MORPHO-PHYSIOLOGICAL RESPONSES OF TOSSA JUTE (*Corchorus olitorius* L.) UNDER DIFFERENT ABIOTIC STRESSES AT VEGETATIVE STAGE

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CERTIFICATE

This is to certify that the thesis entitled ***"MORPHO-PHYSIOLOGICAL RESPONSES OF TOSSA JUTE (Corchorus olitorius L.) UNDER DIFFERENT ABIOTIC STRESSES AT VEGETATIVE STAGE"*** *submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE (MS) in AGRONOMY****, embodies the results of a piece of bonafide research work carried out by* ***KHUSSBOO RAHMAN****, Registration No.* ***13-05594*** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

Dated:

Place: Dhaka, Bangladesh

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ABSTRACT

Abiotic stress has become an alarming issue for plants survivable due to the constant changes in the environment. Stresses such as drought, salt, waterlogging and heavy metals largely influences plant growth and development that finally reduces crop productivity. Focusing this issue, two individual experiment, germination trial (Experiment-I) and pot experiment (Experiment-II) were carried out at Sher-e-Bangla Agricultural University, during the kharif-1 season of 2019 to investigate the responses of tossa jute (*Corchorus olitorius*) cv. O-9897 under different abiotic stresses. Experiment-I and Experiment-II were designed in completely randomized design (CRD) and randomized complete block design (RCBD), respectively. In Experiment-I seeds were exposed in two doses of salt (75 and 100 mM NaCl) and Cd stress (0.5 and 1 mM $CdCl_2$). Germination parameters were affected by salt stress where seedling parameters were highly damaged by Cd stress. At 15th day after sowing (DAS) plants were exposed to salt, cadmium (Cd), waterlogging and water deficit condition for various duration in Experiment-II. Two doses of NaCl (200 and 400 mM) were applied to impose salt stress, while two doses of $CdCl_2$ (2 and 4 mM) were applied for Cd stress. Among two waterlogging treatments, 5 days of waterlogged condition was imposed at 15 and 30 DAS in first one and in second one 20 days of waterlogged condition was imposed. For water deficit, water was withheld from soil for the duration of 10 and 15 days. Morphological parameters like plant height, relative water content, above ground fresh and dry weight, SPAD value, leaf area and stem diameter were decreased upon exposure to salt, water deficit, waterlogging and Cd. These treatments were also resulted in oxidative stress which was evident by the increased levels of lipid peroxidation (MDA), H_2O_2 and electrolyte leakage and decrease in catalase enzyme activity. Results also revealed that waterlogging and drought stress drastically affected plants morpho-physiology, whereas *C. olitorius* could tolerate moderate level of salt (i.e. 200 mM NaCl) and Cd (i.e. 2 mM $CdCl_2$). Considering this fact, it may be concluded that tossa jute become susceptible towards either duration of waterlogging and drought stress, salt stress more than 200 mM NaCl and Cd stress, more than 2 mM $CdCl_2$.

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ABBREVIATIONS AND ACCRONYMS

ANOVA	Analysis of variance
ANS	Abnormal seedling
APX	Ascorbate peroxidase
BJMC	Bangladesh Jute Mill Corporation
BJRI	Bangladesh Jute Research Institute
CAT	Catalase
Chl	Chlorophyll
CRD	Completely Randomized Design
CV	Co-efficient of Variance
cv.	Cultivar
CVG	Co-efficient of Velocity of Germination
DAS	Days after sowing
DW	Dry Weight
et al.	*et alibi* (and others)
EL	Electrolyte Leakage
FC	Field Capacity
FW	Fresh Weight
LSD	Least Significance Difference
MDA	Malondialdehyde
MGR	Mean Germination Rate
MGT	Mean Germination Time
Pro	Proline
RCBD	Randomized Complete Block Design
ROS	Reactive Oxygen Species
RWC	Relative water content
TGI	Timson Germination Index
viz.	Namely

ABBREVIATIONS AND ACCRONYMS (Cont’d)

°C	Degree Celsius
µg	Microgram
cm	Centimeter
g	Gram
ha	Hectare
kg	Kilogram
m	Meter
mg	Milligram
mM	Millimole
t	Ton

Chapter I

INTRODUCTION

Stressful environmental conditions continuously create unfavorable conditions for plants and lead plants toward physiological and biochemical changes (Farooq *et al.,* 2013). Thus, plants show many metabolic dysfunction and ultimately hampered plant growth. When plants are exposed to abiotic stresses such as, drought, waterlogging, extreme temperatures, salinity, metal /metalloid stresses etc. they are negatively affected and reduced plant growth and yield (Gul *et al.,* 2016). Abiotic stresses reduced stomatal conductance, photosynthetic and RuBisCo activity and disrupt energy production of plant (Demirevska *et al.,* 2009). Plants produce excessive reactive oxygen species (ROS) in stress condition and cause damage of photosynthetic apparatus and functional elements of plants (Hasanuzzaman *et al.,* 2012).

Bangladesh is exceptionally vulnerable to climate change due to its geographical position. Bangladesh is now facing with several abiotic stresses like drought, salinity, flood, erosions etc. which may displace large number of people. In 2050, only salinity can displace about 18 million people (EJF, 2019). Most of the coastal areas of Bangladesh are located at low tidal zone about 1-1.5 m above the sea level and cover about 32% of the total area of country (Rahman and Rahman, 2015). In saline condition plants produce excessive ROS which cause damage of chloroplast and reduce photosynthesis rate and ultimately reduced plant growth and yield (Saberi *et al.,* 2011).

Northwestern part region of Bangladesh are considered as the most drought prone region. Rajshahi, Dinajpur, Rangpur, Chapai Nawabganj, Pabna, Bogra, Joypurhat and Naogaon district of northwestern zone along with Jessore, Jhenaidah, and Satkhira districts of south western region are considered as drought affected area in our country (Habiba *et al.*, 2011). Drought stress is considered as the most detrimental factor of crop production which may reduce the crop yield up to 50% (Zlateb and Lidon, 2012). It altered the pathway of photosynthesis, reduced CO_2 assimilation and stomatal conductance and reduced the growth of plant (Hasanuzzaman *et al.*, 2014).

Due to monsoon rain, melting of Himalayan iceberg and other climatic reasons flooding or waterlogged condition was created and create a devastating effect on crop production. Jamalpur, Kurigram, Bogura, Nilphamari, Sunamgonj are the most flood prone area in country. Besides coastal southern zone also facing flooding with saline water (Sarkar *et al.,* 2020). At waterlogged condition plants are unable to uptake water due to hypoxia or anoxia and unable to uptake nutrients which cause yellowing of leaves and reduced photosynthesis (Prasad *et al.,* 2004).

Heavy metals /metalloids are non-biodegradable in nature and may deteriorate human health directly or indirectly (Wang *et al.,* 2013). Arsenic contamination in Bangladesh is one of the well-studied metal pollution in the world. Due to urbanization and rapid industrialization incident of metal contamination is increasing in an alarming rate in our country (Ali *et al.,* 2016). Rivers around Dhaka and Chittagong like, Buriganga, Shitalakhya, Turag, and Karnaphuli are highly contaminated with Pb, Cd and Cr (Banu *et al.,* 2013).

Jute (*Corchorus* spp.) is a tropical crop and cultivated mostly in Asia and African continents. Indo-Bangladesh subcontinent is considered as the best region for commercial jute cultivation (Saleem *et al.,* 2020a). Though jute is a water loving plant at mature stage but at the initial stage it is water sensitive and may be unable to complete its life cycle (Prodhan *et al.,* 2001). At salt stress it reduced plant growth and affected germination at a great extent (Naik *et al.,* 2015). It also cause reduction of stem diameter which ultimately reduced yield and deteriorate fibre quality. Drought stress reduced leaf area of jute and decreased net photosynthesis rate and cause growth reduction (Yumnam *et al.,* 2017). When jute is exposed in Cu contaminated soil, physiological and biochemical process of jute become stunted and produced low quality fibre compared to the control one (Saleem *et al.,* 2019a). Though jute is a number one cash crop in our country but there are very few literatures regarding jute responses to different abiotic stresses. Therefore, we propose a study on the morpho-physiological responses of *Corchorus olitorius* plant to various abiotic stresses such as drought, salinity, waterlogging and cadmium.

The present study keeping in mind the following objectives:

i. To investigate the morpho-physiological responses of *C. olitorius* under salinity, drought, cadmium and waterlogging

ii. To compare the stress responses of *C. olitorius* under salinity, drought, cadmium and waterlogging stress

Chapter II

REVIEW OF LITERATURE

2.1 Jute

Jute is a vegetative fibrous crop (popularly called golden fibre) which belongs to the family of Malvaceae. White jute (*Corchours capsularis*) and tossa jute (*C. olitorius*) are two commercially important species of jute. Jute is an herbaceous annual C_3 plant having a long, soft, golden and shiny fiber. Jute fiber is the second most important fiber source in the world after cotton.

Jute is cultivated in a hot and humid tropical regions especially in Asia and African continents. Commercial jute cultivation is mainly restricted between 80°18′ E–92° E and 21°24′ N–26°30′ N on the Indo-Bangladesh sub-continent. Other major jute producing countries are China, Thailand, Myanmar, Indonesia, Brazil, and Nepal (Mahapatra *et al*., 2014). Due to alluvial type soil and sufficient rainfall delta Ganga is considered as the best region for jute cultivation. With annual 1.968 million tons production India is considered as the largest jute producing country in the world followed by Bangladesh (1.349 million tons/year) and China (29,628 tons /year) (Singh *et al*., 2018).

Bangladesh is the second largest producer of jute in the world with the coverage of 12.35 Lac acre land. Tangail, Jessore, Dhaka, Jamalpur, Bogra, and Faridpur are the main jute producing regions in Bangladesh where 3.5 million farmers are engaged in cultivation of jute (BJRI, 2019). About two hundred and fifty jute mills are present where 0.3 million expert workers are directly engaged in manufacturing process (BJMC, 2019). Bangladesh Jute Research Institute (BJRI) has introduced 50 jute variety. Total 85 percent of Bangladesh's yearly jute fiber output comes from tossa Jute and the remaining 15 percent from white Jute. CVL-1, OM-4, O-9897 and BJRI tossa Pat-4 are the most cultivated jute variety in Bangladesh (BJRI, 2019).

Jute is a cellulosic fibre. It contained cellulose (58-63%), hemicellulose (21-24%), lignin (12-14%), wax (0.4-0.8%), pectin (0.2-0.5%), protine (0.8-2.5%) and mineral matter (0.6-1.2%) (Datta *et al.,* 2016).

2.2 Importance of jute

Jute is a versatile fiber. Jute is not only a major textile fiber but also a raw material for non-traditional and value added non-textile products. Jute plays a vital role in the socio-economic activities of Bangladesh.

Jute leaf is a worldwide popular vegetable. The apical tender shoot and leaves of jute is a nourishing vegetable. It contain 0.025% flavonoid and 0.55% carotenoid which are good for human health and also have a medicinal value (Omenna and Ojo, 2018).

Jute tensile strength ranges between 400-800 MPa which is higher than bamboo (73-505 MPa), sisal (347-378 MPa) and coir (95-118 MPa) and lower moisture absorption capacity (13%) compared to bamboo, sisal and coir (145%, 110% and 93% respectively) which increase its fibre quality (Sen and Reddy, 2011).

Gon *et al*. (2012) reported that jute plant is a good substitute of wood. Due to its higher fibre density and multicellular structure it can be used in combination with thermoplastics to produce wide variety of solid and semi-solid products.

Biofuel can be produced from jute stick through pyrolysis process which calorific value is around 17 to 23 MJ kg^{-1} which would encourage their use as replacements for conventional liquid fuels. Besides jute stick also a good source of fire in village area. (Ferdous and Hossain, 2017).

2.3 Abiotic stress

Due to continuous climate change plants are exposed to different abiotic stress which severely affects agricultural production. Plant growth, physiology, metabolism and productivity- every aspects of crop production are negatively affected by abiotic stress conditions and can reduce yield up to 70% (Arun-Chinnappa *et al.,* 2017). Plant biochemical metabolisms and physiological processes are greatly hampered by abiotic stress and produce prolific amount of ROS those are stress indicators in plants (Xu *et al.,* 2010).

Hasanuzzaman *et al.* (2012) stated that prolonged exposure to stress condition may cause cell death in plants. To avoid cell death and reduced ROS activity plants increased the activity of antioxidant enzyme (viz. APX, CAT, SOD, POD). Non-enzymatic antioxidants viz. tocopherol, glutathione, ascorbate etc. also help to detoxify ROS species ($O_2^{\cdot -}$, H_2O_2, $^{\cdot}OH$ etc.) and minimized cell damage (Jaleel *et al.*, 2009).

2.3.1 Drought stress

Water is one of the most important factors for crop growth and productivity in absence of which morphological and physiological processes and yield development of plants reduced drastically. In water deficit condition 50% plant biomass can be reduced (Chaves *et al.*, 2009).

Tang *et al.* (2019) carried out an experiment with four genotypes of kenaf to investigate the effect of PEG induced drought stress on germination and seedling characters of kenaf. In all genotypes maximum germination was observed under 10% PEG treatment over 20% PEG treatment and control. C2/992, C3/992, C4/992 genotypes showed better germination percentage (90-95%) compared with P3B (81%). Under water deficit condition significant reduction in root growth was observed compared to control but no significant difference was observed in root growth between 10% and 20% PEG conditions. C3/992, P3A, and P3B genotype showed better root growth than C2/992.

Moharramnejad *et al.* (2019) conducted an experiment with two lines of maize and exposed them in water deficit condition which resulted reduction in plant height, shoot fresh weight and dry weight (DW) in both lines. Plant height reduced by 46% for B_{73} and 40% for MO_{17} and DW was also reduced by 25% and 33% for B_{73} and MO_{17}, respectively over control. Similarly, Bhuiyan *et al.* (2019) found significant reduction in plant height, shoot fresh weight (FW) and DW of rapeseed seedlings in drought stress where plant height reduced by 20% over control.

Karmollachaab and Gharineh, (2015) found that in PEG induced (20% PEG) drought condition shoot FW and DW of wheat seedlings reduced by 43% and 50%, respectively but no significant changes were noticed in root FW and DW.

While working with six lines of sunflower Sarvari *et al.* (2017) found that at 60% drought stress condition line LR55 showed no significant reduction in relative water content (RWC) but maximum reduction of RWC (27%) was found in C100 line over control. Ghobadi *et al.* (2013) also found that RWC of sunflower reduced significantly at drought condition. They also stated that in moderate and severe drought condition (irrigation after 15 and 20 days respectively) chl a reduced 15% and 24% and chl b reduced to 18% and 25%, respectively compared to control. But opposite result was found in case of carotenoid and electrolyte leakage (EL). Carotenoid amount increased at 44% and 66% and EL increased at 67% and 81% in both stress condition, respectively over control.

RWC of wheat crop reduced by 16% under water deficit stress (PEG 20%). Drought stress significantly decreased pigment content, Chl a, Chl b and total chlorophyll content decreased to 36, 43 and 38%, respectively over control. On contrary, EL increased by 75% in stress condition (Karmollachaab and Gharineh, 2015). Moharramnejad *et al.* (2019) also found that among two line of maize reduction of RWC was higher in B_{73} (27%) than in MO_{17} (14%) during water deficit condition. In both maize lines leaf Chl b and anthocyanins were not significantly changed but Chl a, total chlorophyll and carotenoids decrease significantly in both maize line and higher in MO_{17} than in B_{73}.

Drought stress caused a significant decrease in grain yield of maize and reduced yield by 67% and 47% for B_{73} and MO_{17}, respectively over control (Moharramnejad *et al.*, 2019).

Sarvari *et al.* (2017) found that in six lines of sunflower proline content increased in the lines C104, LR25, LR55 and decreased in the lines C100, LR4 and RHA266 at 60% drought stress. Malondialdehyde (MDA) content increase significantly in all of the studied line except the line RHA266 where minimum and maximum reduction was observed in lines LR25 (21%) and C104 (35%), respectively compared to control. In water deficit condition, CAT and GR activity increased in the line C100 and RHA266 and decreased in other lines but APX activity significantly increased in the lines C100 and C104 and decreased in other lines. Similarly, Ghobadi *et al.* (2013) also found increased CAT activity in sunflower at severe (28%) and moderate drought (107%) condition over control. Proline (Pro) amount also increased with the severity of water deficit condition.

While working with wheat seedlings under PEG induced drought condition Karmollachaab and Gharineh, (2015) found that soluble sugar content reduced in drought condition (20% PEG). On contrary proline amount increased by 61% in water deficit condition compared to control. Moharramnejad *et al.* (2019) also found that proline concentrations were increased in two maize line during water deficit and significantly higher in line MO_{17} than in B73. Total phenolic content increased and decreased in B_{73} (4%) and MO_{17} (6%), respectively. In B_{73} MDA content is much higher than line MO_{17} but H_2O_2 content in both maize lines was not significantly changed compared to control. CAT and SOD activity were significantly higher in B_{73} and increased by 32% and 76%, respectively.

2.3.2 Salt stress

Salinity is considered as the vital environmental stress which significantly reduced germination, plant vigor and yield of crops (Hasanuzzaman *et al.*, 2013). One fifth of the total arable land is covered with salinity and every year about 1.3 mha land is being loss due to salinity (Rasool *et al*., 2013). Hajlaoui *et al.* (2010) stated that by creating osmotic stress, ionic imbalance and toxicity salt stress severely effect plant physiology and biochemistry.

Seed germination percentage decreased with the increase of salt concentration and at 200 mM NaCl concentration, germination percentage of BARI Gom 25 and BARI Gom 21 reduced by 14 and 18% and increased abnormal seedlings percentage in both variety (Fardus *et al.,* 2018). Akbari *et al.* (2018) also stated that increasing level of salt stress (-0.6 and -1.2 MPa NaCl) decreased germination percentage, seedling FW and DW, radicle and hypocotyl length and radicle DW, but hypocotyl DW increased at the potential -0.6 MPa.

While working with two genotypes of sunflower Noreen *et al.* (2017) found that in exposure of salt stress (120 mM NaCl) maximum reduction in of plant height (19%), shoot FW (18%) and root FW(31%) were observed in genotype LG-56-63 then Hysun-33 (8%, 9% and 7%, respectively) except for root dry weight. Similar results were found by Fardus *et al.* (2018) where he observed maximum reduction of plant height, root and shoot length, shoot and root FW, DW plant^{-1} occurred in BARI Gom 21 compared to BARI Gom 25 at different level of salt stress.

Rahman *et al.* (2016a) stated that upon exposure to salt stress (200 mM NaCl) RWC of rice seedlings reduced by 20% and Chl a and Chl b also reduced by 33 and 38%, respectively compared to control. Similarly, sunflower seedlings exposed to salt stress (120 mM NaCl) also showed reduction in SPAD value at LG-56-63 (4%) and Hysun-33 (16%) genotype over control (Noreen *et al.*, 2017).

In exposure of different level of salt stress, MDA and H_2O_2 amount increased in rapeseed seedlings by 118% and 103% at 100 mM NaCl and 198% and 144% at 200 mM NaCl, respectively over control. Sharp increase was observed in APX, GR and GST activity in both doses of stress but salt stress could not bring any significant changes in GPX activity. Activity of CAT, DHAR and MDHAR reduced in salt stress (Hasanuzzamam *et al.*, 2014). Similarly, Nahar *et al.* (2015) also found that salt stress (200 mM NaCl) sharply increased the amount of MDA (86 and 90%), proline (172 and 407%) and H_2O_2 (48 and 80%) in mungbean seedlings after 24 and 48 h, respectively over control. Due to stress condition the activity of AsA, CAT, DHAR, MDHAR decreased significantly but increasing trend was followed by APX, SOD and MG.

2.3.3 Metal/ metalloid stress

Earth's crust contain varying levels of different metals/metalloids, among these some of are essential for plant cells viz., Cu, Fe, Mn, Ni, Zn at supra-optimum concentrations and some are harmful viz., Ag, Al, As, Cd, Cr, Cs, Hg, Pb, Sr, and U even at low concentrations (Anjum *et al.*, 2015). These toxic elements leads plant towards unusual morphological changes, metabolic disorders and finally decreased yield (Amari *et al.*, 2017). Plants fight against metal/metalloid stress through different defense strategies viz., exclusion, compartmentalization, complexation, chelation etc. (Hassan and Aarts, 2011).

An experiment was carried out by Muhammad *et al.* (2015) where *Vigna radiata* was exposed in different doses of Hg concentration (1, 3, 5 and 7 mM). No significant changes were observed in 1 mM concentration but highest reduction was observed at 7 mM treatment where seed germination, seedling length, root length and seedling DW reduced by 42, 70, 66 and 47%, respectively. Huang *et al.* (2016) also found that germination percentage of wheat reduced by 32, 46, 58, 93 and 96% at 1, 2, 3, 4 and 5 mM $CdCl_2$ concentrations. Length of bud and radicle also decreased sharply over control.

Farooq *et al.* (2013) observed reduction of plant height (40% and 74%), root length (32% and 67%) and leaf area (34% and 62%) in cotton plant during exposure to different level of cadmium (Cd) concentration (1 and 5 μM), respectively compared to control. This phenomenon cause reduction of shoot and root FW and DW also. Rizwan *et al.* (2016a) also found significant reduction in shoot length (31%) and root length (13%) of wheat during exposure to 5 μM Cd stress.

Relative water content of leaf severely affected Cd stress (0.04 mg L^{-1}) and reduced by 35% and 27% at Okmass and S2000 genotype of maize compared to control. Chl a and Chl b content also reduced significantly but carotenoid content did not change over control at stress condition (Gul *et al.*, 2016). Similar results were found by Dubey and Pandey (2011) where they found significant reduction of photosynthetic pigments upon exposure to Ni stress (100 μM) in black gram.

Cd stress (5 μM) increased the amount of MDA, H_2O_2 and EL by 72%, 67% and 77%, respectively in plants which indicate the increased production of oxidative stress in cotton plants over control (Farooq *et al.*, 2013). MDA content also increased in rapeseed seedlings by 37 and 60% at 0.5 and 1 mM $CdCl_2$ stress respectively. DHA, APX, GST, GSH, GSSG and GR activity also increased in stress condition where activity of CAT, MDHAR and DHAR reduced at both stress but GPX activity only increased at mild stress (0.5 mM) (Hasanuzzaman *et al.*, 2017). Similar oxidative changes were also found by Rahman *et al.* (2016b) and Mahmud *et al.* (2019) where rice seedlings were exposed in 0.25 and 0.5 mM $CdCl_2$ and mustard seedlings in 0.5 and 1 mM $CdCl_2$ stress condition, respectively.

2.3.4 Waterlogging

Waterlogging is considered as the main constraints for sustainable agriculture. Waterlogging create hypoxia in soils which leads plants towards growth reduction, leaf senescence, reduction of leaf area, stomatal closure etc. (Wei *et al.,* 2013). Due to the generation of ROS plants face oxidative damage at waterlogged conditions. They damage membrane integrity and minimized the efficiency of photosystem II and causing significant decrease in net photosynthetic rates (Ashraf, 2012). Waterlogging may reduce yield up to 50% in different crops (Bhushan *et al.*, 2007).

Prasanna and Rao (2014) stated that 4 days of waterlogging was more acute in green gram cultivar compared with 2 days waterlogging treatment over the control and reduced the plant height by 33%, number of leaves by 31%, leaf area by 31%, number of branches by 34%, and total dry matter by 30%. Similarly, Kumutha *et al.* (2009) also found that at 2, 4 and 6 days of waterlogging effect of waterlogging was more severe in Pusa 207 than ICP 301. Reduction of plant height at 2nd, 4th and 6th day was 26, 53, 66 % and 4, 14, 20 % in Pusa 207 and ICP 301, respectively over control. Leaf area and dry biomass also reduced at different durations of waterlogging and more severe in Pusa 207 than ICP 301.

Paltaa *et al.* (2010) stated that periodic waterlogging decreased the yield of seed Almaz (kabuli cultivar) and Rupali (desi cultivar) by 44% and 54%. Almaz showed reduction of seed yield resulted from 50% reduction in the number of seeds pod^{-1}. However, Rupali (deshi cultivar) showed less pods number and number of seeds pod^{-1} under waterlogging condition. Tian *et al.* (2019) found that with the increasing duration (3, 6 and 9 days) of waterlogging grain yield reduced significantly in maize cultivar DMY1 and KY16. Maximum grain yield reduction observed in DMY1 and KY16 (approximately 65% and 81%) at 9 days of waterlogging during tasseling stage.

An experiment was carried out by Wei *et al.* (2013) to measure the MDA content of two different cultivars of sesame, one of which is tolerant (ZZM2541) to waterlogging and another intolerant (Ezhi-2). In Ezhi-2 plants MDA content increased by about 1.3-fold and1.8-fold at 2 and 6 day of waterlogging. In ZZM2541, MDA content was notably unaffected by waterlogging stress. Tian *et al.* (2019) also found that MDA content increased with the increase duration of waterlogging. Highest MDA content of DMY1 and KY16 cultivar measured at 9 day of waterlogging during seedling, jointing and tasseling stage.

Ascorbate peroxidase (APX) activities was measured by Wei *et al.* (2013) in two sesame cultivars namely ZZM2541 (tolerant) and Ezhi-2 (sensitive) under different duration of waterlogging up to 8 days. In Ezhi-2, it sharply increased up to the 4th day and then slightly dropped, but in ZZM254, but after 2 days of waterlogging it was about 8-folds higher and 9-folds higher at 6th days. SOD and CAT activities also increased at 8th day of waterlogging. However, CAT activity was highest (2.3-times higher) in the tolerant cultivar at 2nd day of waterlogging. Damanik *et al.* (2010) also measured increased SOD activity and fluctuated CAT, APX and GR activity in rice seedlings at 12 days of waterlogging.

2.4 Jute responses and tolerance to abiotic stress

2.4.1 Drought stress

An experiment was carried out by Shiwachi *et al.* (2009) where they used two varieties of *C. olitorius*- Yaya and Moroheiya to investigate the difference of their responses at three different soil moisture conditions (FCW, field capacity water level >75%, LMS, light moisture stress; 60-50% and AMS, acute moisture stress; 40-30%) after 7 days from transplanting and maintained till harvest. In both varieties plant height reduced in AMS and LMS conditions comparing to FCW. Plant height reduction in cv. Moroheiya at AMS and LMS conditions was recorded as 12% and 23% compared to FCW and in cv. Yaya it was 19% and 26% of FCW, respectively. In both varieties leaf number, branches and leaf area sharply reduced in AMS condition. Similar results were found by Prodhan *et al.* (2001) where they found at constant drought (8-10% soil moisture) condition plant height of tossa jute cultivar O-4 decreased by 35, 45 and 50% at the age of 75, 105 and 120 days compared to control and plant height of white jute cultivar

CVL-1 reduced by 31, 43 and 50% at the age of 60, 75 and 90 days. At constant drought cv. CVL-1 survived up to 90 days but cv. O-4 was able to complete their life cycle.

In order to explore the effect of PEG-6000 induced water stress on seedling characters, Yumnam *et al.* (2017) performed an experiment with twenty genotypes of *C. olitorius* where maximum reduction in shoot length, shoot FW, root length and FW were observed in OEX 008 (13%), OIJ 246 (42%), OIN 986 (22%) and OIN 986 (63%), respectively and minimum reduction were observed in OIJ 213, OIJ 284, OIJ 299 and OIJ 257 compared to control.

2.4.2 Salt stress

Naik *et al.* (2015) conducted an experiment with nine tossa jute varieties to investigate the effect of salt stress on germination. Five different levels of NaCl concentrations (0, 100, 160, 240, and 300 mM) were used where no germination took place above 160 mM. In S 19 variety maximum reduction (14% and 38% at 100 mM and 160 mM NaCl, respectively) of germination took place and minimum in JRO 128 (no reduction at 100 mM and 18% at 160 mM) over control. At 10 days old seedlings, highest and lowest salt tolerance index was found in S 19 (77%) and JRO 128 (30%), respectively at 160 mM NaCl concentration. Shoot and root length reduced with the increase of NaCl concentrations where no root growth found at 160 mM. Similar results were found by Ghosh *et al.* (2013) where shoot length of *C. olitorius* (cv. O-9897) reduced by 39% and 59% at 100 and 200 mM NaCl concentrations, respectively. Root length also reduced by 30% and 60% at 100 mM and 200 mM NaCl concentrations over control.

While working with eight genotypes of jute (viz., CVE-3, C-83, CVL-1, BJC-7370, O-795, O-9897, OM-1 and O-72), exposed in different levels of salt stress (0, 20, 40, 60, 80 and 100 mM NaCl) Bhuyan *et al.* (2018) found that among the genotypes maximum inhibition of plant height (74%) and dry matter (73%) occurred in cv. CVL-1 and minimum reduction of plant height (51%) and plant dry matter (50%) found in cv. O-9897 in 100 mM NaCl stress over the control. They stated jute cv. O-9897 as a most saline tolerant variety then other seven genotypes.

In *C. olitorius* RWC decreased by 9% and 21% at 100 and 200 mM NaCl concentration. But chlorophyll content (SPAD value) increased 5% at 100 mM NaCl but reduced 13% at 200 mM NaCl over control. Similar results were found by Yakoub *et al.* (2019) where they carried out an experiment with tossa jute which was exposed to four different doses of NaCl (control-T_0: 0.2%, T_1: 0.4%, T_2: 0.6% and T_3: 0.8%) concentrations. They measured that stomatal conductance, net photosynthetic rates and transpiration rate of jute decreased in T_3 at 86, 75 and 75%, respectively over control. Leaf area (46% and 69%) and yield parameters i.e. number of pods/plant, lengths of pods (cm), number of seeds/ pods were also decreased in T_2 and T_3, respectively and seed yield reduced 37% at T_3 compared to control.

Yakoub *et al.* (2019) also reported that with the increase of salt concentration amount of proline and soluble sugar increase in tossa jute and in higher salt concentration (8 g L^{-1}) soluble sugar increase up to 72% compared to control.

2.3.3 Metal/metalloid stress

Germination percentage of jute (cv. DaAnQingPi) reduced at different level of Cu concentrations (0, 2, 5, 10, 30 and 50 µmol L^{-1}) and maximum reduction (50%) occurred at 50 µmol L^{-1} Cu concentration. Seedling height, FW and DW also reduced significantly (Saleem *et al.*, 2019a). Saleem *et al.* (2019a) also stated that germination percentage, plant height, FW and dry biomass weight reduced at 50 µmol L^{-1} in Cu sensitive genotypes (GuBaChangJia and ShangHuoMa).

While working on *C. capsularis*, Saleem *et al.* (2020a) found that in four different proportions of Cu in soil (Cu contaminated and natural soil ratio, T_1- 1:4, T_2- 1:2, T_3- 1:1 and T_4-1:0) maximum inhibition of plant height (23%), FW (34%) and DW (41%) observed in T_4. Parveen *et al*. (2020) also found significant reduction in plant height, plant diameter, FW, and DW of *C. capsularis* by 37, 20, 35, and 33%, respectively at 100 µM Cu concentration ($CuSO_4 . 5H_2O$) compared to control.

At different level of Cu stress (50 and 100 µM) total chlorophyll and carotenoid contents reduced significantly in *C. capsularis* and at 100 µM Cu concentration they reduced by 64% and 26%. Cu stress sharply reduced transpiration rate (T*r*), net photosynthetic rate (P*n*), stomatal conductance (G*s*) and intercellular CO_2 (C*i*) at 50 μM (40, 41, 46 and 13%) and 100 μM (58, 67, 77 and 20%) Cu concentration (Parveen *et al.*, 2020). Saleem *et al.* (2020b) also found that at 1:0 ratio of Cu contaminated and natural soil; net photosynthesis rate, intercellular CO_2 concentrations, stomatal conductance and transpiration rate reduced significantly and EL increased up to 900% compared to control.

2.4.4 Waterlogging

An experiment was carried out by Prodhan *et al.* (2001) with four genotypes white jute (cv. D-154 and cv. CVL-1) and tossa jute (cv. O-4 and cv. R-26) to investigate the difference of their responses at different level of standing water (5, 10, 20 and 30 cm). CVL-1 able to survive for 120 days and O-4 for 105 days at 30 cm standing water where plant height reduced by 28% and 42%, respectively. Ghorai *et al*. (2005) also found that at different level of standing water (0, 5, 10, 15, 20, 25 and 30 cm) plant height, basal diameter and tap root DW of tossa jute reduced up to 40, 41 and 80%, respectively compared to control and at severe waterlogging (25-30 cm water regime) plants able to survive for only 20-30 days. Waterlogging severely affect the quality of fiber by reducing fibre length (11-43%) and fibre strength (12-55%).

2.5 Jute responses to oxidative stress

Four genotypes of tossa and white jute were tested in three different level of salt concentrations for the measurement of MDA, different enzymes activities viz., POD, SOD and CAT by Ma *et al.* (2011). MDA content significantly increased in three level of salt concentrations in genotypes Mengyuan and 07-21 over Huang No.1 and 9511. On the other side CAT and SOD amount significantly reduced in genotypes Mengyuan and 07-21. Quite similar amount of POD activity was noticed in 9511 genotype under three salt stress levels which indicate the salt tolerance ability of that genotype.

With the increasing levels of Cu (50 and 100 μM) concentrations MDA contents increased by 108 and 228% and Pro contents increased by 51 and 77%, respectively in *C. capsularis* (Parveen *et al.*, 2020). Saleem *et al.* (2020c) also found that MDA and H_2O_2 amount increased at 409 and 1136%, respectively in white jute at 1:0 ratio of Cu contaminated and natural soil. They also measured SOD, POD, CAT and APX activity those were increased by 678, 491, 223 and 479%, respectively over control.

Saleem *et al.* (2019a) stated that POD and SOD activity increased with increase of Cu concentrations and at 50 µmol L^{-1} Cu concentration their activity increased by 46% and 29%, respectively in Da An Qing Pi variety of jute. MDA and Pro content also increased sharply with the increase level of Cu. Saleem *et al.* (2019b) also reported that maximum activity of MDA, H_2O_2, SOD, POD, CAT and APX were observed at ShangHuoMa genotype when exposed in Cu contaminated (50 µmol L^{-1}) media.

From the above explained review it was evident that abiotic stress has harmful effects on plant growth, physiology and productivity. Abiotic stress creates oxidative stress in crops including jute which leads toward excess production of ROS and ultimately cause cell death. However, very few studies were documented regarding jute responses at different abiotic stresses. Therefore, a higher scope of further research on this aspect should be availed worldwide.

Chapter III

MATERIALS AND METHODS

This chapter presents a concise description about the experimental time, site, climatic condition, seed or planting materials, treatments, experimental design and layout, crop growing procedure, fertilizer application, intercultural operations, data collection and statistical analysis of the experiment.

3.1 Location

There were two independent expreriments in this study, viz. germination trial (Experiment- I) and pot experiment (Experiment-II). Both the experiments were carried out at the Crop Science Laboratory of Sher-e-Bangla Agricultural University, Dhaka (90º 77´ E longitude and 23º 77´ N latitude), Bangladesh, during the kharif-1 season of 2019.

3.2 Characteristics of Soil

In Experiment-I, sand was used and in Experiment-II, properly fertilized sandy loam soil was used as plant growth substrate.

3.3 Climatic condition of experimental site

The area of experiment was under the subtropical climate and was characterized by high temperature (25-35ºC), high humidity (60-77%) and heavy precipitation (155-375mm) with occasional gusty winds during the period from April to June (Appendix II).

3.4. Plant materials

Tossa jute (*Corchorus olitorius*) cv. O-9897 seed was used as planting material in conducting the entire experiment. This variety was released in 1987. Seed color is greenish blue. Crop Duration is 120-150 days with a yield of 2.73 t ha^{-1}.

3.5 Treatments

Experiment-I consisted of five treatments as follows
(i) Control (C)
(ii) Salt, 75 mM NaCl (S_1)
(iii) Salt, 100 mM NaCl (S_2)
(iv) Cadmium, 0.5 mM $CdCl_2$ (Cd_1)
(v) Cadmium, 1 mM $CdCl_2$ (Cd_2)
After giving 160 ml of relevant treatments seeds were sown in 0.5 L earthen pots.

Experiment-II consisted of nine treatments as follows
(i) Control (C)
(ii) Salt, 200 mM NaCl (S_1)
(iii) Salt, 400 mM NaCl (S_2)
(iv) Drought, 10 days of water deficit condition up to 25 DAS (D_1)
(v) Drought, 15 days of water deficit condition up to 30 DAS (D_2)
(vi) Cadmium, 2 mM $CdCl_2$ (Cd_1)
(vii) Cadmium, 4 mM $CdCl_2$ (Cd_2)
(viii) Waterlogging, at 15-20 DAS and 30-35 DAS (W_1)
(ix) Continuous waterlogging, 15-35 DAS (W_2)

Stress treatments were imposed on seedlings at 15 days after sowing (DAS). In control, salt stresses and cadmium stresses first treatments were given at 15 DAS. Than 2nd, 3rd, 4th and 5th treatments were given at 20, 25, 30 and 35 DAS, respectively maintaining the same amount of treatment. In waterlogging treatments, waterlogged condition were imposed at 15 DAS. In first waterlogging treatment waterlogged condition was maintained for 5 days. After recovery again 5 days of waterlogged condition was imposed at 30 DAS. In second waterlogging treatment, waterlogged condition was maintained up to 35 DAS. In drought stresses after maintaining 10 and 15 days of water deficit condition first irrigation was given at 25 DAS and 30 DAS in first and second drought stress, respectively. Morphological data were started taken 6 day after treatments and biochemical assessment was done after 11 days of stress treatments.

3.6 Design and layout of the experiment

Experiment-I and Experiment-II were laid out in a completely randomized design (CRD) and randomized completely block design (RCBD), respectively with three replications.

3.7 Seed collection

Seeds of tossa jute cv. O-9897 were collected from Bangladesh Jute Research Institute (BJRI), Manik Mia Avenue, Dhaka 1207. Seeds from same lot were used in both experiments.

3.8 Preparation of planting media

In Experiment-I, sand was washed with water thoroughly and then sun dried. After that earthen pots of 0.5L capacity were filled with clean and dry sand.

In Experiment-II, the collected soil was sun dried, crushed and sieved. The soil, organic manure and fertilizers were mixed well before placing the soils in the pots. Empty plastic pots (12 liter) were used. Each pot was filled up with 10 kg soil. Then the pots were placed at the experimental shed.

3.9 Fertilizer application

Fertilizers used in Experiment-II were organic manure, urea, triple super phosphate and muriate of potash and used as per recommendation of Islam (2019). During final pot preparation the whole amount of fertilizers were incorporated in soil before sowing.

Fertilizer doses are as follows:

Fertilizers	Dose (kg ha^{-1})
Organic manure	5000
Urea	20
Triple superphosphate	20
Muriate of potash	20

3.10 Intercultural operations

In Experiment-I, no intercultural operations were needed. But in Experiment-II, following intercultural operations were performed:

3.10.1 Thinning

After sowing seeds continuous observation was kept. In Experiment-II, two thinning was done to maintain the population and spacing. First thinning was done at 7 DAS and second one at 12 DAS.

3.10.2 Weeding

Sometimes there were some weeds observed in pots which were uprooted manually.

3.10.3 Irrigation

In control plants tap water was used as irrigation water and in stress treated plants treatments were used as irrigation water. Irrigation was given when needed.

3.10.4 Plant protection measure

While the plant was in seedling condition, root rot disease was observed. At that time Carbendazim 50 WP @ 2g kg^{-1} was applied two times with a seven days interval.

3.11 Data collection

In Experiment- I, germinated seeds were counted for first five days then growth parameters were taken at 15 DAS. Following germination parameters were taken

3.11.1 Germination and seedling parameters

- Germination percentage
- Shoot length
- Root length
- Root: shoot ratio
- Vigor index
- Survivability
- Vigor value
- Co-efficient of velocity of germination
- Timson germination index
- Mean germination time
- Mean germination rate
- Abnormal seedling

In Experiment-II, growth and morphological parameters were collected at 21, 28 and 35 DAS where biochemical parameters were collected at 25 and 35 DAS.
Data were collected on following sequences:

3.11.2 Crop growth parameters

- Plant height
- Leaf area
- Above ground fresh weight plant^{-1}
- Above ground dry matter weight plant^{-1}
- Stem diameter

3.11.3 Physiological parameters

- SPAD value of leaf
- Relative water content
- Electrolyte leakage

3.11.4 Oxidative stress indicators

- Lipid peroxidation
- H_2O_2 content
- Activities of catalase

3.12 Procedure of sampling germination and seedling parameters

3.12.1 Germination percentage

Twenty seeds were placed on each earthen pot. Five day after germination total normal germinated seedlings was counted. A seed was considered to have germinated if the seed coat ruptured and radicle came out up to 0.2 cm or length. Germination percentage was calculated using the following formula (Islam, 2009).

$$\text{Germination (\%)} = \frac{\text{Number of normal germinated seedlings}}{\text{Number of seeds tested}} \times 100$$

3.12.2 Shoot and root length

On 15th day of the germination test seedling shoot and root lengths (cm) were measured. Five seedling samples from each earthen pot were collected randomly. Shoot and root lengths (cm) of individual seedlings were recorded.

3.12.3 Root : shoot ratio

Length basis root: shoot ratio was calculated by following the method of Khandakar (1980) to estimate root efficiency to support production.

$$\text{RSR} = \frac{\text{Root length}}{\text{Shoot length}} \times 100$$

3.12.4 Vigor index

Seedling vigor index was calculated by using the formula of Abdul-Baki *et al.* (1973) in whole number.

Vigor Index (VI) = Germination (%) × Seedling length (cm)

3.12.5 Survivability

At 15th day of germination test number of total survived seedling were counted. Survivability% was calculated using the following formula

$$\text{Survivability\%} = \frac{\text{Number of survived seedling}}{\text{Total number of seed}} \times 100$$

3.12.6 Vigor value

Vigor value was calculated by following formula (Jain and Saha, 1971).

$$\text{Vigor value} = \frac{a}{5} + \frac{b}{5} + \frac{\text{c}}{5} + \frac{\text{d}}{5} + \frac{\text{e}}{5}$$

Where, a, b, c, d and e were the number of seeds germinated after 1, 2, 3, 4 and 5 days. The final count was made at the end of 5th day.

3.12.7 Co-efficient of velocity of germination

It was calculated using the formula of Copeland (1976).

$$\text{CVG} = \frac{A_1+A_2+A_3+A_4+A_5}{A_1T_1+A_2T_2+A_3T_3+A_4T_4+A_5T_5} \times 100$$

Where, A = number of seed germinated, T = time corresponding to A

3.12.8 Timson germination index

It was estimated following the procedure of Khan and Ungar (1984).

Timson germination index (TGI) = $\frac{\Sigma G}{T}$

$\Sigma G/T$, where G is the percentage of seed germinated per day, and T is the germination period.

3.12.9 Mean germination rate

Mean germination rate was calculated following the formula of Ranal *et al.* (2009),

Mean germination rate (MGR) = $\frac{CVG}{100} = \frac{1}{T}$

Where T is mean germination time and CVG: co-efficient of velocity of germination.

3.12.10 Mean germination time

The germinated seeds were counted every day and the germination rate was calculated by using the following mathematical formula of Ellis and Roberts (1980),

$$MGT = \frac{(n1 \times t1) + (n2 \times t2) + (n3 \times t3) + (n4 \times t4)}{T}$$

Where, MGT: Mean germination time (days),n_1, n_2, n_3,n_4 and n_5: 1st, 2nd, 3rd,4th and 5th number of days for each counting of germinated seeds, t_1, t_2, t_3, t_4 and t_5: number of germinated seeds in each counting day and T: Total number of germinated seeds.

3.12.11 Abnormal seedling

Abnormal seedlings were classified according to the rules of the Association of Official Seed Analysts (AOSA, 1981) at 5th day of germination test and abnormal seedling% was calculated following the below formula,

$$ANS\% = \frac{\text{Number of abnormal seedling}}{\text{Total number of seedling}} \times 100$$

3.13 Procedure of sampling growth parameters

3.13.1 Plant height

The height of the plants was recorded after the duration of treatment was completed. From each pot, three plants were selected and height was taken beginning from the ground level up to tip of the leaf. The average height of three plants was considered as the height of the plant for each pot.

3.13.2 Leaf area

For leaf area measurement, first leaf images were taken by a digital camera and the area was calculated using Image-J software (Ahmad *et al.*, 2015).

3.13.3 Fresh weight plant^{-1}

From each pot randomly three sample plants were uprooted. Then the plants were weighed in a balance and averaged them to have FW plant^{-1} and taken after completion of treatment duration.

3.13.4 Dry weight plant^{-1}

Three sample plants were randomly selected from each pot and after weighing for fresh weight were dried them in an electric oven maintaining 80 °C for 48 h. Then the samples were weighed in an electric balance and averaged them to have DW plant^{-1}. The data was taken after completion of treatment duration.

3.13.5 Stem diameter

Stem diameter of plants was recorded after the duration of treatment was completed. Stem diameter was taken by using slide calipers. Slide calipers was set in middle part of plant stem and data was taken in millimeter. Three random plants were selected for measuring stem diameter.

3.14 Procedure of sampling physiological parameters

3.14.1 SPAD value

From each pot, five leaves were randomly selected and with atLEAF (FT Green LLC, USA) atLEAF value was taken. Then it was averaged and SPAD value was measured by the conversion of atLEAF value into SPAD units.

3.14.2 Relative water content

Relative water content (RWC) was measured following the procedure of Barrs and Weatherly (1962).Whole leaf discs were weighed as FW and then floated on distilled water in Petri dishes and kept in a dark place. After 24 h, excess surface water was removed and leaf discs were weighed again which was considered as turgid weight (TW). Then after drying at 80 °C for 48 h DW was measured. Leaf RWC was calculated using the following formula:

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

3.14.3 Electrolyte leakage

To measure EL, standard procedure of Dionisio-Sese and Tobita (1998) was followed. From each pot 0.5 g plant sample was collected and then entered into the 15ml empty falcon tube and then filled up with distilled water. After this falcon tubes were put into water bath at 40 ºC for 60 minutes. After that EC_1 was taken by using the electrical conductivity meter. Then falcon tubes were put in autoclave. After autoclaving falcon tubes were cooled in room temperature then EC_2 was taken. EL was determined by using the following formula

$$\text{EL} = \frac{\text{EC1}}{\text{EC2}} \times 100$$

3.15 Procedure of measuring oxidative stress indicators

3.15.1 Measurement of lipid peroxidation

Through the formula of Heath and Packer (1968) with slight changes by Hasanuzzaman *et al.* (2012) lipid peroxidation level was estimated by measuring malondialdehyde (MDA) content. In 3 mL 5% (w/v) trichloroacetic acid (TCA) leaf samples (0.5 g) were homogenized and after that it was centrifuged at 11,500 × g for 15 min. After that the supernatant (1 mL) was mixed with 4 mL of thiobarbituric acid (TBA) reagent (0.5% of TBA in 20% TCA). The mixture was heated for 30 min at 95 °C in a water bath. After that, sample was cooled in an ice bath. The absorbance of the colored supernatant was measured at 532 nm and was corrected for non-specific absorbance at 600 nm. MDA content was calculated by using extinction coefficient 155 mM^{-1} cm^{-1} and expressed as nmol g^{-1} FW.

3.15.2 Determination of hydrogen peroxide content

H_2O_2 was assayed following the method described by Yu *et al.* (2003). Leaf samples (0.5 g) was homogenized with 3 ml of 50 mM potassium-phosphate (K-P) buffer (pH 6.5) at 4°C and then centrifuged at 11,500× g for 15 min. With 3 ml of supernatant 1 ml of 0.1% $TiCl_4$ in 20% H_2SO_4 (v/v) were mixed and kept in room temperature for 10 min. The optical absorption of the supernatant was measured spectrophotometrically at 410 nm to determine the H_2O_2 content using extinction coefficient 0.28 μM^{-1} cm^{-1} and expressed as nmol g^{-1} fresh weight.

3.15.3 Determination of protein

Through the method of Bradford (1976) protein concentration of each sample was measured. By using Coomassie Brilliant Blue G-250, ethanol and 85% phosphoric acid Bradford reagent was made. Then 5 μL enzyme extracts were mixed in 5 ml Bradford reagent and absorbance was observed in spectrophotometer at 590 nm. For standard, Bovin serum albumin (BSA) was used instead of enzyme extracts.

3.15.4 Enzyme extraction and assays

Leaf sample (0.5 g) was homogenized in 1 ml of 50 mM ice-cold K-P buffer (pH 7.0) containing 100 mM KCl, , 5 mM β-mercaptoethanol, 1 mM ascorbate and 10% (w/v) glycerol with the ice-cooled mortar and pestle. Than centrifuged at 11,500× g for 15 min and the supernatants were used for determination of enzyme activity. Throughout the overall procedure 0−4ºC temperature was maintained.

3.15.4.1 Catalase activity

CAT (EC: 1.11.1.6) activity was measured by using a reaction mixture contained 50 mM K-P buffer (pH 7.0), 15 mM H_2O_2, and enzyme solution in a final volume of 700 μL was used and the decrease in absorbance was observed at 240 nm for 1 min happened due to the defragmentation of H_2O_2. The reaction was initiated with the enzyme extract and activity was calculated using an extinction co-efficient of 39.4 M^{-1} cm^{-1} (Hasanuzzaman *et al*., 2012).

3.16 Statistical analysis

Data accumulated from different parameters were subjected to analysis of variance (ANOVA) using the software CoStat v.6.400 (CoStat, 2008). Correlation analysis was done considering both 1 % and 5% level of significance by using SPSS v.27 (SPSS, 2020).

Chapter IV

RESULTS AND DISCUSSION

4.1 Experiment-I

In the preliminary trials, simulated drought and waterlogging failed to give expected results. That's why in this experiment, only control, salt and Cd stress were considerate.

4.1.1 Germination

Data presented in Figure 1A, reveal that upon exposure to different abiotic stress germination of *C. olitorius* plant reduced sharply. With the increase of salt stress, germination percentage reduced by 23 and 41% at 75 and 100 mM NaCl-induced salt stress and 18 and 30% at moderate and severe Cd stress, respectively corresponding to control.

4.1.2 Shoot length

Shoot length reduced remarkably at different abiotic stresses and it decreased gradually with the severity of stress (Figure 1B). Shoot length decreased by 23 and 29% at 75 and 100 mM NaCl-induced salt stress and 16 and 60% at 0.5 and 1 mM $CdCl_2$ which is more severe than any other stresses.

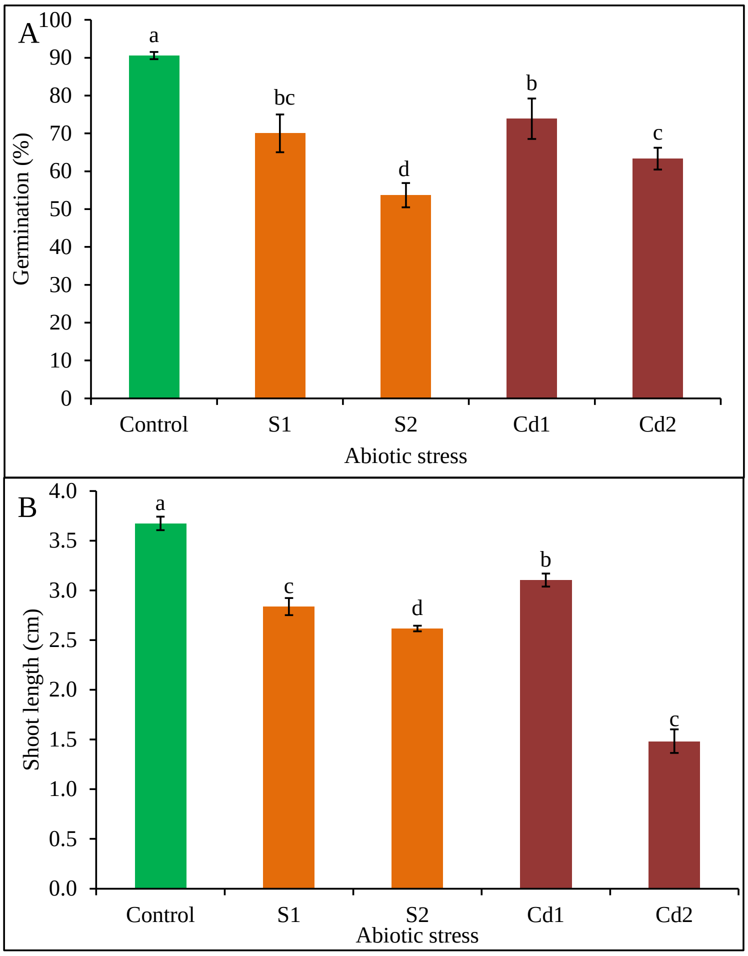

Figure 1. Germination percentage (A), shoot length (B) of *C. olitorius* plants affected by different abiotic stresses. Here, S_1, S_2, Cd_1, Cd_2 represents 75 mM NaCl, 100 mM NaCl, 0.5 mM $CdCl_2$, 1 mM $CdCl_2$. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.1.3 Root length

In this study, a sharp decrease in root length was noticed in both doses of salt treatments (Figure 2A). With the increase of salt stress, root length reduced by 14 and 40% at 75 and 100 mM NaCl-induced salt stress and 78% at severe Cd stress, respectively corresponding to control. However, at moderate Cd stress root length increased by 9% compared to control.

4.1.4 Root: shoot ratio

In this experiment, root shoot ratio increased by 10% at moderate salt stress. However, decreased by 18% at severe stress. Similarly, at moderate Cd stress root shoot ratio increased by 27% but reduced by 46% at severe stress over control. Root: shoot ratio of *C. olitorius* plants were more crucial to Cd stress compared to salt stress (Figure 2B).

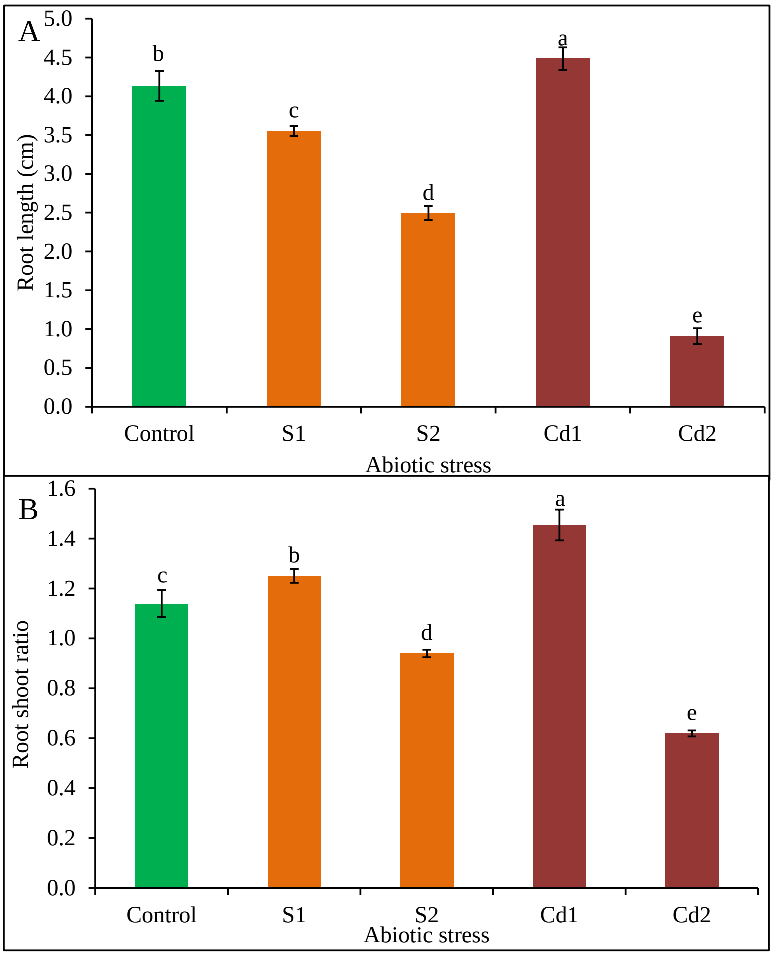

Figure 2. Root length (A), root: shoot ratio (B) of *C. olitorius* plants affected by different abiotic stresses. Here, S_1, S_2, Cd_1, Cd_2 represents 75 mM NaCl, 100 mM NaCl, 0.5 mM $CdCl_2$, 1 mM $CdCl_2$. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.1.5 Vigor index

Vigor index reduced remarkably at different abiotic stresses and it decreased gradually with the severity of stress (Figure 3A). Vigor index decreased by 32 and 47% at 75 and 100 mM NaCl-induced salt stress and 24 and 54% at 0.5 and 1 mM $CdCl_2$.

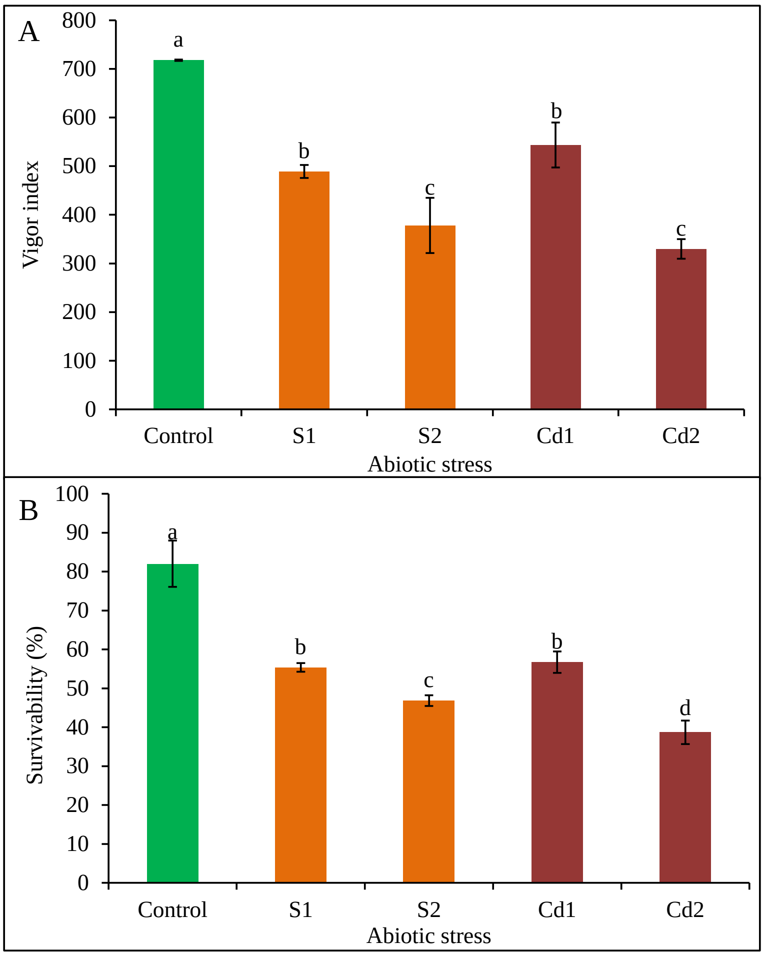

Figure 3. Vigor index (A), survivability (B) of *C. olitorius* plants affected by different abiotic stresses. Here, S_1, S_2, Cd_1, Cd_2 represents 75 mM NaCl, 100 mM NaCl, 0.5 mM $CdCl_2$, 1 mM $CdCl_2$. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.1.6 Survivability

Upon exposure to different abiotic stresses survivability percentage decreased sharply (Figure 3B) With the increase of salt stress, survivability% reduced by 32 and 43% at 75 and 100 mM NaCl-induced salt stress and 31 and 53% at moderate and severe Cd stress, respectively corresponding to control.

4.1.7 Vigor value

In this study, a sharp decrease in vigor value was noticed in all doses of treatments (Figure 4A). With the increase of salt stress, vigor value reduced by 41 and 56% at 75 and 100 mM NaCl-induced salt stress and 28 and 46% at moderate and severe Cd stress, respectively corresponding to control. Severe salt stress (100 mM NaCl) was more crucial for vigor value then severe Cd stress**.**

4.1.8 Co-efficient of velocity of germination

Upon exposure to different abiotic stresses co-efficient of velocity of germination (CVG) decreased sharply (Figure 4B). With the increase of salt stress CVG reduced by 24 and 27% at 75 and 100 mM NaCl-induced salt stress and 11 and 19% at 0.5 and1 mM $CdCl_2$, respectively corresponding to control

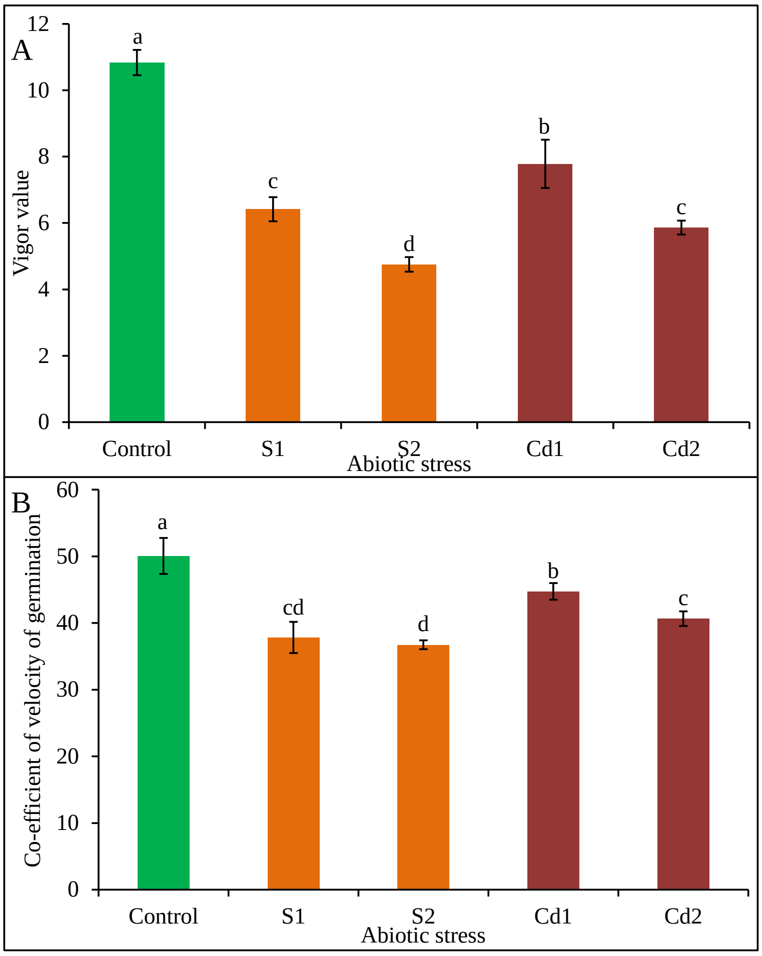

Figure 4. Vigor value (A), co-efficient of velocity of germination (B) of *C. olitorius* plants affected by different abiotic stresses. Here, S_1, S_2, Cd_1, Cd_2 represents 75 mM NaCl, 100 mM NaCl, 0.5 mM $CdCl_2$, 1 mM $CdCl_2$. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.1.9 Timson germination index

In this study, a sharp decrease in TGI was noticed at any doses of treatments (Figure 5A). With the increase of salt stress, TGI reduced by 21 and 38% at 75 and 100 mM NaCl-induced salt stress and 19 and 27% at 0.5 and 1 mM $CdCl_2$, respectively over control. Highest reduction of TGI was observed at 100 mM NaCl-induced salt stress.

4.1.10 Mean germination rate

In this study, mean germination rate reduced upon exposure to different abiotic stresses and decreased gradually with the extent of treatments (Figure 5B). MGR decreased by 24% at S_1 and 10 and 18% at 0.5 and 1 mM $CdCl_2$. Highest reduction was observed at S_2 (26%).

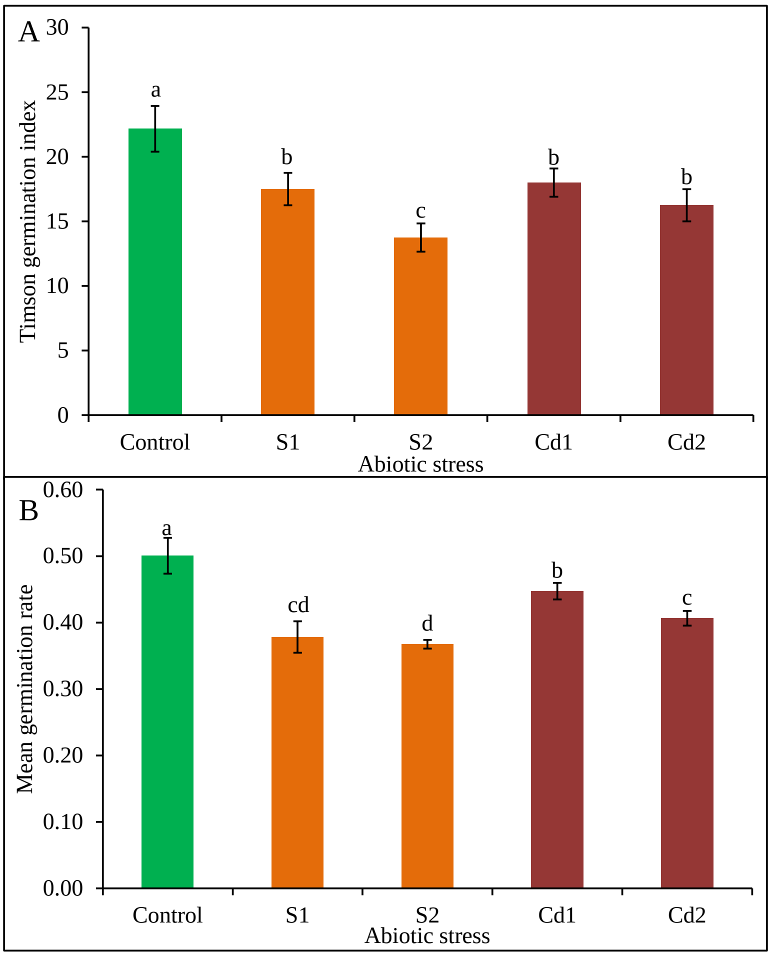

Figure 5. Timson germination index (A), mean germination rate (B) of *C. olitorius* plants affected by different abiotic stresses. Here, S_1, S_2, Cd_1, Cd_2 represents 75 mM NaCl, 100 mM NaCl, 0.5 mM $CdCl_2$, 1 mM $CdCl_2$. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.1.11 Mean germination time

Mean germination time (MGT) of tossa jute remarkably increased at all extent of treatments (Figure 6A). MGT increased by 32 and 36% at moderate and severe salt stress and 12 and 23% at 0.5 and 1 mM $CdCl_2$. Salt was more crucial for germination compared to Cd stress.

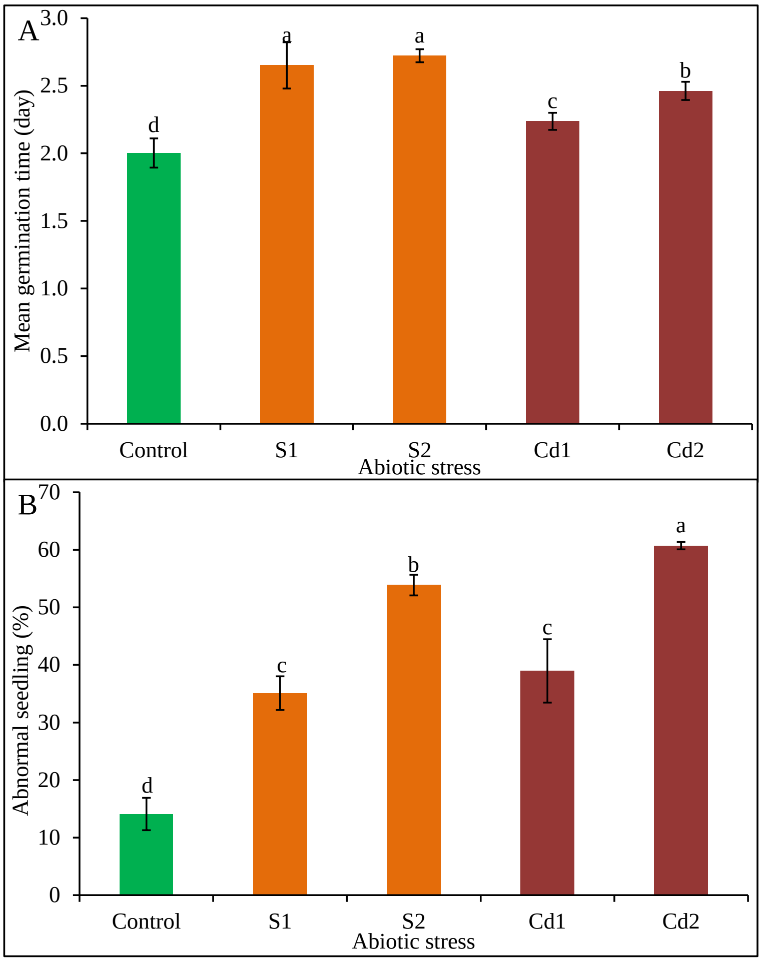

Figure 6. Mean germination time (A) and abnormal seedling (B) of *C. olitorius* plants affected by different abiotic stresses. Here, S_1, S_2, Cd_1, Cd_2 represents 75 mM NaCl, 100 mM NaCl, 0.5 mM $CdCl_2$, 1 mM $CdCl_2$. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.1.12 Abnormal seedling

In this experiment, abnormal seedling% increased sharply at all doses of treatments (Figure 6B). However, remarkable increase was noticed in severe Cd stress compared to other doses of treatments. Abnormal seedling% increased by 149 and 282 % at moderate and severe salt stress and 176, 330% at 0.5 and 1 mM $CdCl_2$.

Germination is termed as a crucial phase in plant life cycle during which different physiological, morphological and biochemical changes take place. But the higher concentration of salt and metal/metalloids impose stress condition on seeds and hinder their growth and metabolic processes. Among the metal/metalloids Cd has a drastic effect on seed growth which delayed germination, caused abnormalities, reduced shoot and root elongation and leads towards seed toxicity and productivity loss (Sethy and Ghosh, 2013). In stress condition Cd limited the water uptake of embryo, induced starch immobilization of endosperm and hinder translocation of soluble sugars (Kuriakose and Prasad, 2008; Vijayaragavan *et al*., 2011). It also inhibits alpha-amylase and invertase enzyme which ultimately leads toward embryo starvation and reduced germination percentage (Ahsan *et al*., 2007). In our present study, we observed reduction in germination percentage (18-30%) with the increase of Cd stress in tossa jute (Figure 1A). Similar results were also found in case of Cu stress where germination percentage of *C. capsularis* reduced with the increase of Cu concentration in soil (Saleem *et al*., 2020a). In salt stress plants faced osmotic potential which create water deficit condition in plants and availability of Na^+ and Cl^- ions create cell toxicity and caused embryo cell damage and ultimately reduced germination (Gill *et al*., 2002). In *C. capsularis* and *C. olitorius* plants germination percentage decrease sharply with the increase of salt stress (Ghosh *et al*., 2013). Due to this reduction and delayed germination some germination parameters viz., CVG (Figure 4B), TGI (Figure 5A) and MGR (Figure 5B) also reduced in salt and Cd stress in a dose dependent manner. This delayed germination also increased the mean germination time of seed which indicates that seeds need higher time to germinate at stress condition (Figure 6A). In *C. olitorius* with the increase of salt stress MGT increased which cause delayed germination reduced seed vigor (Naik *et al*., 2015). Ahmad *et al*. (2012) also observed the drastic effect of Cd on MGT of wheat seedlings. They also noticed reduction in shoot and root length where at 50 μmol L^{-1} Cd concentration shoot length reduced by 48%. Excess Cd concentration create physiological imbalance in plants which may reduce plant growth, create root necrosis and bronzing (Rizwan *et al*., 2016b). This shoot and root length reduction (Figure 1B and Figure 2A) may also occur due to the nutrient imbalance caused by metal toxicity. Similar results were also found in case of wheat (Ahmad *et al*., 2012) and maize (Akhter *et al*., 2017) seedlings. On the other hand salinity retard plant growth, immobilize reserve food and reduce hypocotyl length (Rahman *et al*., 2008). Naik *et al*. (2015) observed reduction of root and shoot length with increase of salt stress in *C. olitorius* plant. Similar results were also found by Ghosh *et al*. (2013) in *C. capsularis* and *C. olitorius* plant where root and shoot length decrease with the severity of salt stress. Due to these reduction root: shoot ratio also reduced (Figure 2B). In basil seedlings root: shoot ratio decreased with increase of salt stress which means root growth reduced more than shoot length (Çamlica and Yaldiz, 2017). In this present study, under moderate stress higher root: shoot ratio was recorded and in severe stress reduction in root-shoot were compared to control. Similar results were also found in wheat seedlings where higher root-shoot ratio was observed in low Cd concentration (25 ppm) corresponding to control (Siddique, 2018). They also found that with the increase concentration of Cd seed vigor of wheat seeds reduced sharply compared to control. Our present study showed the similar results where negative relation of Cd stress and vigor index were found (Figure 3A). This may occur due to the continuous breakdown of reserve food material (Raziuddin *et al*., 2011). Reduction of vigor index indicates the abnormal physiological and morphological changes in seedlings which reduced the survivability% of seedlings (Figure 3B). Higher the severity of stress, lower the chance of survivable for plants. In *C. olitorius* plant salt stress reduced water uptake, transpiration and photosynthesis rate and increased soluble sugars, proline and MDA content and thus plants failed to complete normal life cycle, produce more ROS and showed abnormal characteristics (Yakoub *et al*., 2019). When seeds are sown in metal/metalloid contaminated media, they faced oxidative stress due to increase ROS activity which cause membrane damage, genetic mutation and growth inhibition in plants (Hossain *et al*., 2012). Due to these incidents in our present study number of normal seedlings decreased and abnormal seedlings increased (Figure 6B) in both level of salt and Cd stresses.

4.2 Experiment-II

4.2.1 Crop growth parameters

4.2.1.1 Plant height

Plant height of *C. olitorius* decreased upon exposure to different abiotic stresses (Figure 7). At 21 DAS, moderate and severe salt stress caused 11 and 25% reduction in plant height, respectively. Similarly, moderate and severe drought stress reduced plant height by 12 and 19%, respectively. On the other hand, moderate and severe doses of Cd decreased plant height by 18 and 21%. Waterlogging was the most crucial for *C. olitorius* plant for reducing plant height and it was observed that plant height was reduced by 42 and 44% when the plants were exposed to short and long term waterlogging. At 28 DAS, plant height decreased by 8 and 28% in moderate and severe doses of salt stress. However, 13 and 16% reduction in plant height was also observed in mild and severe stress of drought. Moderate (8%) and severe (25%) doses of Cd also caused reduction in plant height. In moderate waterlogging stress plant height reduced by 38%, however maximum reduction (41%) was observed in severe waterlogging stress. Moderate and severe doses of salt stress decreased plant height by 29 and 31% corresponding to control at 35 DAS. Plant height reduced by 29, 37, 28 and 36% at moderate and severe stress of drought and cadmium respectively. Plant height also reduced at 5 day of waterlogging (16%) but maximum reduction was observed at 20 day of waterlogging (40%) over control.

In this experiment, plant height was decreased due to different abiotic stresses in a dose dependent manner at any ages of plant growth (Figure 7). After imposing abiotic stress on plant, growth arrest is the first response of plant which cause metabolic reduction and gradually reduced shoot growth (Gull *et al.*, 2019). At constant drought (8-10% soil moisture) condition plant height of tossa jute cultivar O-4 decreased by 50% (Prodhan *et al.*, 2001). Bhuiyan *et al*. (2019) also noticed reduction in plant height under water deficit condition in rapeseed seedlings. Moderate and severe doses of salt about threshold level of plants cause reduction in plant height (Darwish *et al.*, 2009). At 100 mM NaCl -induced plant height of tossa jute cv. O-9897 reduced by 51% corresponding to control (Bhuyan *et al.*, 2018). However, plant height reduction also noticed in metal stress. Plant height of cotton plant reduced by 40 and 74% at 1 and 5 µM Cd concentration (Farooq *et al.*, 2013). Waterlogging cause nutrient deficiencies in plant which disrupts physiological process (Zhang *et al.*, 2019). Ghorai *et al*. (2005) also found 41% reduction in plant height of tossa jute at 30 cm water regime.

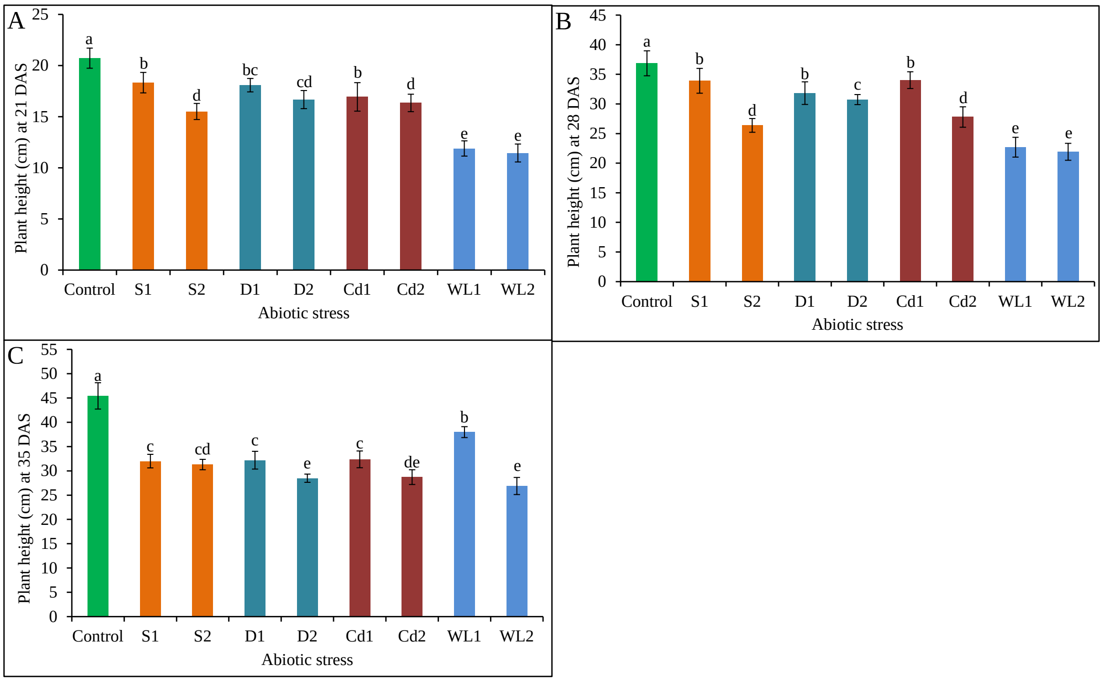

Figure 7. Plant height of *C. olitorius* plants affected by different abiotic stresses. S_1, S_2, D_1, D_2, Cd_1, Cd_2, WL_1, WL_2 represents 200 mM NaCl, 400 mM NaCl, 10 days of water deficit, 15 days of water deficit, 2 mM $CdCl_2$, 4 mM $CdCl_2$, 5 days of waterlogging and 20 days of waterlogging. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.2.1.2 Leaf area

Leaf area of tossa jute showed completely different responses at different abiotic stresses (Figure 8). Upon exposure to different level of Cd stress, leaf area showed no sharp changes. Only severe level of salinity and waterlogging sharply reduced leaf area by 28 and 21%, respectively. However, only drought stress reduced leaf area at any level of stress (16 and 36%).

Leaf area is an indication of plant growth, which can be affected by different abiotic stresses (Figure 8). According to Duan *et al.* (2017) leaf is the main part of plant photosynthesis which can directly affect the strength of plant metabolism. In their study, leaf area of wheat seedling decreased at 15%-25% PEG-6000. Under drought stress palisade tissue cell become gradually shorter which cause shortening of leaf (Zhang *et al.*, 2017). Due to these reasons leaf area of *C. olitorius* plant reduced by 2 and 4 fold at 70 and 40% FC, respectively (Yakoub *et al.*, 2016). Qados (2011) observed

18 and 26% reduction in *Vicia faba* at 120 and 240 mM NaCl- induced salt stress. These results agree with what Jamil *et al.* (2007) reported that, with increase of salt stress (50, 100 and 150 mM) leaf area of *Beta vulgaris* decrease proportionately. Uptake of Cd by living cells leads the plant towards cell death depending on the concentration of metal dose and time of exposure (Vitoria *et al.,* 2001). El-Beltagi *et al.* (2010) found decrease in leaf area of radish with the increase concentration of Cd. Prasanna and Rao (2014) found 31% reduction in leaf area of green gram at 4 day of waterlogging. Kumutha *et al.* (2009) also found similar results in pigeon pea at 2, 4 and 6 day of waterlogging.

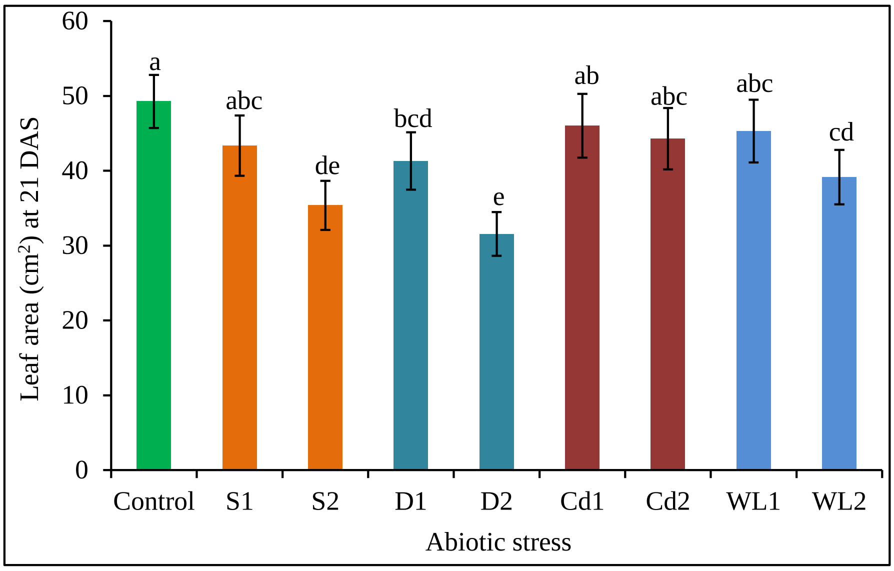

Figure 8. Leaf area of *C. olitorius* plants affected by different abiotic stresses. S_1, S_2, D_1, D_2, Cd_1, Cd_2, WL_1, WL_2 represents 200 mM NaCl, 400 mM NaCl, 10 days of water deficit, 15 days of water deficit, 2 mM $CdCl_2$, 4 mM $CdCl_2$, 5 days of waterlogging and 20 days of waterlogging. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.2.1.3 Above ground fresh weight plant^{-1}

In this study, a sharp decrease in above ground FW was noticed in both doses of treatments. At 21 DAS, moderate level of salt, drought, Cd and waterlogged stresses reduced by 11, 32, 20 and 42% and 30, 46, 24 and 46% in severe doses, respectively over control (Figure 9A). During exposure to salt, drought and cadmium stress above ground FW decreased by 16, 22 and 20% in moderate level and 35, 30 and 26% in severe level of stress, respectively over control at 28 DAS (Figure 9B). Above ground FW reduced by 35 and 38% in WL_1 and WL_2.

In this experiment, above ground FW plant^{-1} was decreased due to different abiotic stresses in a dose dependent manner at any ages of plant growth (Figure 9). Due to water unavailability in drought stress, plants metabolism and physiological process greatly hampered. As a results, net photosynthesis reduced and growth stunted which ultimately leads towards reduction of FW (Demir *et al.,* 2013). In PEG-6000 induced water stress shoot FW of tossa jute reduced remarkably (Yumnam *et al.,* 2017). In Figure 9, FW had negative relation with salt stress. On contrary, Qados *et al.* (2011) found both positive and negative effect on shoot FW of *Vicia faba* at 60 and 120 mM NaCl, respectively corresponding to control. Many studies have shown salt stress either have positive or negative impact on shoot FW (Huang *et al.*, 2009, Memon *et al.*, 2010). Upon exposure to 1 and 5 µM Cd stress reduction in shoot FW of cotton plant was noticed by Farooq *et al.* (2013). Vaculík *et al.* (2009) also found the same results in maize plant, upon exposure to 5 µM Cd stress. In waterlogged condition plants are unable to uptake water as water deficit condition is created which leads plant towards nutrient deficiencies and reduce the biomass of plant (Steffens *et al.*, 2005). *C. olitorius plants* were unable to withstand waterlogged condition at early growth stages and caused drastic growth reduction (Bisaria and Saraswat, 1983). Wollmer *et al.* (2018) observed drastic reduction in shoot FW of maize hybrids under waterlogged condition.

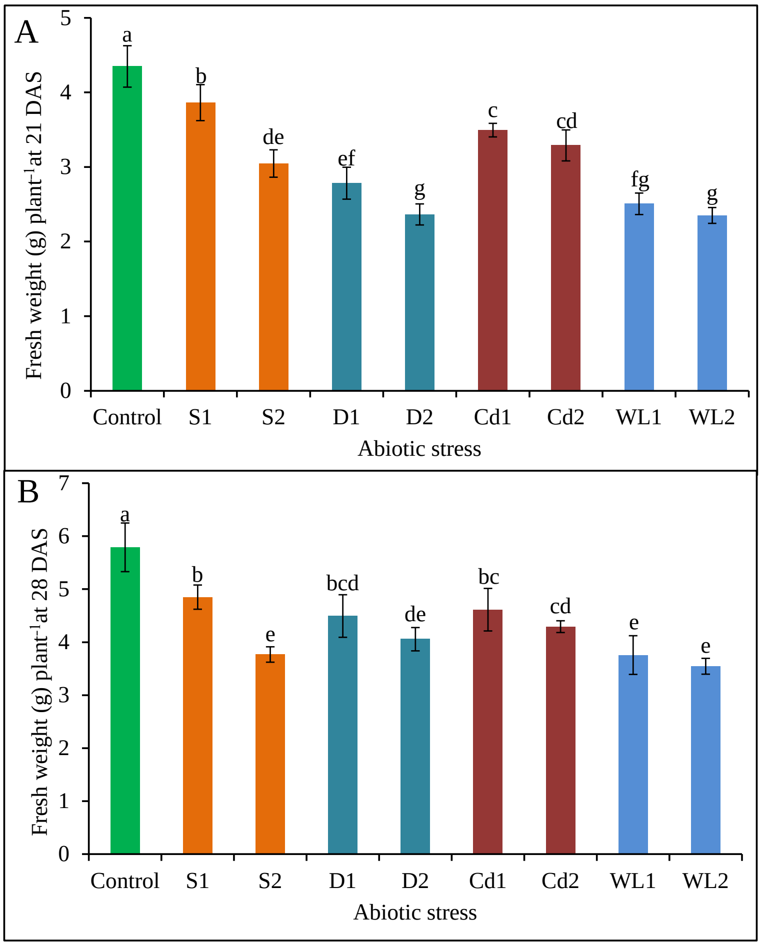

Figure 9. Above ground fresh weight plant^{-1} affected by different abiotic stresses. S_1, S_2, D_1, D_2, Cd_1, Cd_2, WL_1, WL_2 represents 200 mM NaCl, 400 mM NaCl, 10 days of water deficit, 15 days of water deficit, 2 mM $CdCl_2$, 4 mM $CdCl_2$, 5 days of waterlogging and 20 days of waterlogging. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.2.1.4 Above ground dry weight plant^{-1}

Above ground DW was decreased sharply only at severe salt stress at 21 DAS (Figure 10A). However, moderate and severe levels of drought and Cd stress decreased DW by 19, 22, 30, 16 and 21%, respectively corresponding to control. In short and long term of waterlogged condition, DW was decreased by 46 and 50% and had no sharp difference between them. At 28 DAS, a sharp decrease in above ground DW was noticed in both doses of treatments. Moderate level of salt, drought and Cd stresses reduced DW by 8, 11 and 16%, respectively compared to control. Severe level of salt, drought and Cd stresses reduced DW by 26, 16 and 19%, respectively over control

(Figure 10B). Above ground DW reduced by 45 and 48% in WL_1 and WL_2 and both were statistically similar.

Under drought condition, most plants are unable to uptake water which inhibit normal plant physiology and as a result plant growth stunted (Manivannan *et al.,* 2007). Shoot DW reduced by 0.8 and 1.5 fold at 10 and 20% PEG-induced drought stress in maize plants (Raj *et al.,* 2019). Shiwachi *et al.* (2009) also noticed 2.4 fold reduction of DW in *C. olitorius* plant at heavy moisture stress (<30%). In saline stress, Wang *et al.* (2020) noticed overproduction of ROS, due to which chloroplast badly damaged and reduce photosynthesis rate which ultimately reduced plant biomass. They also observed reduction in maize DW at 100 mM NaCl -induced salt stress. Naik *et al.* (2015) also found the same results in different varieties of *C. olitorius* plant at 100 and 160 mM NaCl -induced salt stress. Metal stress leads plant towards unusual morphological changes and metabolic disorders (Amari *et al.,* 2017). Muhammad *et al.* (2015) noticed 47% reduction of shoot DW in *Vigna radiata* upon exposure to 7 mM Hg concentration. Similar results were found by Rizwan *et al.* (2016a) at wheat in 5 µM Cd stress and Gul *et al.* (2016) at maize upon exposure to Cd stress (0.04 mg L^{-1}). In *C. capsularis* plant, DW also reduced at 2, 5, 10, 20 and 50 µM Cu stress (Saleem *et al*., 2019a). At waterlogged condition, plant metabolic process altered and create nutrient unavailability in plant which caused growth reduction and decreased dry matter production in plant (Bailey-Serres *et al*., 2012). Men *et al.* (2020) observed reduction in dry matter of rapeseed at 6 and 9 day of waterlogging. In the present study we also observed decreased of above ground DW $plant^{-1}$ at different abiotic stresses in a dose dependent manner at any ages of plant growth (Figure 10).

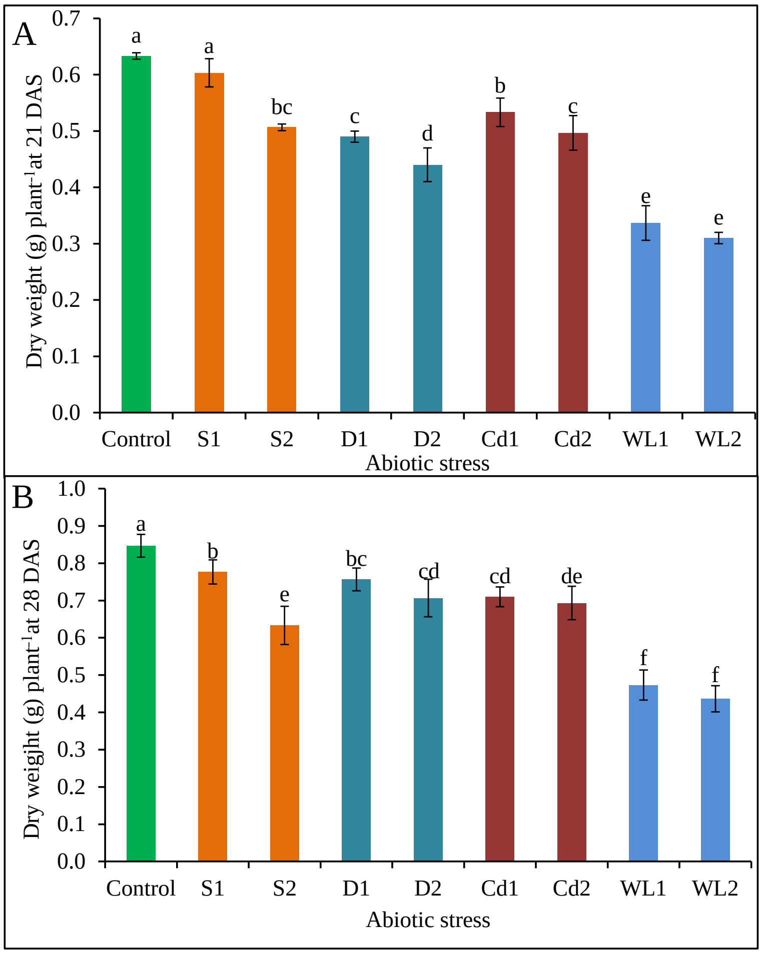

Figure 10. Above ground dry weight plant^{-1} affected by different abiotic stresses. S$_1$, S$_2$, D$_1$, D$_2$, Cd$_1$, Cd$_2$, WL$_1$, WL$_2$ represents 200 mM NaCl, 400 mM NaCl, 10 days of water deficit, 15 days of water deficit, 2 mM CdCl$_2$, 4 mM CdCl$_2$, 5 days of waterlogging and 20 days of waterlogging. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.2.1.5 Stem diameters

In this study, stem diameter reduced upon exposure to different abiotic stresses (Figure 11). Stem diameter reduced sharply at severe doses of treatments than the moderate one. Stem diameter decreased by 50, 39, 48 and 33% in moderate level of salt, drought, Cd and waterlogging stress and by 56, 61, 57 and 58% in severe levels of salt, drought, cadmium and waterlogging stress, respectively corresponding to control.

In this experiment, stem diameter decreased upon exposure to different abiotic stresses at dose-dependent manner (Figure 11). Salt stress stunted plant growth specially shoot growth. With the decrease of shoot growth reduction in stem diameter was also observed. Some studies pointed that these growth reduction proportionately related with the increase of Na (Kaya *et al.*, 2003). In sorghum, stem diameter reduced up to 6% at 15 ds m^{-1} (Saberi *et al.*, 2011). At water deficit condition radius growth reduced and main stem growth stunted and cause a sharp decrease in stem diameter of sunflower (Nezami *et al.,* 2008). Visual symptoms of Cd stress was first observed in upper part of plants. It stunted shoot growth and reduced dry matter which cause reduction in stem diameter (Nikolič *et al.*, 2008). In hybrid poplar, shoot mass reduced by 76% at 10^{-4} M Cd. Parveen *et al.* (2020) also noticed reduction of stem diameter at 50 and 100 µM Cu stress in *C. capsularis* plant. Waterlogging stress reduced fibre yield and marketable grade of jute (Ghorai *et al*., 2005). They noticed reduction in stem diameter with increase of water regime. Similar results were also observed in soybean plant (Andrade *et al.*, 2018).

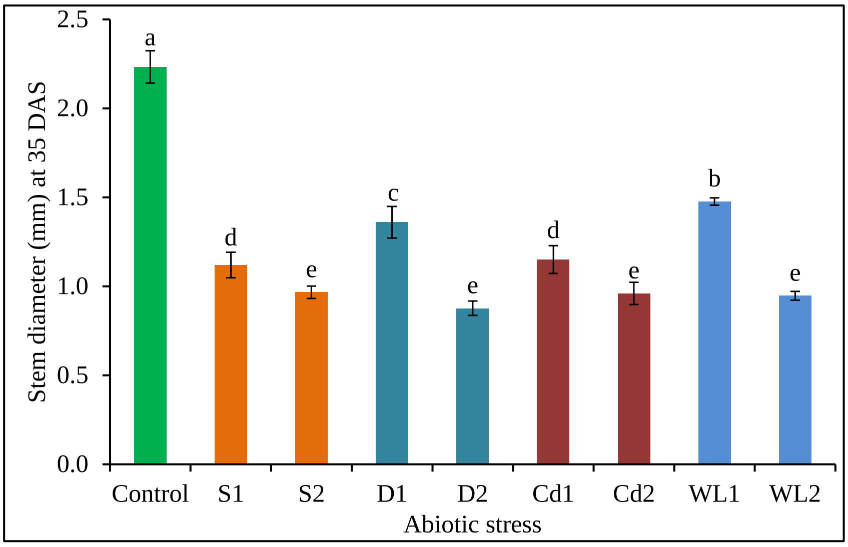

Figure 11. Stem diameter of *C. olitorius* plants affected by different abiotic stresses. S_1, S_2, D_1, D_2, Cd_1, Cd_2, WL_1, WL_2 represents 200 mM NaCl, 400 mM NaCl, 10 days of water deficit, 15 days of water deficit, 2 mM $CdCl_2$, 4 mM $CdCl_2$, 5 days of waterlogging and 20 days of waterlogging. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.2.2 Physiological parameters

4.2.2.1 SPAD value

Reduction in SPAD value was observed in all abiotic stress (Figure 12). However, remarkable reduction was observed in WL_2 compared to other stresses. At Figure 12A, SPAD value of Cd_1 and Cd_2 reduced by 7 and 8%. Upon exposure to salt stress no sharp changes were observed. However, drought stress reduced SPAD value by 5% and had no sharp changes among moderate and severe level of stress. In Cd_1, Cd_2, WL_1 and WL_2 value decreased by 7, 8, 11 and 13%, respectively corresponding to control. At Figure 12B, SPAD value decreased by 6 and 9% at short and long term of water deficit condition. SPAD value decreased by 10, 17, 29 and 31% in Cd_1, Cd_2, WL_1 and WL_2, respectively over control. Only severe salt stress sharply reduced by 8 and 13% at 28 and 35 DAS, respectively. Moderate and severe level of drought and Cd stress reduced SPAD value by 9, 11, 17 and 19%, respectively, corresponding to control (Figure 12C). In this experiment, it was observed that SPAD value was reduced by 17 and 24% when the plants were exposed to short and long term of waterlogging.

In this experiment, SPAD value was decreased due to different abiotic stresses in a dose dependent manner at any ages of plant growth (Figure 12). Drought stress damaged photosynthetic pigments due to oxidation of pigments and reduced photosynthesis rate. It also cause reduction of Chl a and Chl b in plants (Anjum *et al.*, 2011). At 40% field capacity total chlorophyll content of tossa jute reduced by 59% (Yakoub *et al.*, 2016). Similar results were found by Hasanuzzaman *et al.* (2018a) where at PEG –induced drought stress SPAD of *Brassica* species reduced by 14-27%. In salt stress Na^+ and Cl^- ions concentration increase in cell which inhibit net photosynthesis rate (Hasanuzzaman *et al.*, 2013). During exposure to salt stress transpiration rate, stomatal conductance, sub-stomatal CO_2 concentration and net CO_2 assimilation rate greatly reduced in wheat plant (Arfan *et al.*, 2007). In *C. olitorius*, Bhuyan *et al*. (2018) found 13% reduction of SPAD value at 200 mM –induce NaCl. Cd stress reduced water use efficiency of plant and decreased photosynthesis rate and stomatal conductance (Ahmad *et al.,* 2012). Photosynthetic parameters greatly reduced by Cd stress in cucumber, tomato and rapeseed plants (Sun *et al.,* 2017, Alyemeni *et al.,* 2018 and Rossi *et al.,* 2019). Saleem *et al.* (2020b) also found reduction in total chlorophyll content in *C. capsularis* plant with the increase level of Cu stress. Leaf yellowing occur at waterlogging stress which reduced photosynthesis rate and chlorophyll content (Prasad *et al.*, 2004). Collaku and Harrison (2002), Mensah *et al.* (2006) and Yiu *et al.* (2009) found negative correlation of waterlogging and gas exchange parameters in wheat, sesame and onion, respectively.

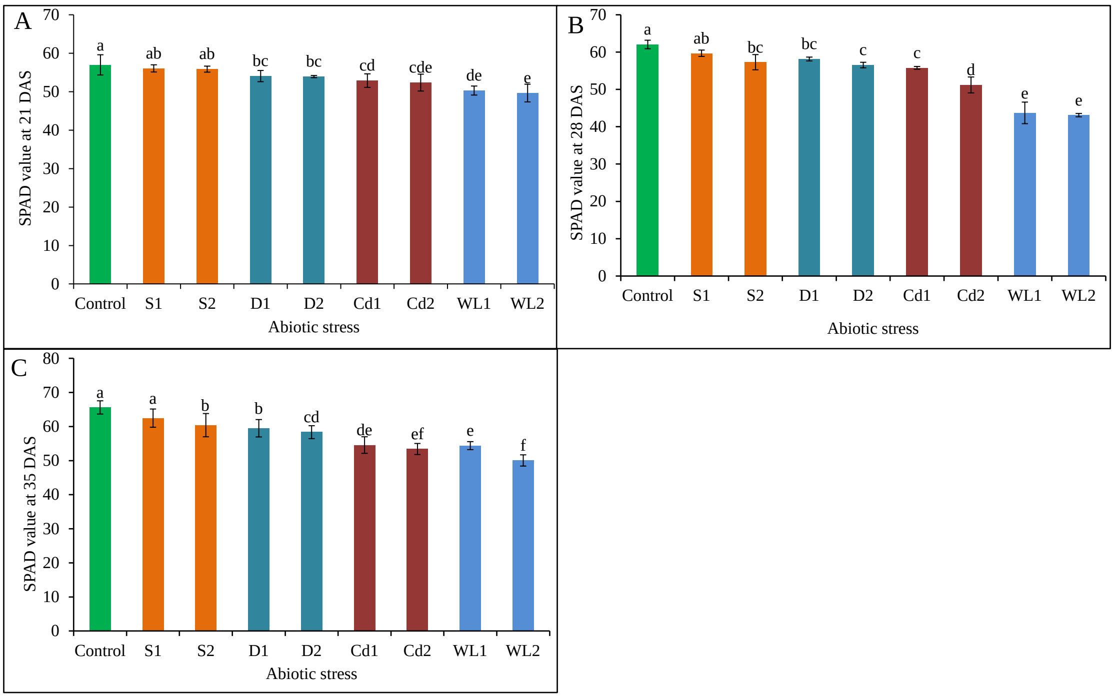

Figure 12. SPAD value of *C. olitorius* plants affected by different abiotic stresses. S_1, S_2, D_1, D_2, Cd_1, Cd_2, WL_1, WL_2 represents 200 mM NaCl, 400 mM NaCl, 10 days of water deficit, 15 days of water deficit, 2 mM $CdCl_2$, 4 mM $CdCl_2$, 5 days of waterlogging and 20 days of waterlogging. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.2.2.2 Relative water content

Exposure to different abiotic stresses resulted in decreases in RWC of plant leaf (Figure 13). At moderate and severe stress level, RWC of both drought and waterlogging stress decreased by 15, 12, 12 and 13% following the same trend. Severe stress of Cd decreased (10%) RWC more sharply than the moderate one at 23 DAS. Salt stress at any level reduced RWC by 16 and 23%, respectively over control. At 30 DAS, RWC of leaf reduced sharply in all abiotic stresses, at any level of treatments (except in WL_1). Both D_1 and D_2 reduced RWC by 16 and 23%. However, RWC reduced by 16, 11 and

8% in Cd_1, Cd_2 and WL_2. Drastic reduction was observed in both salt stress by 23 and 25%.

Cellular damage during stress exposure is assessed by RWC. In this experiment, RWC was decreased due to different abiotic stresses in a dose dependent manner at any ages of plant growth (Figure 13). Drought stress diversely effect plants physiological process and metabolism (Hasanuzzaman *et al.,* 2012). It reduced water content in leaf, create oxidative stress and stunted cell growth (Mahmood *et al.,* 2012). Upon exposure to PEG –induced drought stress relative water content of *Brassica* species reduced greatly (Hasanuzzaman *et al.,* 2014; Bhuyian *et al*., 2019). Similar results were also noticed in *C. olitorius* plant where RWC reduced by 53% at 40% moisture condition (Yakoub *et al.*, 2016). Higher salt concentration create osmotic stress in plants and ultimately leads towards low water potential and thus reduced RWC by 3 and 7% in both salt tolerant and sensitive cultivar of wheat at 100 mM NaCl –induced salt stress (Hasanuzzaman *et al.,* 2017). Relative water content of wheat also reduced at 16 dS m^{-1} and 150 mM NaCl (Poustini *et al.,* 2007; Wahid *et al*., 2007). Ghosh *et al*. (2013) also observed reduction in RWC at 100 and 200 mM NaCl- induced salt stress in *Corchorus* spp. which also cause reduction of leaf area. At high concentration of Cd plants morpho-physiology greatly affected and reduced plants water content, imbibition, transpiration rate, osmotic pressure and ultimately RWC. In tomato seedlings, RWC greatly reduced with the increase doses of Cd (Carvalho *et al.,* 2018). In some studies of Saleem *et al.* (2019a, 2020b) they mention the reduction of leaf number, leaf area and photosynthesis of *C. capsularis* plant at different level of Cu stress which ultimately reduced the water content in leaf. Reduction in leaf RWC denoted less water availability for cell growth. Due to hypoxic or anoxic condition, plants faced unavailability of water in spite of being excess availability of water in flooded condition. In sesame plants, Anee *et al.* (2019) observed 75% reduction of RWC at 8 day of waterlogging. However, same results were also found by Min *et al.* (2005) and Kumar *et al.* (2013) during experimenting with pineapple and mung bean.

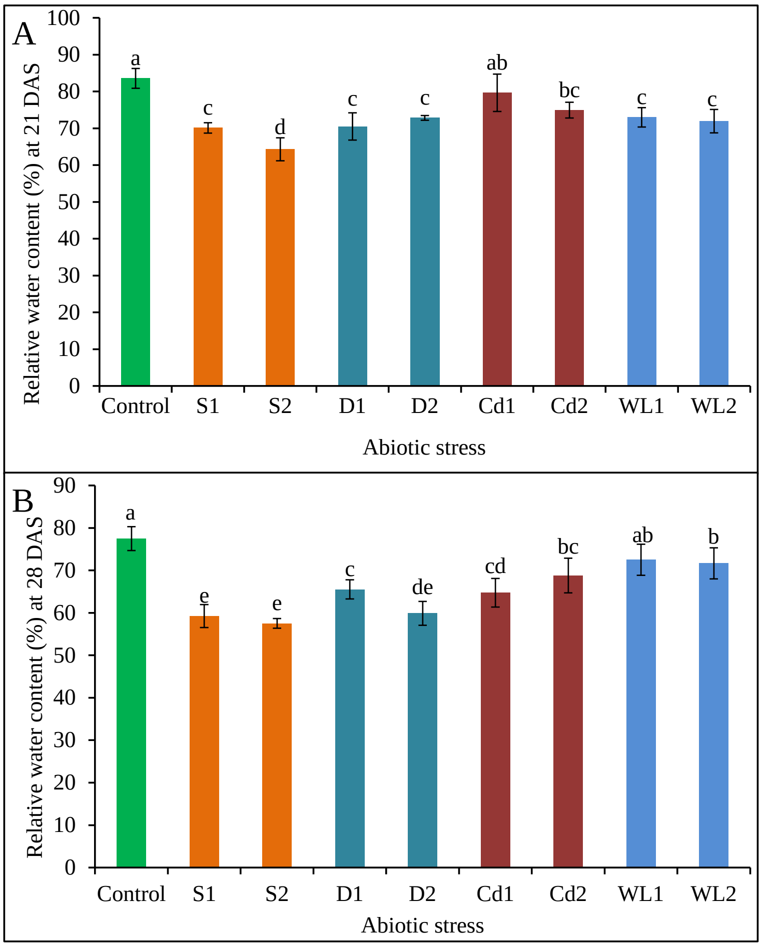

Figure 13. RWC of *C. olitorius* plants affected by different abiotic stresses. S_1, S_2, D_1, D_2, Cd_1, Cd_2, WL_1, WL_2 represents 200 mM NaCl, 400 mM NaCl, 10 days of water deficit, 15 days of water deficit, 2 mM $CdCl_2$, 4 mM $CdCl_2$, 5 days of waterlogging and 20 days of waterlogging. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.2.2.3 Electrolyte leakage

Electrolyte leakage (EL) of tossa jute remarkably increased in all extent of treatments, except in moderate cadmium stress (Figure 14). In moderate level of salt, drought, and waterlogging stress, electrolyte leakage increased by 43, 126, and 34% and 153, 54 and 52% in severe doses of salt, cadmium and waterlogging stress, respectively corresponding to control. Highest amount of EL was observed in severe drought stress (251%).

Ion- specific salt injury occurred first at plasma membrane (Mansour *et al.*, 2005). That's why electrolyte leakage is the most important parameter to know the salt tolerance of crops (Ashraf and Ali, 2008). With the increase of salt stress EL of lettuce root and leaves increased proportionately (Mahmoudi *et al.*, 2011). Similar results were also reported by Demidchik *et al.* (2014), Hniličková *et al.* (2019) on different crop species. The increased EL indicating the damage of plasma membrane caused by drought stress. In faba bean EL increased positively with the increased of drought stress which cause severe membrane damage and efflux K^+ (Siddiqui *et al.*, 2015). Cd toxicity caused membrane damage in tobacco plant and increased EL in a dose dependent manner. They also found positive correlation between EL and MDA in tobacco plants (Wang *et al.*, 2008). Similar results were also observed in white jute by Saleem *et al.* (2019b). The degree of membrane integrity was assessed by the electrolyte leakage of maize plants where 48% increase of EL was noticed at 120 h of flooding (Yordanova and Popova, 2007). In this experiment, EL increased at different abiotic stresses in a dose dependent manner (Figure 14).

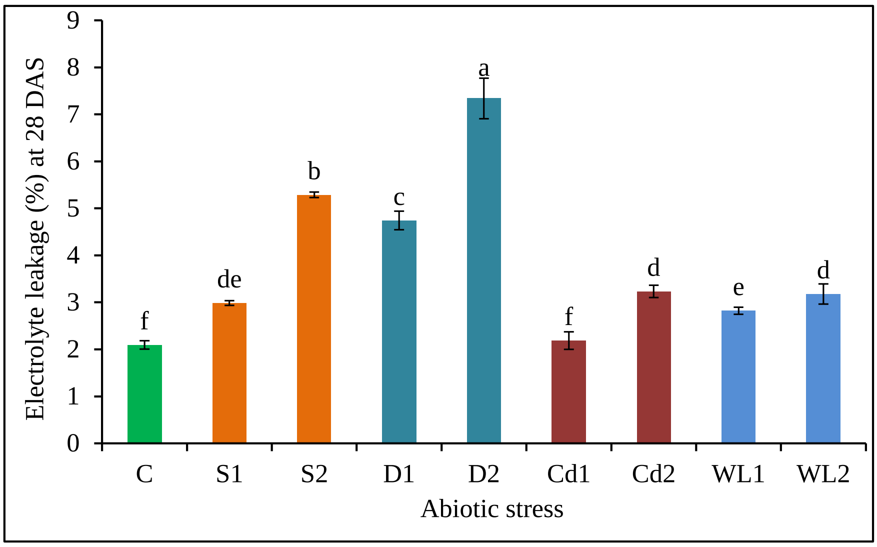

Figure 14. Electrolyte leakage of *C. olitorius* plants affected by different abiotic stresses. S_1, S_2, D_1 Cd_1, Cd_2, D_2, , WL_1, WL_2 represents 200 mM NaCl, 400 mM NaCl, 10 days of water deficit, 15 days of water deficit, 2 mM $CdCl_2$, 4 mM $CdCl_2$, 5 days of waterlogging and 20 days of waterlogging. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.2.3 Oxidative stress indicators

4.2.3.1 Lipid peroxidation (MDA content)

In this study, a sharp increase in MDA content was noticed in all treatments (except in severe waterlogging at 25 DAS). Highest amount of MDA was found in severe drought stress. At 25 DAS, MDA content increased by 75, 118, 40 and 52% in moderate level of salt, drought and cadmium stress and 113, 48 and 15 % in severe doses of salt, cadmium and waterlogging stress, respectively compared to control (Figure 15A). Highest amount of MDA was observed in severe drought stress (137%). Similar results were also found in MDA content at 35 DAS where increased MDA content were noticed in all doses of treatments and highest MDA amount was found in severe drought stress (142%) over control (Figure 15B).

Drought stress cause overproduction of ROS which leads towards protein denaturation, reduced carboxylation efficiency and inactivation of TCA cycle enzymes and ultimately increased MDA (Hasanuzzaman *et al.*, 2018a). At 60% FC condition MDA content of *Solanum lycopersicum* increased by 83% (Rady *et al.,* 2020). Saha *et al.* (2018) also noticed 1.66 fold increase of MDA in rice seedlings at 8 day of water withheld condition. Plants are affected by salinity and imposing many complications like osmotic stress, nutritional deficiency, ion toxicity etc. and resulting excessive production of ROS (Munns and Tester, 2008). Salinity may leads plants towards stomatal closure which reduce CO_2 availability in plants and cause chloroplast degeneration and ultimately produced excessive amount of ROS (Parida and Das, 2005 and Hasanuzzaman *et al.,* 2018b). Ma *et al.* (2011) observed increase trend of MDA with the increase of salinity in both *C. capsularis* and *C. olitorius* plant. However, similar results were also found by Rao *et al.* (2013) in wheat where MDA content increased in a dose dependent manner (2, 4, 8, and 16 EC). Toxic metal/metalloids produced excessive amount of ROS which cause chloroplastic damage and interfere with mitochondrial activity. Ahanger *et al.* (2020) reported increase of MDA content in *V. angularis* at 100 µM $CdCl_2$. Similar results were also found by Saleem *et al.* (2020b) in *C. capsularis* plant at different level of Cu stress. Due to hypoxic and anoxic condition at waterlogging plants produced toxic compounds which generate excessive ROS and increase lipid peroxidation (Loreti *et al.*, 2016). While working with sesame at different duration of waterlogging, Anee *et al.*, (2019) observed the increase of MDA in a dose dependent manner. Similarly, in the present study, MDA increased at different abiotic stresses in a dose dependent manner at any ages of plant (Figure 15).

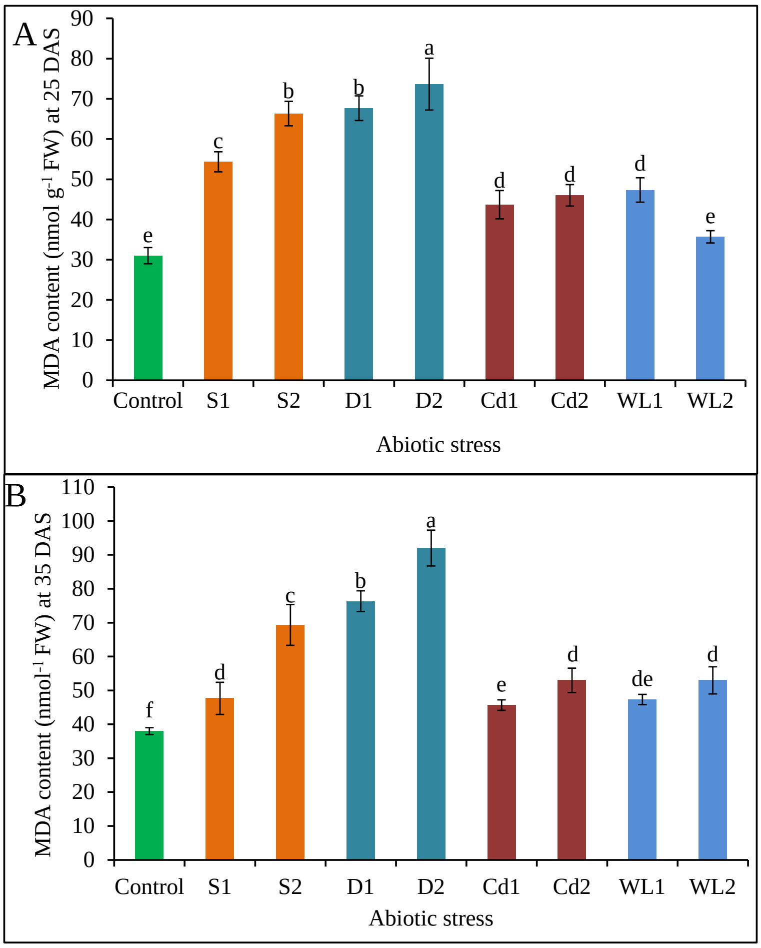

Figure 15. MDA content of *C. olitorius* plants affected by different abiotic stresses. S_1, S_2, D_1, D_2, Cd_1, Cd_2, WL_1, WL_2 represents 200 mM NaCl, 400 mM NaCl, 10 days of water deficit, 15 days of water deficit, 2 mM $CdCl_2$, 4 mM $CdCl_2$, 5 days of waterlogging and 20 days of waterlogging. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.2.3.2 H_2O_2 content

In this experiment, H_2O_2 content was increased sharply upon exposure to different abiotic stresses (Figure 16). At 25 DAS, highest H_2O_2 content was noticed in drought stress and it increased with the severity of stress (83 and 101%). In S_1, S_2, Cd_1 and Cd_2, H_2O_2 content increased by 58, 68, 36 and 46%, respectively corresponding to control.

During short term waterlogged condition H_2O_2 content increased by 30% which was statistically similar with long term waterlogged condition (40%). At 35 DAS, H_2O_2 content increased in both moderate and severe doses of treatments. Upon exposure to salt stress, H_2O_2 content increased by 54 and 72% in S_1 and S_2, respectively. In Cd_1, H_2O_2 content increased by 51% which was statistically similar with Cd_2 (47%). During short and long term of water deficit and waterlogged condition H_2O_2 content increased by 20, 77, 121 and 83%, respectively over control.

Drought initiates stomatal closure, reduced CO_2 assimilation and impaired light production which cause excessive production of ROS (Hasanuzzaman *et al.*, 2019). Nahar *et al.* (2017) observed increase of H_2O_2 in mung bean plant upon exposure to water deficit condition. Similar results were also found by Hasanuzzaman *et al.* (2018a) in *Brassica rapus* seedlings. Salinity create ion toxicity, nutrient deficiency and osmotic imbalance in plant due to which overproduction of ROS occurred (Munns and Tester, 2008). In mung bean plant, H_2O_2 increased by 2- fold at 100 mM NaCl-induced salt stress which indicating the overproduction of ROS in stress condition (Ahmad *et al.*, 2019). Lalarukh and Shahbaz (2020) observed 77% increase in H_2O_2 content in sunflower variety at 120 mM NaCl− induced salt stress. Metal stress increased lipid peroxidation and create membrane toxicity in plant, due to which H_2O_2 content increased in *C. capsularis* plant with the increase level of Cu stress (Saleem *et al.*, 2020c). In case of Cd stress ((100 µM $CdCl_2$) similar results were found in *Arabidopsis thaliana* (Gupta *et al*., 2017). At waterlogging stress, plants generate toxic compounds, due to which H_2O_2 content increased in sorghum with the duration of waterlogged condition (Zhang *et al*., 2019). In this experiment, H_2O_2 content was increased sharply upon exposure to different abiotic stresses (Figure 16).

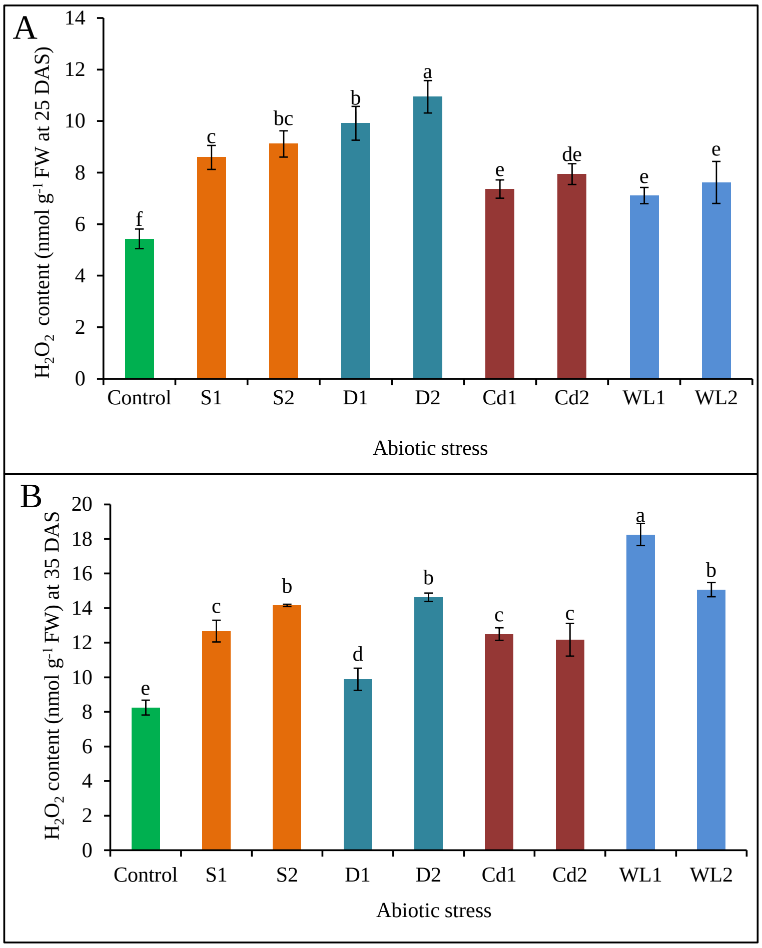

Figure 16. H_2O_2 content of *C. olitorius* plants affected by different abiotic stresses. S_1, S_2, D_1, D_2, Cd_1, Cd_2, WL_1, WL_2 represents 200 mM NaCl, 400 mM NaCl, 10 days of water deficit, 15 days of water deficit, 2 mM $CdCl_2$, 4 mM $CdCl_2$, 5 days of waterlogging and 20 days of waterlogging. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.2.3.3 Catalase activity

In this study, a sharp decrease in CAT activity was noticed in both doses of treatments (except in Cd_1). In severe Cd stress CAT activity decreased by 52% (Figure 17). In moderate and severe salt stress CAT activity decreased by 17 and 16%, respectively and both were statistically similar. In water deficit and waterlogged condition, CAT activity decreased in both moderate and severe doses of treatments by 19, 53, 31 and 68%, respectively corresponding to control.

When plants are exposed in stress condition, antioxidant enzymes help to reduce ROS production and remove toxic compounds (Carocho and Ferreira, 2013). In sweet sorghum, H_2O_2 content increased with the severity of water deficit condition but CAT activity decreased (Guo *et al.*, 2018). Similar results were found in canola cultivar where increased H_2O_2 content decreased CAT activity at 60% FC (Akram *et al.*, 2018). Ma *et al.* (2011) found higher CAT activity in salt resistant jute variety (cv. Huang No.1) compared to the salt susceptible (cv. Menguyan) one. Here, higher CAT activity helps plants to mitigate salt stress. Similar results were also found by Alsahli *et al.* (2019) in wheat seedlings. Heavy metal promotes lipid peroxidation through destroying the equilibrium of ROS production and cause membrane damage in plant cell (Thounaojam *et al.*, 2012). Metal resistant variety reduce lipid peroxidation by increasing the CAT activity in kenaf plant (Li *et al.*, 2013). Saleem *et al.* (2019b) found the similar results in *C. capsularis* plant. Similarly, waterlogging stress tolerant maize variety show higher CAT activity compared to the resistant one which indicate their ability of reduce ROS production (Li *et al.*, 2018). In the present study, sharp decrease of CAT activity was observed in severe stresses (Figure 17).

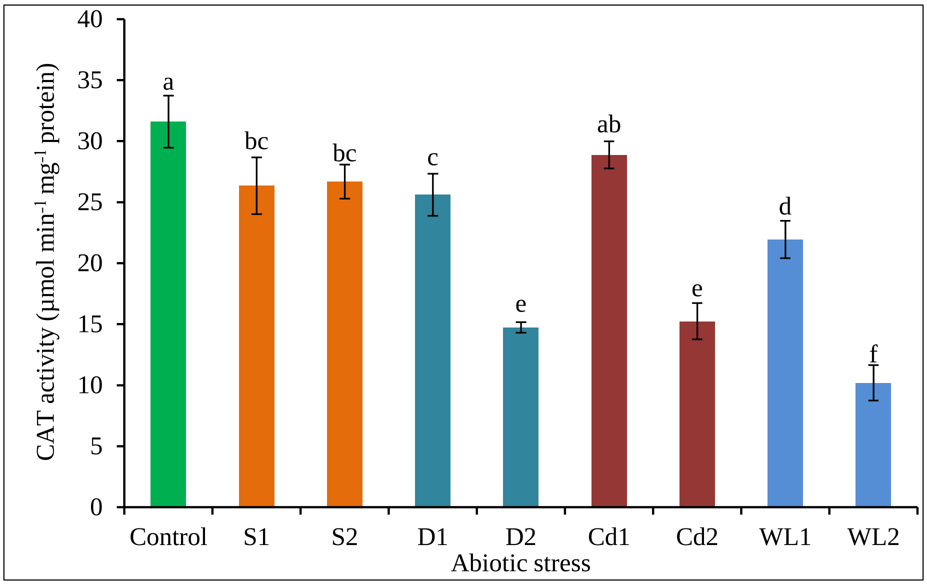

Figure 17. CAT activity of *C. olitorius* plants affected by different abiotic stresses. S_1, S_2, D_1, D_2, Cd_1, Cd_2, WL_1, WL_2 represents 200 mM NaCl, 400 mM NaCl, 10 day of water deficit, 15 day of water deficit, 2 mM $CdCl_2$, 4 mM $CdCl_2$, 5 day of waterlogging and 20 day of waterlogging. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.2.4 Correlation analysis

It was clear from the correlation study that oxidative stress indicators viz., EL, MDA and H_2O_2 were negatively correlated with most of the growth (plant height, leaf area, FW, DW and stem diameters) and physiological parameters (SPAD value, RWC) and antioxidant enzyme (CAT). Similarly, the negative correlation of stress markers with growth and physiological parameters were also observed in different studies (Hasanuzzaman *et al.* 2018a; Saleem *et al.* 2020b).

Table 1. Correlation matrix (n=27) of different observed parameters of *C. olitorius* affected by different abiotic stresses

Variables	**PHT**	**LA**	**FW**	**DW**	**SDM**	**SPAD**	**RWC**	**EL**	**MDA**	**H_2O_2**	**CAT**
PHT	1										
LA	0.572**	1									
FW	0.632**	0.572**	1								
DW	0.362^{ns}	0.228^{ns}	0.779**	1							
SDM	0.933**	0.571**	0.665**	0.389*	1						
SPAD	0.551**	0.040^{ns}	0.575**	0.721**	0.539**	1					
RWC	0.499*	0.548**	0.283^{ns}	-0.138^{ns}	0.612**	-0.141^{ns}	1				
EL	-0.465*	-0.806**	-0.387*	-0.007^{ns}	-0.493**	0.088^{ns}	-0.578**	1			
MDA	-0.495**	0.751**	-0.394*	0.027^{ns}	-0.501**	0.017^{ns}	-0.549**	0.956**	1		
H_2O_2	-0.348^{ns}	-0.334^{ns}	-0.773**	-0.820**	-0.492**	-0.529**	-0.138^{ns}	0.196^{ns}	0.124^{ns}	1	
CAT	0.677**	0.444*	0.629**	0.597**	0.613**	0.664**	-0.024^{ns}	-0.344	-0.324^{ns}	-0.489**	1

*Singnificant at $p \leq 0.05$; **Significant at $p \leq 0.01$

PHT−Plant height; LA−Leaf area; FW−Fresh weight; DW−Dry weight; SDM−Stem diameter; SPAD; RWC−Relative water content; EL−Electrolyte leakage; MDA−Malondialdehyde; H_2O_2 –Hydrogen peroxide content; RWC−Relative water content; CAT−Catalase activity

Chapter V

SUMMARY AND CONCLUSION

The present study contained two experiments and were performed at Crop Science Laboratory of Sher-e-Bangla Agricultural University, Dhaka during the period from April to June, 2019 to investigate the morpho-physiological responses of *C. olitorius* under different abiotic stresses.

Germination trial and pot experiment were conducted in CRD and RCBD design with three replications. Experiment-I consisted of 5 treatments and were applied before sowing. The treatments were control; salt, 75 mM; salt, 100 mM NaCl; cadmium, 0.5 mM $CdCl_2$ and cadmium, 1 mM $CdCl_2$. Experiment-II consisted of 9 treatments. The treatments were control; salt, 200 mM NaCl; salt, 400 mM NaCl; moderate drought, 10 days of water deficit condition at 15-25 DAS; severe drought, 15 days of water deficit condition at 15-30 DAS; cadmium, 2 mM $CdCl_2$; cadmium, 4 mM $CdCl_2$; moderate waterlogging, at 15-20 DAS and 30-35 DAS and severe waterlogging at 15-35 DAS. Stress treatments were imposed on seedlings at 15 days after sowing (DAS). Salinity and Cd treatments were given at 15, 20, 25, 30 and 35 DAS.

Germination parameters viz. germination percentage, mean germination rate, vigor index, Timson germination index, co efficient of velocity of germination, mean germination time, normal seedlings, abnormal seedlings were measured at 5 DAS to observe the effects of stress treatments on seed germination and seedling quality. Seedling parameters e.g. shoot length, root length, root shoot ratio and vigor value were estimated at 15 DAS to evaluate the morphological responses of seedling in stress condition. In Experiment-II, growth parameters (plant height, above ground fresh weight plant^{-1}, above ground dry weight plant^{-1}, leaf area and stem diameter) and physiological parameters (RWC, SPAD value and electrolyte leakage) were measured at 21, 28 and 35 DAS. Biochemical parameters including lipid peroxidation (MDA content), H_2O_2 and CAT activity were measured at 25 and 35 DAS.

Salt and Cd stress sharply reduced germination%, mean germination rate, vigor index, Timson germination index, co efficient of velocity of germination and mean germination time. However, salt stress is more severe than Cd stress and highest reduction was observed in severe salt stress (100 mM NaCl). Upon exposure to salt and Cd stress, shoot and root length of seedlings drastically reduced but this reduction is more severe in Cd stress compared to salt stress. Due to reduction in shoot and root length, root: shoot ratio and vigor index also reduced and highest reduction were observed in Cd stress (1 mM $CdCl_2$). Survivability% of seedlings reduced sharply in stress condition. The intensity of reduction is more prominent in severe Cd stress.

Abiotic stress sharply decreased plant height in a dose dependent manner. The reduction was more severe in waterlogging stress. Similar results were found in above ground fresh weight and dry weight plant^{-1} and highest reduction were observed in waterlogging followed by drought stress corresponding to control. Upon exposure to different level of Cd stress, leaf area showed no changes. Only severe level of salinity and waterlogging sharply reduced leaf area. Among the stresses, drought reduced leaf area at any level of stress. In this study, stem diameter reduced upon exposure to different abiotic stresses. However, stem diameter reduced sharply at severe doses of treatments than the moderate one.

Chlorophyll was decreased upon exposure to different abiotic stresses in dose-dependent manner. The highest reduction was observed in waterlogging stress with increasing duration of waterlogging which verifies the damaging effect of stress on leaf photosynthetic apparatus. Reduction in RWC was observed in all abiotic stress. However, the highest reduction in leaf RWC was observed in salinity stress corresponding to other stress. In this study, EL of tossa jute remarkably increased in all extent of treatments. It represents the membrane injury caused by the leakage of excessive ions. The highest EL was measured in severe drought stress compared to other stress.

Abiotic stress induced oxidative damage results in a drastic increase in MDA and H_2O_2 contents and increased with the severity of stress. The damage is more severe in drought stress compared to other stress. Abiotic stress also reduced CAT activity in a dose dependent manner and fasten the damage process.

From the findings of this study, it was observed that *C. olitorius* response differently in different abiotic stresses. These reduction were occurred in a dose dependent manner. Germination parameters were highly affected by salt stress compared to Cd stress. Seed germination was more affected by salt stress than Cd stress while seedling parameters viz. root and shoot length were highly damaged by Cd stress compared to salt stress. Growth parameters and SPAD value were highly sensitive to waterlogging followed by drought. However, drought stress caused severe oxidative damage and electrolyte leakage compare to any other stresses. This can be a promising phenomenon to create a further scope of investigation at molecular level to tailor stress tolerance parameters of *C. olitorius* and eventually tolerant varieties.

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PLATES

Plates 1. Effect of different abiotic stress on shoot and root growth of *C. olitorius* seedlings (here, Plate 1A refers control, S_1 and S_2 and Plate 1B refers control, Cd_1 and Cd_2, starting from left to right)

Plates 2. Morphological changes of *C. olitorius* seedlings under different abiotic stresses (here, Plate 2A refers control, S_1 and S_2 and Plate 2B refers control, Cd_1 and Cd_2, starting from left to right)

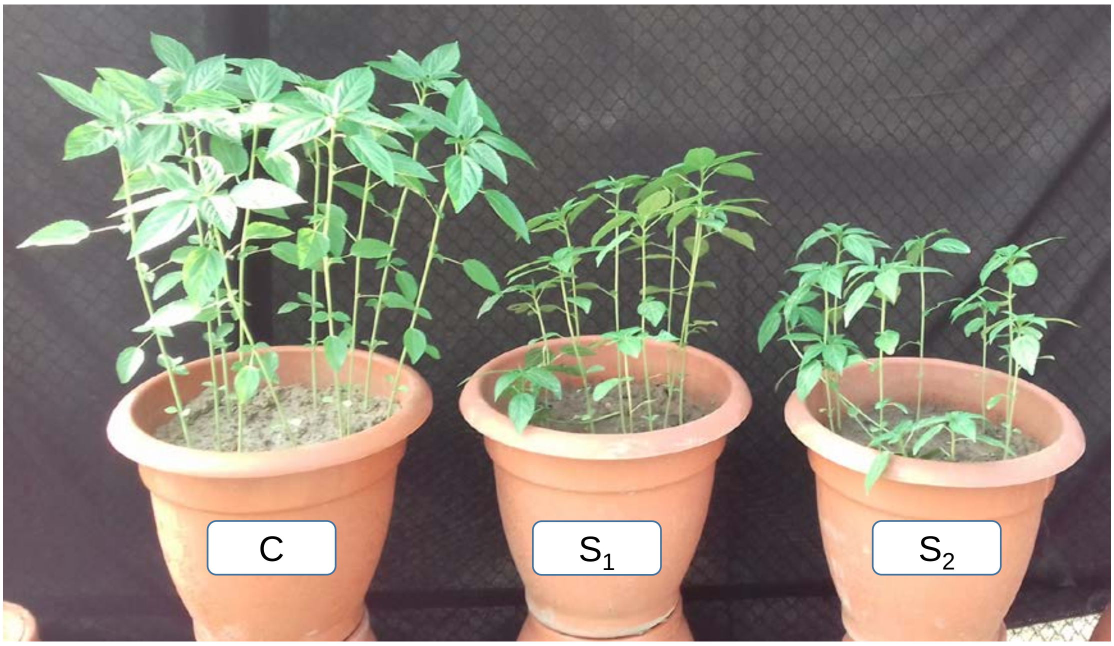


Plate 3. Effect of different levels of salt stress on *C. olitorius* plants. Here, C = control, S_1= 200 mM NaCl, S_2= 400 mM NaCl

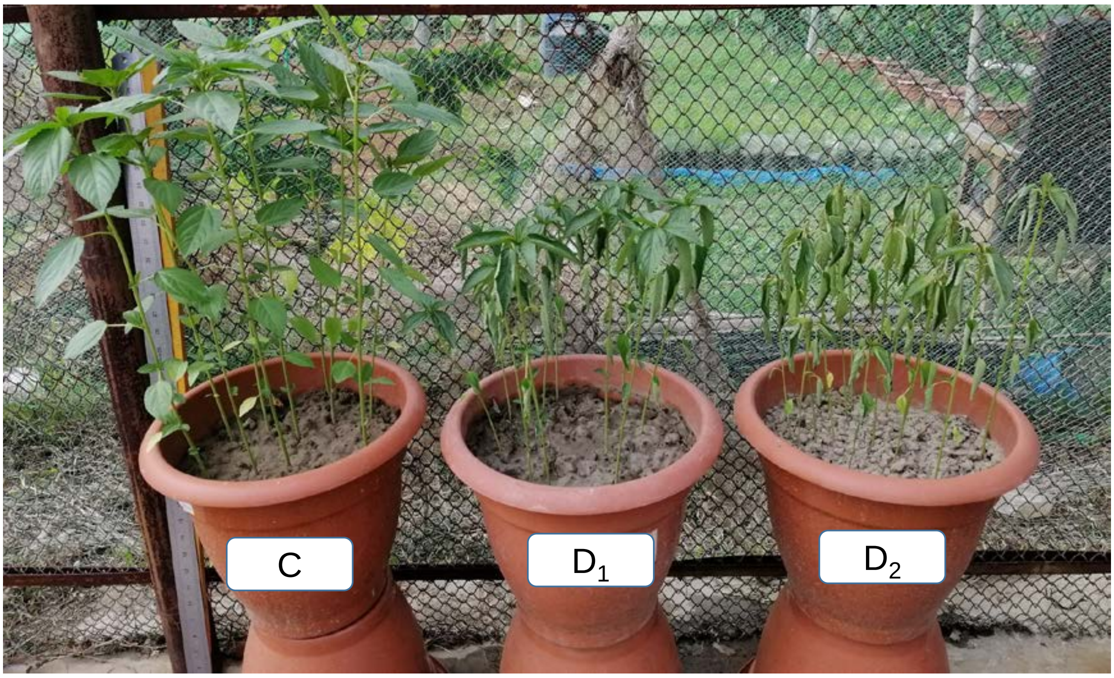


Plate 4. Effect of different levels of drought stress on *C. olitorius* plants. Here, C = control, D_1= 10 days of water deficit, D_2= 15 days of water deficit

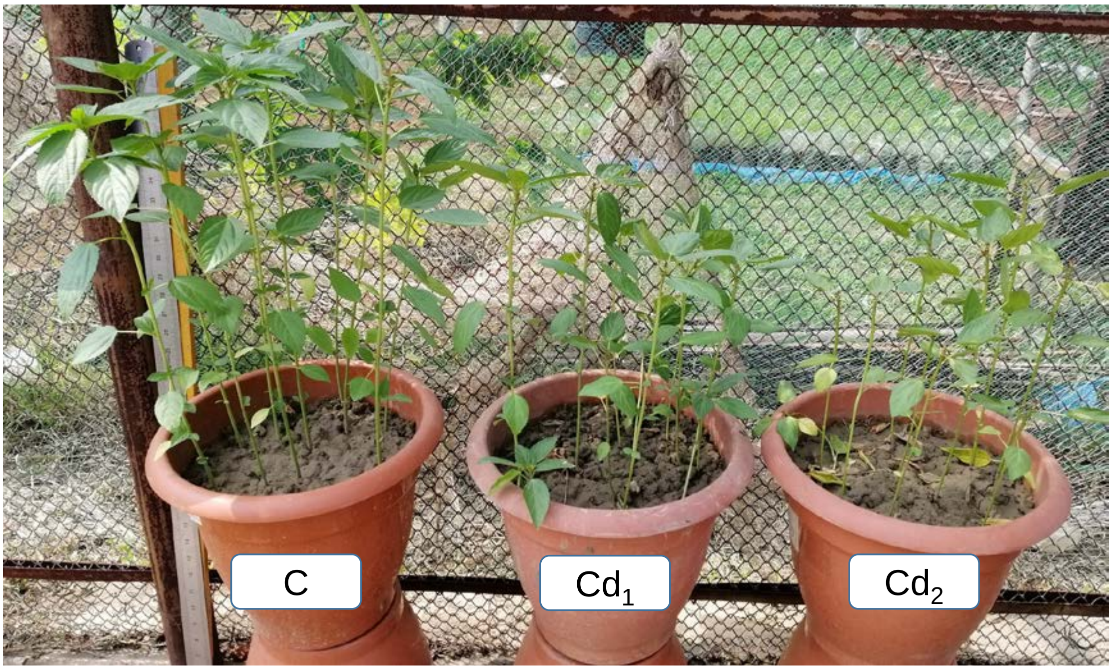


Plate 5. Effect of different levels of Cd stress on *C. olitorius* plants Here, C = control, Cd_1= 2mM $CdCl_2$, Cd_2= 4 mM $CdCl_2$

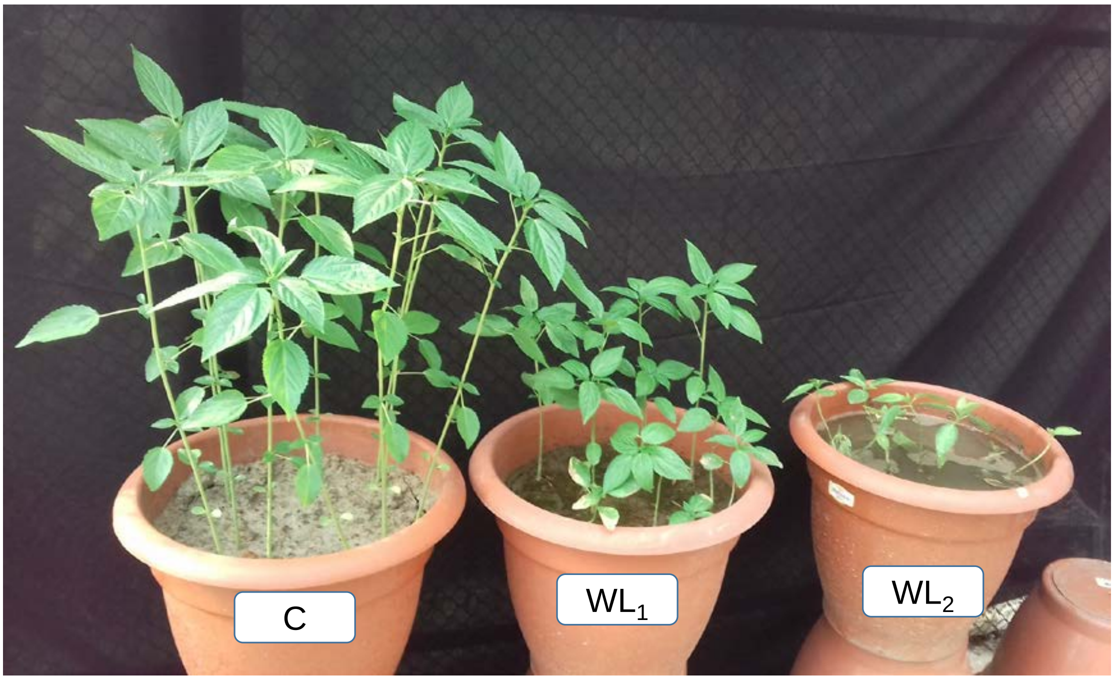
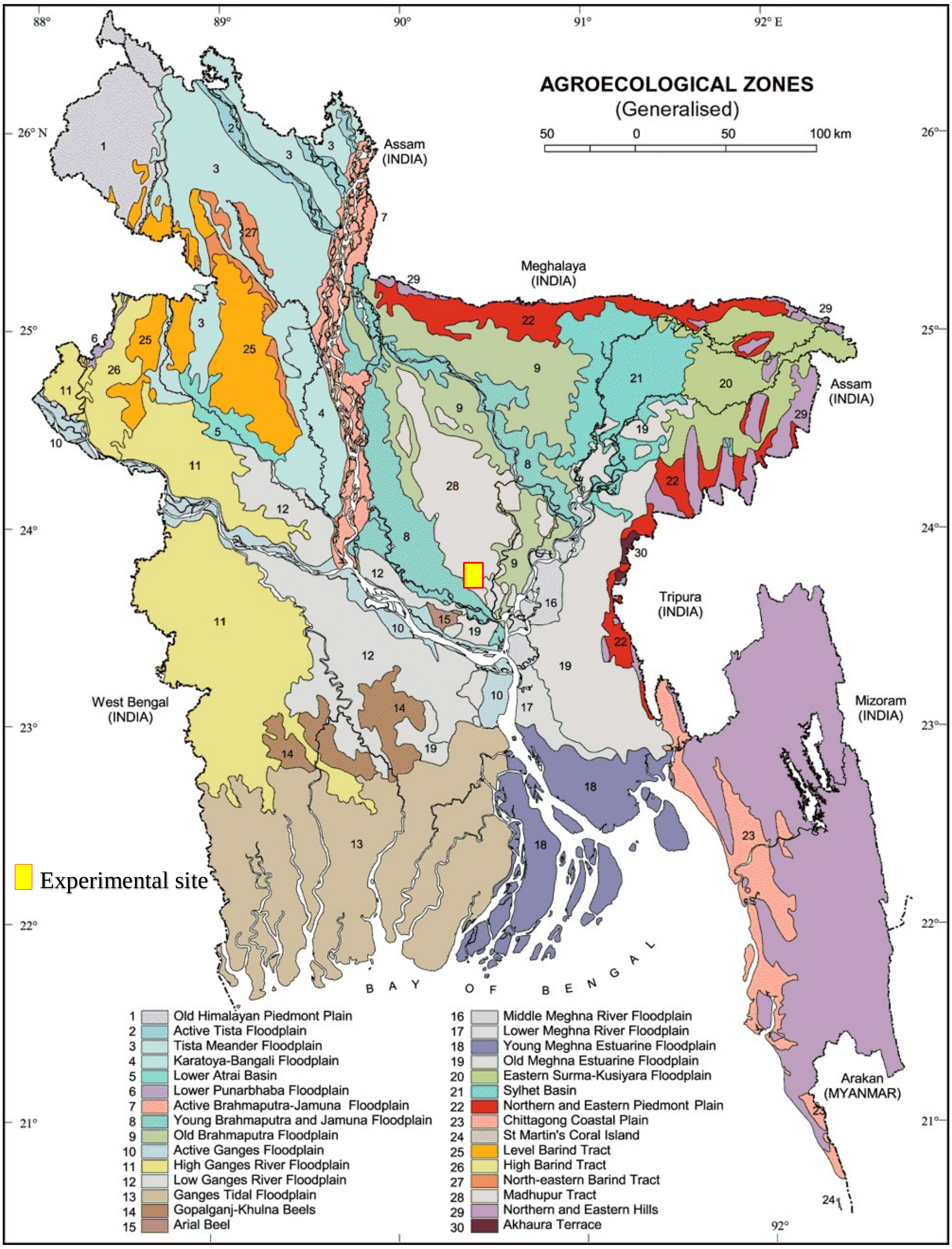


Plate 6. Effect of different levels of waterlogging stress on *C. olitorius* plants Here, C = control, WL_1= 5 days of waterlogging, WL_2= 20 days of waterlogging

APPENDICES

Appendix I. Map showing the location of experiment



Appendix II. Monthly average air temperature, rainfall and relative humidity of the experiment site during the period from April 2019 to June 2019

Month	Air temperature (°C)		Relative humidity (%)	Total Rainfall (mm)
	Maximum	Minimum		
April, 2019	33.7	23.6	42	156.3
May, 2019	32.9	24.5	59	339.4
June, 2019	32.1	26.1	72	340.4

Appendix III. Mean square values and degree of freedom (DF) of germination%, shoot length, root length and root: shoot ratio of *C. olitorius* under different abiotic stresses

Source of variation	DF	Mean square value of			
		Germination%	Shoot length	Root length	Root: shoot ratio
Treatment	4	561.344	1.955	6.262	0.303
Error	10	14.659	0.006	0.016	0.002

Appendix IV. Mean square values and degree of freedom (DF) of vigor index, survivability, vigor value, co efficient of velocity and Timson germinaton index of *C. olitorius* under different abiotic stresses

Source of variation	DF	Mean square value of				
		Vigor index	Survivability	Vigor value	CVG	TGI
Treatment	4	69661.378	795.211	16.438	89.326	28.235
Error	10	1192.209	11.104	1.801	3.207	1.729

Appendix V. Mean square values and degree of freedom (DF) of mean germination time, mean germination rate and abnormal seedling of *C. olitorius* under different abiotic stresses

Source of variation	DF	Mean square value of		
		MGR	MGT	Abnormal seedling
Treatment	4	0.009	0.266	986.984
Error	10	0.001	0.010	10.080

Appendix VI. Mean square values and degree of freedom (DF) of plant height at 21, 28 and 35 DAS and leaf area of *C. olitorius* under different abiotic stresses

Source of variation	DF	Mean square value of			
		PHT			Leaf area 21 DAS
		21 DAS	28 DAS	35 DAS	
Treatment	8	26.615	81.529	97.603	92.902
Error	18	0.868	2.713	2.670	14.283

Appendix VII. Mean square values and degree of freedom (DF) of FW and DW at 21 and 28 DAS and stem diameter of *C. olitorius* under different abiotic stresses

Source of variation	DF	Mean square value of				
		Above ground FW		Above ground DW		Stem diameter 35 DAS
		21 DAS	28 DAS	21 DAS	28 DAS	
Treatment	8	13.216	12.916	0.313	0.485	0.544
Error	18	0.321	0.818	0.004	0.013	0.004

Appendix VIII. Mean square values and degree of freedom (DF) of SPAD 21, 28 and 35 DAS, EL and RWC at 21 and 28 DAS of *C. olitorius* under different abiotic stresses

Source of variation	DF	Mean square value of					
		SPAD			RWC		EL
		21 DAS	28 DAS	35 DAS	21 DAS	28 DAS	
Treatment	8	154.317	137.731	72.562	93.443	138.608	8.801
Error	18	50.156	2.238	4.994	8.965	9.370	0.038

Appendix IX. Mean square values and degree of freedom (DF) of MDA and H_2O_2 SPAD 21, 28 DAS and CAT of *C. olitorius* under different abiotic stresses

Source of variation	DF	Mean square value of				
		MDA		H_2O_2		CAT
		25 DAS	35 DAS	25 DAS	35 DAS	
Treatment	8	660.065	916.204	8.037	25.946	161.127
Error	18	11.259	14.519	0.277	0.295	2.544