 64, 82, and 111%, respectively compared to the control. Supplementation of BC reduced the Pro content by 23, 19, and 15%, and CHT by 8, 10, and 10%, respectively in plants treated with 0.5, 1.0 and 2.0 mM Ni.

In comparison to the control, the 0.5, 1.0, and 2.0 mM Ni stress increased the MDA content by 46, 85, and 119%, respectively in plants. The BC supplementation lowered the MDA generation by 29, 23, and 13%, whereas CHT reduced it by 17, 12, and 8% in plants treated with 0.5, 1.0, and 2.0 mM Ni.

When plants were subjected to 0.5, 1.0, and 2.0 mM Ni, the H_2O_2 content was accelerated by 48, 80, and 99%, respectively over control. However, the supplementation with BC reduced the H_2O_2 content by 19, 18, and 7% in plants treated with 0.5, 1.0, and 2.0 mM Ni, respectively, while the CHT application reduced it by 11, 15, and 4%, respectively, in comparison to the Ni stressed plants only.

Compared to the controls, the EL of roots and shoots were increased under 0.5, 1.0, and 2.0 mM Ni stress. Moreover, the BC and CHT application mitigated the EL of the roots and shoots of rice.

Upon exposure to 0.5, 1.0, and 2.0 mM Ni stress, the content of AsA reduced, whereas increased the DHA, GSH, and GSSG, thus AsA/DHA and GSH/GSSG were declined in the rice. With BC and CHT application, the AsA and GSH further increased with the reduction of DHA and GSSG levels, thus restoring AsA/DHA and GSH/GSSG ratios.

Different yield attributes were declined under 0.5, 1.0, and 2.0 mM Ni stress compared to the control. However, the BC and CHT application improved these parameters. Whereas the BC notably increased the yield parameters than CHT.

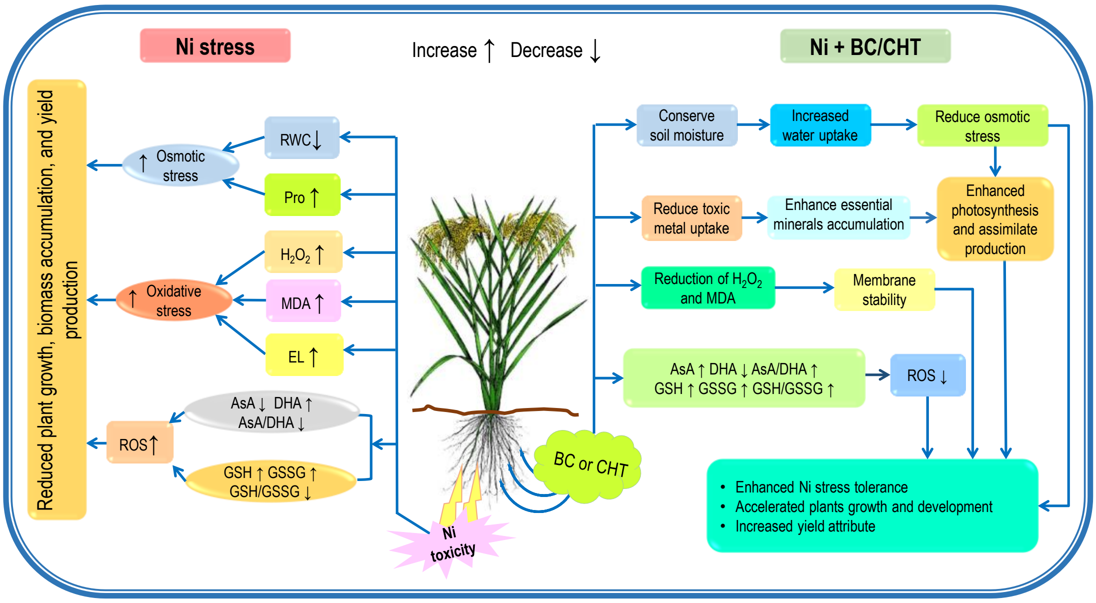


Figure 19. Possible mechanisms of BC and CHT in mitigating Ni stress in rice plants

Considering all the above-mentioned phenomena, it can be stated that upon exposure to Ni stress the growth and yield attributes of rice were adversely affected with the increase of Ni concentrations. The Ni stressed plants showed a higher accumulation of MDA, H_2O_2, and EL which is responsible for the induction of oxidative stress in plants. However, the AsA content of the Ni stressed plants was declined with the increase of DHA, GSH, and GSSG contents, thus the ratio of AsA/DHA and GSH/GSSG were decreased which is an indication of inefficient ROS scavenging within the AsA-GSH cycle. Another effect of Ni is the osmotic stress in plants identified with the reduced RWC in plants and to mitigate this osmotic shock plants accumulate a higher amount of Pro. In contrast, when the BC and CHT were applied to the Ni stressed plants, they mitigated the oxidative stress identified with reduced MDA, H_2O_2, and EL levels, whereas the ratio of AsA/DHA and GSH/GSSG were enhanced. The BC and CHT application also increased the RWC of the plants thus reduced the Pro accumulation. Therefore, mitigation of oxidative stress and osmotic shock ultimately improved the growth and yield attributes of the Ni stressed rice. However, further experiments are to be conducted to elucidate the complex mechanisms of BC and CHT in different biochemical pathways of the plants under Ni stress.

REFERENCES

Abbas, T., Rizwan, M., Ali, S., Zia-ur-Rehman, M., Qayyum, M.F., Abbas, F., Hannan, F., Rinklebe, J. and Ok, Y.S. (2017). Effect of biochar on cadmium bioavailability and uptake in wheat (*Triticum aestivum* L.) grown in a soil with aged contamination. *Ecotoxicol. Environ. Saf.* **140**: 37-47.

Abd_Allah, E.F., Hashem, A., Alam, P. and Ahmad, P. (2019). Silicon alleviates nickel-induced oxidative stress by regulating antioxidant defense and glyoxalase systems in mustard plants. *J. Plant Growth Regul.* **38**(4): 1260-1273.

Abideen, Z., Koyro, H-W., Huchzermeyer, B., Gul, B. and Khan, M.A. (2020). Impact of a biochar or a biochar–compost mixture on water relation, nutrient uptake and photosynthesis of *Phragmites karka*. *Pedosphere.* **30**: 466–477.

Akhtar, S.S., Andersen, M.N. and Liu, F. (2015). Biochar mitigates salinity stress in potato. *J. Agron. Crop Sci.* **201**: 368–378.

Alharby, H.F. and Fahad, S. (2020). Melatonin application enhances biochar efficiency for drought tolerance in maize varieties: Modifications in physio-biochemical machinery. *Agron. J.* **112**: 2826–2847.

Ali, M.A., Asghar, H.N., Khan, M.Y., Saleem, M., Naveed, M. and Niazi, N.K. (2015). Alleviation of nickel-induced stress in mungbean through application of gibberellic acid. *Int. J. Agric. Biol.* **17**: 990-994.

Ali, S., Rizwan, M., Bano, R., Bharwana, S.A., Hussain, M.B. and Al-Wabel, M.I. (2018). Effects of biochar on growth, photosynthesis, and chromium (Cr) uptake in *Brassica rapa* L. under Cr stress. *Arab. J. Geosci.* **11**(17): 1-9.

Amjad, M., Raza, H., Murtaza, B., Abbas, G., Imran, M., Shahid, M., Naeem, M.A., Zakir, A. and Iqbal, M.M. (2019). Nickel toxicity induced changes in nutrient dynamics and antioxidant profiling in two maize (*Zea mays* L.) hybrids. *Plants.* **9**(1): 5.

Anee, T.I., Islam, M.N.N., Hassan, M.M., Masud, A.A.C., Alam, M.M. and Hasanuzzaman, M. (2022). Organic amendments improve plant morpho-physiology and antioxidant metabolism in mitigating drought stress in bread wheat (*Triticum aestivum* L.). *Phyton Int. J. Exp. Bot.* doi:10.32604/phyton.2022.021137.

Anjum, S.A., Ashraf, U., Khan, I., Saleem, M.F. and Wang, L.C. (2016). Chromium toxicity induced alterations in growth, photosynthesis, gas exchange attributes and yield formation in maize. *Pak. J. Agric. Sci.* **53**(4): 751-757.

Aqeel, M., Khalid, N., Tufail, A., Ahmad, R.Z., Akhter, M.S., Luqman, M., Javed, M.T., Irshad, M.K., Alamri, S., Hashem, M. and Noman, A. (2021). Elucidating the distinct interactive impact of cadmium and nickel on growth, photosynthesis, metal-homeostasis, and yield responses of mung bean (*Vigna radiata* L.) varieties. *Environ. Sci. Pollut. Res.* **28**(21): 27376-27390.

Audet, P. and Charest, C. (2008). Allocation plasticity and plant–metal partitioning: Metal-analytical perspectives in phytoremediation. *Environ. Pollut.* **156**: 290–296.

Awasthi, K. and Sinha, P. (2013). Nickel stress induced antioxidant defence system in sponge gourd (*Luffa cylindrica* L.). *J. Plant Physiol. Pathol.* **1**: 1.

Azeem, U. (2018). Ameliorating nickel stress by jasmonic acid treatment in *Zea mays* L. *Rus. Agric. Sci.* **44**(3): 209-215.

Aziz, H., Sabir, M., Ahmad, H.R., Aziz, T., Zia-ur-Rehman, M., Hakeem, K.R. and Ozturk, M. (2015). Alleviating effect of calcium on nickel toxicity in rice. *Clean–Soil. Air. Water.* **43**(6): 901-909.

Bai, C., Reilly, C.C. and Wood, B.W. (2006). Nickel deficiency disrupts metabolism of ureids, amino acids and organic acids of young pecan foliage. *Plant Physiol.* **140**: 433–443.

Baligarx, V.C. (2012). Mechanisms of nickel uptake and hyperaccumulation by plants and implications for soil remediation. *Adv. Agron.* **117**: 117–189.

Barrs, H.D. and Weatherley, P.E. (1962). A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Aust. J. Biol. Sci.* **15**: 413–428.

Bashir, A., Rizwan, M., ur Rehman, M.Z., Zubair, M., Riaz, M., Qayyum, M.F., Alharby, H.F., Bamagoos, A.A. and Ali, S. (2020). Application of co-composted farm manure and biochar increased the wheat growth and decreased cadmium accumulation in plants under different water regimes. *Chemosphere.* **246**: 125809.

Bates, L.S., Waldren, R.P. and Teari, D. (1973). Rapid determination of free proline for water stress studies. *Plant Soil.* **39**: 205–207.

Behboudi, F., Sarvestani, Z.T., Kassaee, M.Z., Sanavi, S.A.M.M., Sorooshzadeh, A. and Ahmadi, S.B. (2018). Evaluation of chitosan nanoparticles effects on yield and yield components of barley (*Hordeum vulgare* L.) under late season drought stress. *J. Water Environ. Nanotechnol.* **3**(1): 22-39.

Bhalerao, S.A., Sharma, A.S. and Poojari, A.C. (2015). Toxicity of nickel in plants. *Int. J. Pure Appl. Biosci.* **3**(2): 345-55.

Bhardwaj, A., Sita, K., Sehgal, A., Bhandari, K., Kumar, S., Prasad, P.V.V., Jha, U., Kumar, J., Siddique, K.H.M. and Nayyar, H. (2021). Heat priming of lentil (*Lens culinaris* Medik.) seeds and foliar treatment with γ-aminobutyric acid (GABA), confers protection to reproductive function and yield traits under high-temperature stress environments. *Int. J. Mol. Sci.* **22**: 5825.

Borges, B.M.M.N., Strauss, M., Camelo, P.A., Sohi, S.P. and Franco, H.C.J. (2020). Re-use of sugarcane residue as a novel biochar fertilizer– Increased phosphorus use efficiency and plant yield. *J. Cleaner Prod.* **262**: 121406

BRRI (Bangladesh Rice Research Institute). (2020). Adhunik Dhaner Chash. Joydebpur, Dhaka- 1701. p. 106.

Bulgari, R., Franzoni, G. and Ferrante, A. (2019). Biostimulants application in horticultural crops under abiotic stress conditions. *Agronomy.* **9**: 306.

CoStat. (2008). CoStat- Statistics Software Version 6.400. CoHort Software, 798 Lighthouse Ave. PMB 320. Monterey, CA, 93940, USA.

Derbali, W., Goussi, R., Koyro, H.W., Abdelly, C. and Manaa, A. (2020). Physiological and biochemical markers for screening salt tolerant quinoa genotypes at early seedling stage. *J. Plant Interact.* **15**(1): 27-38.

Dionisio-Sese, M.L. and Tobita, S. (1998). Antioxidant responses of rice seedlings to salinity stress. *Plant Sci.* **135**: 1–9.

Domagalska, M.A. and Leyser, O. (2011). Signal integration in the control of shoot branching. *Nat. Rev. Mol. Cell Biol.* **12**: 211–221.

Dubey, R.S. and Pessarakli, M. (2002). Physiological mechanisms of nitrogen absorption and assimilating in plants under stressful conditions. **In**: Handbook of Crop Physiology. M. Pessarakli, (ed.). Marcel Dekker, New York. pp. 637–655.

Duda-Chodak, A. and Baszczyk, U. (2008). The impact of nickel on human health. *J. Elementol.* **13**(4): 685–696.

ElSayed, A.I., Rafudeen, M.S., Gomaa, A.M. and Hasanuzzaman, M. (2021). Exogenous melatonin enhances the reactive oxygen species metabolism, antioxidant defense-related gene expression, and photosynthetic capacity of *Phaseolus vulgaris* L. to confer salt stress tolerance. *Physiol. Plant.* **173**(4): 1369-1381

Fahr, M., Laplaze, L., Bendaou, N., Hocher, V., El Mzibri, M., Bogusz, D. and Smouni, A. (2013). Effect of lead on root growth. *Front. Plant Sci.* **4**: 175.

FAO (Food and Agriculture Organization). (2022). Food Outlook – Biannual Report on Global Food Markets. Rome. p. 161.

FAOSTAT. (2021). World Food and Agriculture - Statistical Yearbook 2021. Rome. p. 353.

Fiala, R., Fialová, I., Vaculík, M. and Luxová, M. (2021). Effect of silicon on the young maize plants exposed to nickel stress. *Plant Physiol. Biochem.* **166**: 645-656.

Gajewska, E., Bernat, P., Długoński, J. and Skłodowska, M. (2012). Effect of nickel on membrane integrity, lipid peroxidation and fatty acid composition in wheat seedlings. *J. Agron. Crop Sci.* **198**(4): 286-294.

Gill, S.S. and Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.* **48**: 909–930.

Gupta, V., Jatav, P.K., Verma, R., Kothari, S.L. and Kachhwaha, S. (2017). Nickel accumulation and its effect on growth, physiological and biochemical parameters in millets and oats. *Environ. Sci. Pollut. Res.* **24:** 23915–23925.

Hafeez, Y., Iqbal, S., Jabeen, K., Shahzad, S., Jahan, S. and Rasul, F. (2017). Effect of biochar application on seed germination and seedlings growth of (*Glycine max* L.) Meer. under drought stress. *Pak. J. Bot.* **49**: 7–13.

Hafez, Y., Attia, K., Alamery, S., Ghazy, A., Al-Doss, A., Ibrahim, E., Rashwan, E., El-Maghraby, L., Awad, A. and Abdelaal, K. (2020). Beneficial effects of biochar and chitosan on antioxidative capacity, osmolytes accumulation, and anatomical characters of water-stressed barley plants. *Agronomy*. **10**(5): 630.

Haider, M.Z., Hussain, S., Ramzani, P.M.A., Iqbal, M., Iqbal, M., Shahzad, T., Fatima, M., Khan, S.A., Khan, I., Shahid, M., Ibrahim, M., Tanzeem-ul-Haq, H.S. and Mahmood, F. (2019). Bentonite and biochar mitigate Pb toxicity in *Pisum sativum* by reducing plant oxidative stress and Pb translocation. *Plants*. **8**: 571.

Hamsa, N., Yogesh, G.S., Koushik, U. and Patil, L. (2017). Nitrogen transformation in soil: Effect of heavy metals. *Int. J. Curr. Microbiol. Appl. Sci.* **6**(5): 816–832.

Harada, E., Kim, J.A., Meyer, A.J., Hell, R., Clemens, S. and Choi, Y.E. (2010). Expression profiling of tobacco leaf trichomes identifies genes for biotic and abiotic stresses. *Plant Cell Physiol.* **51**: 1627–1637

Hasanuzzaman, M. and Fujita, M. (2012). Heavy metals in the environment current status, toxic effects on plants and phytoremediation. **In**: Phytotechnologies: Remediation of Environmental Contaminants. N.A. Anjum, M.E. Pereira, I. Ahmad, A.C. Duarte, S. Umar, N.A. Khan, (eds.). CRC Press: Boca Raton. pp. 7–73.

Hasanuzzaman, M., Ahmed, N., Saha, T., Rahman, M., Rahman, K., Alam, M.M., Rohman, M.M. and Nahar, K. (2022). Exogenous salicylic acid and kinetin modulate reactive oxygen species metabolism and glyoxalase system to confer waterlogging stress tolerance in soybean (*Glycine max* L.). *Plant Stress.* **3**: 100057.

Hasanuzzaman, M., Alam, M., Nahar, K., Mohsin, S.M., Bhuyan, M.H.M., Parvin, K., Hawrylak-Nowak, B. and Fujita, M. (2019). Silicon-induced antioxidant defense and methylglyoxal detoxification works coordinately in alleviating nickel toxicity in *Oryza sativa* L. *Ecotoxicology.* **28**: 261-276.

Hasanuzzaman, M., Bhuyan, M.H.M.B., Zulfiqar, F., Raza, A., Mohsin, S.M., Mahmud, J.A., Fujita, M. and Fotopoulos, V. (2020). Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. *Antioxidants.* **9**: 681.

Hasanuzzaman, M., Nahar, K., Anee, T.I., Khan, M.I.R. and Fujita, M. (2018). Silicon-mediated regulation of antioxidant defense and glyoxalase systems confers drought stress tolerance in *Brassica napus* L. *South Afr. J. Bot.* **115**: 50–57.

Hasanuzzaman, M., Raihan, M.R.H., Khojah, E., Samra, B.N., Fujita, M. and Nahar, K. (2021). Biochar and chitosan regulate antioxidant defense and methylglyoxal detoxification systems and enhance salt tolerance in jute (*Corchorus olitorius* L.). *Antioxidants.* **10**: 2017.

Hassan, M.U., Chattha, M.U., Khan, I., Chattha, M.B., Aamer, M., Nawaz, M., Ali, A., Khan, M.A.U. and Khan, T.A. (2019). Nickel toxicity in plants: Reasons, toxic effects, tolerance mechanisms, and remediation possibilities- A review. *Environ. Sci. Pollut. Res.* **26**: 12673–12688.

Hauser, M.T. (2014). Molecular basis of natural variation and environmental control of trichome patterning. *Front. Plant. Sci.* **5**: 1–7.

He, M., He, C.Q. and Ding, N.Z. (2018). Abiotic stresses: General defenses of land plants and chances for engineering multi stress tolerance. *Front. Plant Sci.* **9**: 1771.

Heath, R.L. and Packer, L. (1968). Photoperoxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* **125**: 189–198.

Heidari, J., Amooaghaie, R. and Kiani, S. (2020). Impact of chitosan on nickel bioavailability in soil, the accumulation and tolerance of nickel in *Calendula tripterocarpa*. *Int. J. Phytoremediat.* **22**(11): 1175-1184.

Helaly, M.N., Farouk, S., Arafa, S.A. and Amhimmid, N.B. (2018). Inducing salinity tolerance of rosemary (*Rosmarinus officinalis* L.) plants by chitosan or zeolite application. *Asian J. Adv. Agric. Res.* **5**(4): 1-20.

Helaoui, S., Boughattas, I., Hattab, S., Mkhinini, M., Alphonse, V., Livet, A., Bousserrhine, N. and Banni, M. (2020). Physiological, biochemical and transcriptomic responses of *Medicago sativa* to nickel exposure. *Chemosphere.* **249**: 126121.

Hidangmayum, A., Dwivedi, P., Katiyar, D. and Hemantaranjan, A. (2019). Application of chitosan on plant responses with special reference to abiotic stress. *Physiol. Mol. Biol. Plants.* **25**(2): 313-326.

Huang, C., He, W., Guo, J., Chang, X., Su, P. and Zhang, L. (2005). Increased sensitivity to salt stress in ascorbate-deficient *Arabidopsis* mutant. *J. Exp. Bot.* **56**: 3041–3049.

Huang, M., Zhang, Z., Zhai, Y., Lu, P. and Zhu, C. (2019). Effect of straw biochar on soil properties and wheat production under saline water irrigation. *Agronomy*. **9**: 457.

Imran, S., Sarker, P., Hoque, M.N., Paul, N.C., Mahamud, M.A., Chakrobortty, J., Tahjib-Ul-Arif, M., Latef, A.A.H.A., Hasanuzzaman, M. and Rhaman, M.S. (2022). Biochar actions for the mitigation of plant abiotic stress. *Crop Past. Sci.* doi:10.1071/CP21486

Imrul, M.H., Jahan, M.A., Rabin, M.H., Siddik, M.A., Islam, S. and Yeasmin, M. (2016). Influence of nitrogen and phosphorus on the growth and yield of BRRI dhan57. *Plant Sci. Today*. **3**(2): 175-185.

IRRI (International Rice Research Institute). (2022). http://www.knowledgebank.irri.org. accessed on December 18, 2022.

Ivanishchev, V.V. and Abramova, E.A. (2015). Accumulation of nickel ions in seedlings of *Vicia sativa* L. and manifestations of oxidative stress. *Environ. Sci. Pollut. Res.* **22**: 7897–7905.

Jamil, M., Zeb, S., Anees, M., Roohi, A., Ahmed, I., ur Rehman, S. and Rha, E.S. (2014). Role of *Bacillus licheniformis* in phytoremediation of nickel contaminated soil cultivated with rice. *Int. J. Phytoremediat.* **16**(6): 554-571.

Kanwal, S., Ilyas, N., Shabir, S., Saeed, M., Gul, R., Zahoor, M., Batool, N. and Mazhar, R. (2018). Application of biochar in mitigation of negative effects of salinity stress in wheat (*Triticum aestivum* L.). *J. Plant Nutri.* **41**(4): 526-538.

Katerova, Z., Sergiev, I., Todorova, D., Shopova, E., Dimitrova, L. and Brankova, L. (2021). Physiological responses of wheat seedlings to soil waterlogging applied after treatment with selective herbicide. *Plants*. **10**: 1195.

Katiyar, D., Hemantaranjan, A. and Singh, B. (2015). Chitosan as a promising natural compound to enhance potential physiological responses in plant: A review. *Ind. J. Plant Physiol.* **20**: 1–9.

Kaushal, N., Bhandari, K., Siddique, K.H. and Nayyar, H. (2016). Food crops face rising temperatures: An overview of responses, adaptive mechanisms, and approaches to improve heat tolerance. *Cogent. Food Agric.* **2**: 1–42.

Khair, K.U., Farid, M., Ashraf, U., Zubair, M., Rizwan, M., Farid, S., Ishaq, H.K., Iftikhar, U. and Ali, S. (2020). Citric acid enhanced phytoextraction of nickel (Ni) and alleviate *Mentha piperita* (L.) from Ni-induced physiological and biochemical damages. *Environ. Sci. Pollut. Res.* **27**(21): 27010-27022.

Khan, F., Hussain, S., Khan, S. and Geng, M. (2020b). Seed priming improved antioxidant defense system and alleviated Ni-induced adversities in rice seedlings under N, P, or K deprivation. *Front. Plant Sci.* **11**: 565647.

Khan, M.I.R., Jahan, B., AlAjmi, M.F., Rehman, M.T. and Khan, N.A. (2020a). Ethephon mitigates nickel stress by modulating antioxidant system, glyoxalase system and proline metabolism in Indian mustard. *Physiol. Mol. Biol. Plants.* **26**(6): 201-1213.

Khan, M.I.R., Khan, N.A., Masood, A., Per, T.S. and Asgher, M. (2016). Hydrogen peroxide alleviates nickel-inhibited photosynthetic responses through increase in use-efficiency of nitrogen and sulfur, and glutathione production in mustard. *Front. Plant Sci.* **7**: 44.

Kupper, H. and Kroneck, P.M.H. (2007). Nickel in the environment and its role in the metabolism of plants and cyanobacteria. **In**: Metal Ions in Life Sciences. A. Sigel, H. Sigel, R.K.O. Sigel, (eds.). Wiley, Chichester. pp 31–62

Kurita, K. (2006). Chitin and chitosan: Functional biopolymers from marine crustaceans. *Mar. Biotechnol.* **8**: 203–226.

Leng, L., Huang, H., Li, H., Li, J. and Zhou, W. (2019). Biochar stability assessment methods: A review. *Sci. Total Environ.* **647**: 210–222.

Liu, J., Gai, L. and Zong, H. (2021). Foliage application of chitosan alleviates the adverse effects of cadmium stress in wheat seedlings (*Triticum aestivum* L.). *Plant Physiol. Biochem.* **164**: 115-121.

Malerba, M. and Cerana, R. (2015). Reactive oxygen and nitrogen species in defense/stress responses activated by chitosan in sycamore cultured cells. *Int. J. Mol. Sci.* **16**: 3019–3034.

McGrath, S.P. (1995). Chromium and Nickel. **In:** In heavy metals in soils. B.J. Alloway, (ed.). Blackie Academic and Professional, London. pp. 152–174.

McIlveen, W.D. and Negusantil, J.J. (1994). Nickel in the terrestrial environment. *Sci. Total Environ.* **148**: 109–138.

Mehmood, S., Ahmed, W., Ikram, M., Imtiaz, M., Mahmood, S., Tu, S. and Chen, D. (2020). Chitosan modified biochar increases soybean (*Glycine max* L.) resistance to salt-stress by augmenting root morphology, antioxidant defense mechanisms and the expression of stress-responsive genes. *Plants.* **9**(9): 1173.

Mir, M.A., Sirhindi, G., Alyemeni, M.N., Alam, P. and Ahmad, P. (2018). Jasmonic acid improves growth performance of soybean under nickel toxicity by regulating nickel uptake, redox balance, and oxidative stress metabolism. *J. Plant Growth Regul.* **37**(4): 1195-1209.

Mishra, P. and Dubey, R.S. (2011). Nickel and Al-excess inhibit nitrate reductase but upregulate activities of aminating glutamate dehydrogenase and aminotransferases in growing rice seedlings. *Plant Growth Regul.* **64**(3): 251-261.

Mosa, A., El-Banna, M.F. and Gao, B. (2016). Biochar filters reduced the toxic effects of nickel on tomato (*Lycopersicon esculentum* L.) grown in nutrient film technique hydroponic system. *Chemosphere.* **149**: 254-262.

Naheed, N., Abbas, G., Naeem, M.A., Hussain, M., Shabbir, R., Alamri, S., Siddiqui, M.H. and Mumtaz, M.Z. (2022). Nickel tolerance and phytoremediation potential of quinoa are modulated under salinity: Multivariate comparison of physiological and biochemical attributes. *Environ. Geochem. Health.* **44**(4): 1409-1424.

Namgay, T., Singh, B. and Singh, B.P. (2010). Influence of biochar application to soil on the availability of As, Cd, Cu, Pb, and Zn to maize (*Zea mays* L.). *Soil Res.* **48**(6–7): 638–647.

Naveed, M., Ramzan, N., Mustafa, A., Samad, A., Niamat, B., Yaseen, M., Ahmad, Z., Hasanuzzaman, M., Sun, N., Shi, W. and Xu, M. (2020). Alleviation of salinity induced oxidative stress in *Chenopodium quinoa* by Fe biofortification and biochar—endophyte interaction. *Agronomy*. **10**(2): 168.

Nikolaou, G., Neocleous, D., Christou, A., Kitta, E. and Katsoulas, N. (2020). Implementing sustainable irrigation in water-scarce regions under the impact of climate change. *Agronomy*. **10**: 1120.

Niu, K., Zhang, R., Zhu, R., Wang, Y., Zhang, D. and Ma, H. (2021). Cadmium stress suppresses the tillering of perennial ryegrass and is associated with the transcriptional regulation of genes controlling axillary bud outgrowth. *Ecotoxicol. Environ. Saf.* **212**: 112002.

Ozfidan-Konakci, C., Yildiztugay, E., Bahtiyar, M. and Kucukoduk, M. (2018). The humic acid-induced changes in the water status, chlorophyll fluorescence and antioxidant defense systems of wheat leaves with cadmium stress. *Ecotoxicol. Environ. Saf.* **155**: 66-75.

Pan, J., Sharif, R., Xu, X. and Chen, X. (2021). Mechanisms of waterlogging tolerance in plants: Research progress and prospects. *Front. Plant Sci.* **11**: 627331.

Pandey, N. and Sharma, C.P. (2002). Effect of heavy metals Co^{2+}, Ni^{2+} and Cd^{2+} on growth and metabolism of cabbage. *Plant Sci.* **163**: 753758.

Parkash, V. and Singh, S. (2020). Potential of biochar application to mitigate salinity stress in eggplant. *Hort. Sci.* **55**: 1946–1955.

Parlak, K.U. (2016). Effect of nickel on growth and biochemical characteristics of wheat (*Triticum aestivum* L.) seedlings. *Wagenin. J. Life Sci.* **76**(1): 1-5.

Parveen, A., Liu, W., Hussain, S., Asghar, J., Perveen, S. and Xiong, Y. (2019). Silicon priming regulates morpho-physiological growth and oxidative metabolism in maize under drought stress. *Plants*. **8**(10): 431.

Qu, D.Y., Gu, W.R., Zhang, L.G., Li, C.F., Chen, X.C., Li, J., Li, L.J., Xie, T.L. and Wei, S. (2019). Role of chitosan in the regulation of the growth, antioxidant system and photosynthetic characteristics of maize seedlings under cadmium stress. *Rus. J. Plant Physiol.* **66**(1): 140-151.

Quitadamo, F., De Simone, V., Beleggia, R. and Trono, D. (2021). Chitosan-induced activation of the antioxidant defense system counteracts the adverse effects of salinity in durum wheat. *Plants.* **10**(7): 1365.

Rabêlo, V.M., Magalhães, P.C., Bressanin, L.A., Carvalho, D.T., Reis, C.O.D., Karam, D., Doriguetto, A.C., Santos, M.H.D., Santos Filho, P.R.D.S. and Souza, T.C.D. (2019). The foliar application of a mixture of semisynthetic chitosan derivatives induces tolerance to water deficit in maize, improving the antioxidant system and increasing photosynthesis and grain yield. *Sci. Rep.* **9**(1): 1-13.

Ragsdale, S.W. (1998). Nickel biochemistry. *Curr. Opin. Chem. Biol.* **2**: 208–215.

Rajpoot, R., Rani, A., Srivastava, R.K., Pandey, P. and Dubey, R.S. (2016). *Terminalia arjuna* bark extract alleviates nickel toxicity by suppressing its uptake and modulating antioxidative defence in rice seedlings. *Protoplasma*. **253**(6): 1449-1462.

Ravichanthiran, K., Ma, Z.F., Zhang, H., Cao, Y., Wang, C.W., Muhammad, S., Aglago, E.K., Zhang, Y., Jin, Y. and Pan, B. (2018). Phytochemical profile of brown rice and its nutrigenomic implications. *Antioxidants*. **7**: 71.

Rehman, M.Z.U., Rizwan, M., Ali, S., Fatima, N., Yousaf, B., Naeem, A., Sabir, M., Ahmad, H.R. and Ok, Y.S. (2016). Contrasting effects of biochar, compost and farm manure on alleviation of nickel toxicity in maize (*Zea mays* L.) in relation to plant growth, photosynthesis and metal uptake. *Ecotoxicol. Environ. Saf.* **133**: 218-225.

Rizwan, M., Ali, S., Abbas, T., Adrees, M., Zia-ur-Rehman, M., Ibrahim, M., Abbas, F., Qayyum, M.F. and Nawaz, R. (2018b). Residual effects of biochar on growth, photosynthesis and cadmium uptake in rice (*Oryza sativa* L.) under Cd stress with different water conditions. *J. Environ. Manag.* **206**: 676-683.

Rizwan, M., Ali, S., ur Rehman, M.Z., Adrees, M., Arshad, M., Qayyum, M.F., Ali, L., Hussain, A., Chatha, S.A.S. and Imran, M. (2019). Alleviation of cadmium accumulation in maize (*Zea mays* L.) by foliar spray of zinc oxide nanoparticles and biochar to contaminated soil. *Environ. Pollut.* **248**: 358-367.

Rizwan, M., Imtiaz, M., Dai, Z., Mehmood, S., Adeel, M., Liu, J. and Tu, S. (2017). Nickel stressed responses of rice in Ni subcellular distribution, antioxidant production, and osmolyte accumulation. *Environ. Sci. Pollut. Res.* **24**: 20587–20598.

Rizwan, M., Mostofa, M.G., Ahmad, M.Z., Imtiaz, M., Mehmood, S., Adeel, M., Dai, Z., Li, Z., Aziz, O., Zhang, Y. and Tu, S. (2018a). Nitric oxide induces rice tolerance to excessive nickel by regulating nickel uptake, reactive oxygen species detoxification and defense-related gene expression. *Chemosphere.* **191**: 23-35.

Sachdev, S., Ansari, S.A., Ansari, M.I., Fujita, M. and Hasanuzzaman, M. (2021). Abiotic stress and reactive oxygen species: Generation, signaling, and defense mechanisms. *Antioxidants*. **10**: 277.

Sadeghipour, O. (2021). Chitosan application improves nickel toxicity tolerance in soybean. *J. Soil Sci. Plant Nutri.* **21**(3): 2096-2104.

Saini, P., Gani, M., Kaur, J.J., Godara, L.C., Singh, C. Chauhan, S.S., Francies, R.M., Bhardwaj, A., Kumar, N.B. and Ghosh, M.K. (2018). Reactive oxygen species (ROS): A way to stress survival in plants. **In**: Abiotic Stress-Mediated Sensing and Signaling in Plants: An Omics Perspective. S.M. Zargar, M.Y. Zargar, (eds.). Springer, Singapore. pp. 127–153.

Saroy, K. and Garg, N. (2021). Relative effectiveness of arbuscular mycorrhiza and polyamines in modulating ROS generation and ascorbate-glutathione cycle in *Cajanus cajan* under nickel stress. *Environ. Sci. Pollut. Res.* **28**(35): 48872-48889.

Shahbaz, A.K., Ramzani, P.M.A., Saeed, R., Turan, V., Iqbal, M., Lewińska, K., Abbas, F., Saqib, M., Tauqeer, H.M., Iqbal, M. and Fatima, M. (2019). Effects of biochar and zeolite soil amendments with foliar proline spray on nickel immobilization, nutritional quality and nickel concentrations in wheat. *Ecotoxicol. Environ. Saf.* **173**: 182-191.

Shaheen, S.M., Rinklebe, J. and Selim, M.H. (2015). Impact of various amendments on immobilization and phytoavailability of nickel and zinc in a contaminated floodplain soil. *Int. J. Environ. Sci. Tech.* **12**: 2765–2776.

Shin, R., Berg, R.H. and Schachtman, D.P. (2005). Reactive oxygen species and root hairs in *Arabidopsis* root response to nitrogen, phosphorus and potassium deficiency. *Plant Cell Physiol.* **46**: 1350–1357.

Singh, A., Kumar, A., Yadav, S. and Singh, I.K. (2019). Reactive oxygen species-mediated signaling during abiotic stress. *Plant Gene*. **18**: 100173.

Sirhindi, G., Mir, M.A., Sharma, P., Gill, S.S., Kaur, H. and Mushtaq, R. (2015). Modulatory role of jasmonic acid on photosynthetic pigments, antioxidants and stress markers of *Glycine max* L. under nickel stress. *Physiol. Mol. Biol. Plants.* **21**: 559–565.

Sofy, M., Mohamed, H., Dawood, M., Abu-Elsaoud, A. and Soliman, M. (2022). Integrated usage of *Trichoderma harzianum* and biochar to ameliorate salt stress on spinach plants. *Arch. Agron. Soil Sci.* **68**(14): 2005-2026.

Soliman, M.H., Alnusairi, G.S., Khan, A.A., Alnusaire, T.S., Fakhr, M.A., Abdulmajeed, A.M., Aldesuquy, H.S., Yahya, M. and Najeeb, U. (2022). Biochar and selenium nanoparticles induce water transporter genes for sustaining carbon assimilation and grain production in salt-stressed wheat. *J. Plant Growth Regul.* doi:10.1007/s00344-022-10636-y.

Tanzeem-ul-Haq, H.S., Rasool, B., Ehtisham-ul-Haque, S., Saif, S., Zafar, S., Younis, T., Akhtar, I., Jafri, L., Iqbal, N., Masood, N. and Lewińska, K. (2021). Chitosan with bentonite and biochar in Ni-affected soil reduces grain Ni concentrations, improves soil enzymes and grain quality in lentil. *Minerals.* **11**: 11.

Tränkner, M., Tavakol, E. and Jákli, B. (2018). Functioning of potassium and magnesium in photosynthesis, photosynthate translocation and photoprotection. *Physiol. Plant.* **163**: 414–431.

Turan, V., Khan, S.A., Iqbal, M., Ramzani, P.M.A. and Fatima, M. (2018a). Promoting the productivity and quality of brinjal aligned with heavy metals immobilization in a wastewater irrigated heavy metal polluted soil with biochar and chitosan. *Ecotoxicol. Environ. Saf.* **161**: 409-419.

Turan, V., Ramzani, P.M.A., Ali, Q., Abbas, F., Iqbal, M., Irum, A. and Khan, W.U.D. (2018b). Alleviation of nickel toxicity and an improvement in zinc bioavailability in sunflower seed with chitosan and biochar application in pH adjusted nickel contaminated soil. *Arch. Agron. Soil Sci.* **64**: 1053-1067.

Turk, H. (2019). Chitosan-induced enhanced expression and activation of alternative oxidase confer tolerance to salt stress in maize seedlings. *Plant Physiol. Biochem.* **141**: 415-422.

Turunen, M., Hyväluoma, J., Heikkinen, J., Keskinen, R., Kaseva, J., Hannula, M. and Rasa, K. (2020). Quantifying the pore structure of different biochars and their impacts on the water retention properties of *Sphagnum* moss growing media. *Biosys. Engineer.* **191**: 96–106.

Valivand, M. and Amooaghaie, R. (2021). Foliar spray with sodium hydrosulfide and calcium chloride advances dynamic of critical elements and efficiency of nitrogen metabolism in *Cucurbita pepo* L. under nickel stress. *Sci. Hortic.* **283**: 110052.

Vinterhalter, B. and D. Vinterhalter (2005). Nickel hyperaccumulation in shoot cultures of *Alyssum markgrafii*. *Biol. Plant.* **49**: 121124.

Wai, A.H. and An, G. (2017). Axillary meristem initiation and bud growth in rice. *J. Plant Biol.* **60**: 440–451.

Wang, M. and Zhou, Q. (2005). Single and joint toxicity of chlorimuron-ethyl, cadmium, and copper acting on wheat *Triticum aestivum*. *Ecotoxicol. Environ. Saf.* **60**: 169–175.

Wang, Y. and Jiao, Y. (2018). Axillary meristem initiation - a way to branch out. *Curr. Opin. Plant Biol.* **41**: 61–66.

Wood, B.W. and Reilly, C.C. (2007). Interaction of nickel and plant disease. **In**: Mineral Nutrition and Plant Disease. L.E. Datnoff, W.H. Elmer, D. M. Huber, (eds.). American Phytopathological Society Press, Minneapolis, MN. pp. 217–247.

Yoshida, S. (1981). Yield and Yield Components. **In**: *Fundamentals of Rice Crop Science*. IRRI, Manila, Philippines. pp. 60-63.

Younas, H.S., Abid, M., Shaaban, M. and Ashraf, M. (2021). Influence of silicon and chitosan on growth and physiological attributes of maize in a saline field. *Physiol. Mol. Biol. Plants.* **27**(2): 387-397.

Yu, C.W., Murphy, T.M. and Lin, C.H. (2003). Hydrogen peroxide induced chilling tolerance in mung beans mediated through ABA-independent glutathione accumulation. *Funct. Plant Biol.* **30**: 955–963.

Yusuf, M., Fariduddin, Q., Hayat, S. and Ahmad, A. (2011). Nickel: An overview of uptake, essentiality and toxicity in plants. *Bull. Environ. Contam. Toxicol.* **86**: 1–17.

Zaheer, M.S., Ali, H.H., Soufan, W., Iqbal, R., Habib-ur-Rahman, M., Iqbal, J., Israr, M. and El Sabagh, A. (2021). Potential effects of biochar application for improving wheat (*Triticum aestivum* L.) growth and soil biochemical properties under drought stress conditions. *Land*. **10**: 1125.

Zaid, A., Mohammad, F., Wani, S.H. and Siddique, K.M. (2019). Salicylic acid enhances nickel stress tolerance by up-regulating antioxidant defense and glyoxalase systems in mustard plants. *Ecotoxicol. Environ. Saf.* **180**: 575-587.

Zhuang, L., Ge, Y., Wang, J., Yu, J., Yang, Z. and Huang, B. (2019). Gibberellic acid inhibition of tillering in tall fescue involving crosstalks with cytokinins and transcriptional regulation of genes controlling axillary bud outgrowth. *Plant Sci.* **287**: 110168.

Zong, H., Liu, S., Xing, R., Chen, X. and Li, P. (2017). Protective effect of chitosan on photosynthesis and antioxidative defense system in edible rape (*Brassica rapa* L.) in the presence of cadmium. *Ecotoxicol. Environ. Saf.* **138**: 271-278.

Zulfiqar, B., Raza, M.A.S., Saleem, M.F., Aslam, M.U., Iqbal, R., Muhammad, F., Amin, J., Ibrahim, M.A. and Khan, I.H. (2022). Biochar enhances wheat crop productivity by mitigating the effects of drought: Insights into physiological and antioxidant defense mechanisms. *PLoS ONE*. **17**(4): e0267819.

PLATES

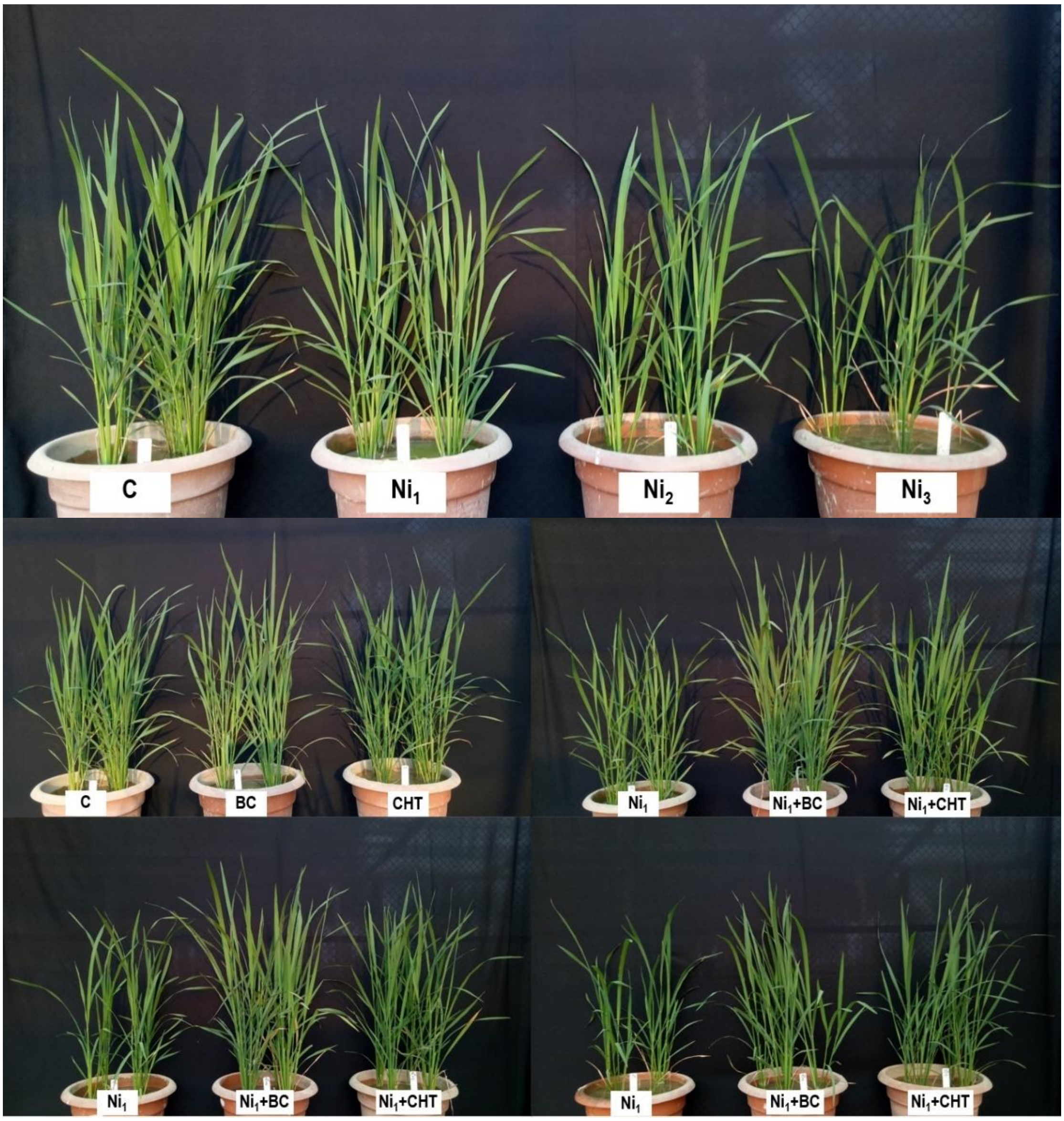
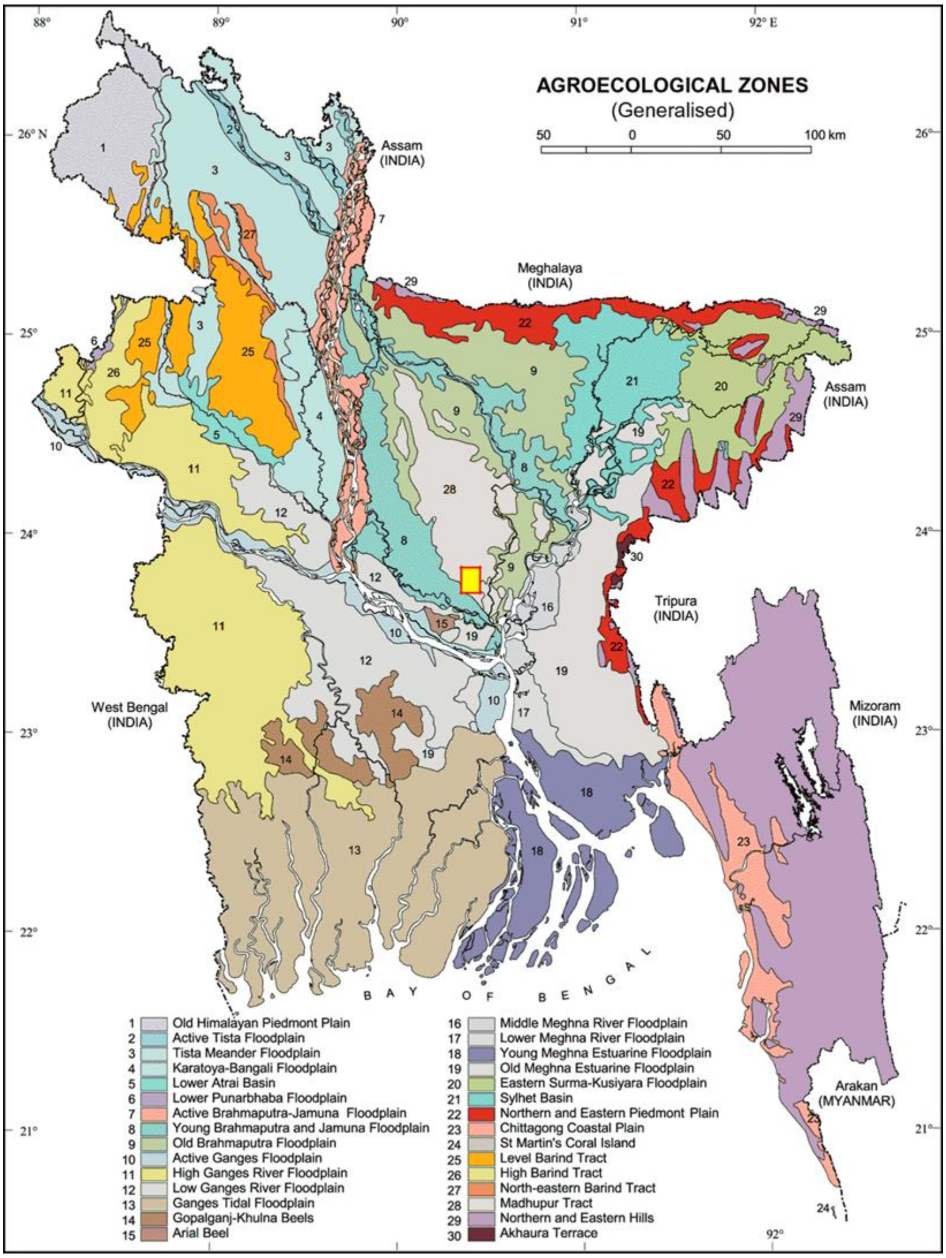


Plate 1. Phenotypic appearance of rice plants (*Oryza sativa* L. cv. BRRI dhan96) under different treatment combinations. Here, C= control, Ni_1= 0.5 mM $NiSO_4 \bullet 6H_2O$, Ni_2= 1.0 mM $NiSO_4 \bullet 6H_2O$, Ni_3= 2.0 mM $NiSO_4 \bullet 6H_2O$, BC= 0.5 g biochar kg^{-1} soil as soil incorporation, CHT= 200 mg chitosan-100 L^{-1} as foliar spray

APPENDICES

Appendix I. Map showing the location of experiment



Appendix II. Monthly average air temperature, relative humidity, and rainfall of the experiment site during the period from December 2020 to April 2021

Months	Air temperature (°C)		Relative humidity (%)	Total rainfall (mm)
	Maximum	Minimum		
December, 2020	19.4	11.1	50	12.8
January, 2021	20.4	12.7	46	7.7
February, 2021	25.1	16.5	37	28.9
March, 2021	29.1	20.4	38	65.8
April, 2021	30. 4	24.5	58	40.7

Appendix III. Mean square values and degree of freedom (DF) of the plant height at 35, 49, 63, 77 DAT, and harvest of rice plants as influenced by BC and CHT application under different levels of nickel stress

Source of variance	DF	Mean square values				
		Plant height				
		35 DAT	49 DAT	63 DAT	77 DAT	Harvest
Treatments	11	35.444	49.263	121.335	65.765	51.860
Error	24	1.249	3.114	5.849	3.035	7.551

Appendix IV. Mean square values and degree of freedom (DF) of the number of tillers hill^{-1} at 49, 63, 77 DAT, and harvest of rice plants as influenced by BC and CHT application under different levels of nickel stress

Source of variance	DF	Mean square values			
		Tillers hill^{-1}			
		49 DAT	63 DAT	77 DAT	Harvest
Treatments	11	0.954	0.689	0.557	0.719
Error	24	0.042	0.154	0.230	0.183

Appendix V. Mean square values and degree of freedom (DF) of the leaf area at 49, 63, and 77 DAT of rice plants as influenced by BC and CHT application under different levels of nickel stress

Source of variance	DF	Mean square values		
		Leaf area		
		49 DAT	63 DAT	77 DAT
Treatments	11	5.814	20.047	18.448
Error	24	0.758	1.186	2.147

Appendix VI. Mean square values and degree of freedom (DF) of the root FW, shoot FW, root DW, and shoot DW of rice plants as influenced by BC and CHT application under different levels of nickel stress

Source of variance	DF	Mean square values			
		Root FW	Shoot FW	Root DW	Shoot DW
Treatments	11	1.683	8.803	0.201	0.691
Error	24	0.013	0.290	0.003	0.007

Appendix VII. Mean square values and degree of freedom (DF) of the soil and plant analysis development (SPAD) value at 35, 49, 63, and 77 DAT of rice plants as influenced by BC and CHT application under different levels of nickel stress

Source of variance	DF	Mean square values			
		SPAD value			
		35 DAT	49 DAT	63 DAT	77 DAT
Treatments	11	14.380	22.729	24.269	16.613
Error	24	0.663	1.102	1.571	0.813

Appendix VIII. Mean square values and degree of freedom (DF) of the RWC, Pro content, MDA content, H_2O_2 content, root EL, and shoot EL of rice plants as influenced by BC and CHT application under different levels of nickel stress

Source of variance	DF	Mean square values					
		RWC	Pro	MDA	H_2O_2	Root EL	Shoot EL
Treatments	11	191.444	0.819	106.970	268.656	334.677	179.220
Error	24	10.592	0.004	1.267	2.263	2.571	1.033

Appendix IX. Mean square values and degree of freedom (DF) of the AsA content, DHA content, AsA/DHA ratio, GSH content, GSSG content, GSH/GSSG ratio of rice plants as influenced by BC and CHT application under different levels of nickel stress

Source of variance	DF	Mean square values					
		AsA	DHA	AsA/ DHA	GSH	GSSG	GSH/ GSSG
Treatments	11	723412.009	12913.081	17.507	1555.056	42.775	15.034
Error	24	6073.992	140.969	0.077	13.763	0.505	0.153

Appendix X. Mean square values and degree of freedom (DF) of the effective tillers hill^{-1}, ineffective tillers hill^{-1}, panicle length, and rachis panicle^{-1} of rice plants as influenced by BC and CHT application under different levels of nickel stress

Source of variance	DF	Mean square values			
		Effective tillers hill^{-1}	Ineffective tillers hill^{-1}	Panicle length	Rachis panicle^{-1}
Treatments	11	4.314	1.705	6.540	0.622
Error	24	0.157	0.007	0.876	0.091

Appendix XI. Mean square values and degree of freedom (DF) of the filled grains panicle^{-1}, unfilled grains panicle^{-1}, and total number of grains panicle^{-1} of rice plants as influenced by BC and CHT application under different levels of nickel stress

Source of variance	DF	Mean square values		
		Filled grains panicle^{-1}	Unfilled grains panicle^{-1}	Total number of grains panicle^{-1}
Treatments	11	386.111	19.975	236.437
Error	24	52.533	1.747	49.462

Appendix XII. Mean square values and degree of freedom (DF) of the 1000-grain weight, grain yield hill^{-1}, and straw yield hill^{-1} of rice plants as influenced by BC and CHT application under different levels of nickel stress

Source of variance	DF	Mean square values		
		1000-grain weight	Grain yield hill^{-1}	Straw yield hill^{-1}
Treatments	11	5.814	20.047	18.448
Error	24	0.758	1.186	2.147