# PERFORMANCE OF DIFFERENT ABIOTIC STRESSES ON MORPHO-PHYSIOLOGICAL RESPONSES OF JUTE (*Chorchorus capsularis*) GROWN IN BANGLADESH


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# *Certificate*

*This is to certify that the thesis entitled **"PERFORMANCE OF DIFFERENT ABIOTIC STRESSES ON MORPHO-PHYSIOLOGICAL RESPONSES OF JUTE (Chorchorus capsularis) GROWN IN BANGLADESH"** submitted to the Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE in AGRONOMY**, embodies the result of a piece of bonafide research work carried out by **NAZMIN SULTANA,** Registration No. **13-05745** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

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- Author

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## ABSTRACT

Abiotic stresses like water logging or flooding, drought, excessive salinity, metal or metalloid toxicity causes serious injuries to environment and thus reduced the entire crop productivity. To investigate the morpho-physiological responses of deshi jute (CVL-1) under different abiotic stresses, two experiments were carried out during the period of April 2019 to June 2019 at the crop science laboratory, Sher-e-Bangla Agricultural University, Bangladesh. Experiment -I consists of total of six treatments considering salt stress (50, 75 and 100 mM NaCl) and Cadmium (Cd) stress (0.50 and 1 mM $CdCl_2$). A total of nine treatments were used combined including salt stress (200 and 400 mM NaCl), Cd stress (2 and 4 mM $CdCl_2$), drought and waterlogging at seedlings and vegetative stage. All the stress treatments were applied at 15 days after sowing (DAS). Drought was imposed at 15 DAS continued for 10 days till 25 DAS at moderate drought stress condition whereas under severe drought stress drought was carried out for 15 days till 30 DAS. Waterlogging condition was maintained for 5 days at 15 DAS and 30 DAS while in other waterlogged condition 20 days of continuous waterlogged condition till 35 DAS was maintained to create waterlogged stress condition. Different germination parameter like germination percentage, mean germination time, root-shoot ratio, vigor index, survivability percentage, vigor value, co-efficient of velocity of germination, mean germination rate were measured. Results revealed that severe salt stress and cadmium stress showed significant effect on all the germination parameter. Physiological and biochemical parameter like plant height, leaf chlorophyll content, fresh weight (FW) and dry weight (DW) of different plant parts, leaf relative water content (RWC) along with MDA and $H_2O_2$ content were measured. The examined results indicated that plant height, chlorophyll content, FW, DW and RWC of leaf remarkably decreased under drought and salt stresses both at seedling and vegetative stages compared to other stress conditions. However, under Cd and waterlogged stress plant showed comparatively better tolerance than the other stresses. Likewise, *C. capsularis* showed more positive response in waterlogged stress than the cadmium stress. The findings of the present study thus conclude that drought and salinity are the potential stresses that could hamper jute (Deshi pat) production seriously and recommended large scale, field level and nation wide further intensive researches.

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## ABBREVIATIONS AND ACCRONYMS

| | | |
|---|---|---|
| ABA | : | Abscisic acid |
| AEZ | : | Agroecological zone |
| AISH | : | International Association of Hydrological Sciences |
| ANOVA | : | Analysis of variance |
| AOSA | : | Association of official seed analysis |
| As | : | Arsenic |
| APX | : | Ascorbate peroxidase |
| BJRI | : | Bangladesh Jute Research Institute |
| CA | : | Citric acid |
| CAT | : | Catalase |
| Cd | : | Cadmium |
| cm | : | centimeter |
| Cr | : | Chromium |
| DAS | : | Days after sowing |
| DEG | : | Differentially express genes |
| DNA | : | Deoxyribonucleic acid |
| ds/m | : | Decisiemens per meter |
| DW | : | Dry weight |
| EL | : | Electrolyte leakage |
| *et.al.* | : | et alibi (and others) |
| FW | : | Fresh weight |
| gm | : | gram |
| GM | : | Genetically modified |
| GP | : | Germination percentage |
| GR | : | Glutathione reductase |
| ha | : | hectare |
| $H_2O_2$ | : | Hydrogen peroxide |
| HMs | : | Heavy metals |
| Kg | : | Kilogram |
| LSD | : | Least significance difference |

| | | |
|---|---|---|
| MDA | : | Malondialdehyde |
| MGR | : | Mean germination rate |
| MGT | : | Mean germination time |
| mg | : | Milligram |
| ml | : | Milliliter |
| mM | : | Millimole |
| mm | : | Millimeter |
| nM | : | Nanometer |
| PCR | : | Photosynthetic carbon assimilation |
| Pb | : | Lead |
| PEG | : | Polyethylene glycol |
| PKs | : | Protein kinases |
| POD | : | Peroxidase |
| RCBD | : | Randomized Complete Block Design |
| RH | : | Relative humidity |
| ROS | : | Reactive oxygen species |
| RWC | : | Relative water content |
| SC | : | Stomatal conductance |
| SOD | : | Superoxide dismutase |
| TR | : | Transpiration rate |
| TGI | : | Timson germination index |
| VPD | : | vapor pressure deficit |
| WUE | : | Water use efficiency |
| WDS | : | Water deficit stress |
| µL | : | Microliter |
| µM | : | Micrometer |
| % | : | Percentages |

# CHAPTER-1

# INTRODUCTION

Jute is generally familiar as a golden fiber of Bangladesh which is also known as fiber of future for its cheap price as well as several socio-economic importance. This is generally produced from *Corchorus olitorius* or *Corchorus capsularis*, which are widely cultivated in China, India and Bangladesh. The scope of global jute cultivation and use as a source of fiber is second only to that of cotton. Bangladesh is exporting jute and jute product and earns approximately 12-13% of total foreign currency. This significant cash crop grow during the period of March-May based on water availability and usually picked in between June-September and grows almost all of the districts of Bangladesh (Alim, 1978; Islam, 2010).

Many essential chemical compounds are enriched with jute leaves which signify its potentials in both local and international market. An estimation of seventeen nutrititive elements has been recorded in jute plant. However, with the environmental and climatic changes in conjunction with different abiotic stresses deteriorated the quantity as well as the eminence of this crop in Bangladesh. It was calculated that approximately more than fifty per cent of yield reduction is directly related to the abiotic stresses (Rodríguez *et al.*, 2005). Plant metabolism is modified with the abiotic stress s leading to destructive effects on productivity. Types of stressor and prevailing period can create variation on plant stress. One of the most common procedures at molecular level in plants growths underneath salinity stress is the substantial upsurges in reactive oxygen species (ROS). Drought is considered as another main abiotic stresses that badly disturbs harvest yield and thus reducing plant through put over the whole world (Hasanuzzaman *et al.,* 2010; Hasanuzzaman and Fujita, 2011; Hasanuzzaman *et al.*, 2012a).

Numerous physical and biological mechanisms in plants harmfully pretentious by the drought stress (Hasanuzzaman *et al.,* 2012b). Drought stress results water content reduction, turgor loss and diminution of cell quality (Jaleel *et al.,* 2009). Heavy metals (HMs) disrupt the different biological and metabolic processes of jute plants (Dubey, 2011). Among all the heavy meals, Cd is the utmost broadly deliberate in plants. The availability of Cd causes unnecessary manufacture of ROS which leading cell demise (Gill and Tuteja, 2010; Gill *et al.*, 2011a). Furthermore, waterlogging reduces the availablity of oxygen for floras (Capon *et al.*, 2009). Several anthropogenic causes i.e., improper drainage practices, failure of dame – are also sometimes responsible for this unwanted occurrence of flood which further leads to waterlogged conditions (Hasanuzzaman *et al.*, 2012a). Although, *Corchorus capsularis* can tolerate moderate level of briny stress with the increase of salinity level it becomes susceptible. In jute, several physiological changes occurred as a consequences of $H_2O_2$ absorption due to drought stress (Chowdhury and Choudhuri, 1985). High enzyme action and low hydrogen peroxide and malondialdehyde helped *Chorchorus. capsularis* to withstand waterlogging strins by depressing salts effluxity (Chowdhury *et al.,* 1985).

Besides, *Chorchorus capsularis* tolerated HMs stress by ROS formation in conjunction with antioxidative resistance methods (Abubakari *et al.*, 2017; Saleem *et al.*, 2020). Moreover, physiological and morphological characteristics of *Chorchorus. capsularis* are said to be resoponsible for its hyperaccumulative behavior of HMs and this properties are essential for phytostabilization process of HMs (Saleem *et al.,* 2020). Although, *Corchorus capsularis* is an important fiber crop, according to the scientific literature and to the best our knowledge there is no structured scientific investigation about the responses of abiotic stress on jute plant in Bangladesh. Considering the concerns of decreasing jute production due to environmental stresses and inadequate scientific knowledge on *Corchorus capsularis* reponses to these stress s in Bangladesh, the present investigation aims to address the objectives as follows:

i. Investigating the morphophysiological responses of *Corchorus capsularis* under different abiotic stresses.

ii. To study the adaptive mechanisms of *Corchorus capsularis* under salinity, drought, cadmium and waterlogging stress.

# CHAPTER-2
# REVIEW OF LITERATURE

## 2.1 Jute

Jute is a significant monytery crop in Bangladesh termed as "golden fiber". There are two jute species in Bangladesh, i.e., Deshi Paat (*C. capsularis*) and *Tossa Paat* (*C. olitorius*). Jute is usually known as "pat" or "nalita" in the western and central provinces of Bengal; "pat" or "koshta" in Eastern part of Bengal; "pata" or "marapata" in Assam; "ihot", "jhout" or "jhuta" in Orissa; and "patua" in Bihar. The species *C. capsularis* is generally known as "Tita-pat" or "Guti-pat" and C. olitorius is called "Suti", "Bogi", "Tossa", "Deshi" or "Mitha" pat. Moreover, jute is called "Jews mallow"in English which is originate from the former name "Olusjudiacum".

### 2.1.1 Current world status of Jute

Jahan (2019) conducted an experiment to study about natural fibers and the world economy. The natural fiber production was calculated about 33 MT in 2013 all over the world. Among these, 3.3 MT of jute, 1.2 MT of clean wool, 900,000 T of coir, 1.6 million T of other natural fibers such as ramie, sisal, silk, abaca, flax and hemp. In 2013 the natural fiber production value in farm was accounted approximately US$60 billion. Therefore, in the natural fiber industries, total engagement is around 60 M families or 300 M individuals, corresponding to about 4% of the earth populace (Sahoo *et al.*, 2003; Kalita and Bhuyan, 2013; Gill, 2014).

### 2.1.2 Current scenario of jute: Bangladesh perspective

Sheheli and Roy (2014) directed an experimentation to explore the current status and practice of jute cultivation in Bangladesh. The research study was conducted in the Kishoregonj sadar upazila, Kishoregonj district (Islam and Ali, 2017). The research study also showed that the main problems associated with the jute production and processing were the dearth of training facilities, inadequate credit lines, the dearth of quality seeds, the sky-scraping cost of jute production, the high infestation of diseases, the high price of inputs, the shortage of water consumed, the matters of weeds infestation, the unstable price of jute and also the shortage of manpower labour within the peak period (Chowdhury and Rashed, 2015).

### 2.1.3 Nutritional composition of jute

By analyzing the *Corchorus* leaf in search of nutritional compositions, the author found proteins, iron, β-carotene, potassium. Some are a decent source of mucilage, fatty acids, glycosides, saponins and steroids (Ahmed and Nizam, 2008)

## 2.2. Types of Jute species

### 2.2.1 Available jute species in Bangladesh

Islam *et al.* (2017) said that *Corchorus olitorius* and *Corchorus capsularis, these two types* are most commonly cultivated in Bangladesh. The fibers are propagated from the secondary phloem and extracted from *C. olitorius* which is golden in color. *C. capsularis* that yields white fibers are inferior to *C. olitorius* in tensile strength. Commercially, they are known as Tossa and also as White jute. According to Rowell and Stout (2007), only two species of *Corchorus* are grown commercially. They are *C. capsularis* L. and *C. olitorius* L., around 40 wild species are altough known. *H. sabdariffa* L. is also marketed as kenaf sometimes. These plants are woody-stemmed berbaceous dicotyledons and they grow in the tropical and subtropical areas.

### 2.2.2 Varieties of *Corchorus capsularis* in Bangladesh like CVL-1, CVL-2 and others

Shamsuzzaman *et al.* (1999) developed a new variety of jute (*Corchorus. capsularis*) "BINADINESSAT-2" through mutagenesis with sodium azide. Based on late-night blossoming, only 200 plants have been selected for planting since 1st March. The C-278 stress (from the 12 mM NaN3 treatment) was true in later generations and offers a better response in terms of flowering and fiber performance than the CVL-1 parent stress .

### 2.2.3 Current scenario of *Corchorus capsularis* in Bangladesh

Fluctuations in the cultivated area are mainly responsible for production fluctuations. According to the authors, although India and Bangladesh can maintain their position on the world stage, the growth results of both countries are not satisfactory. The researcher underlined the understanding of the advantages of the world market in India and Bangladesh and suggested, to improve the quality, the diversification of the products from this natural fiber. Kalita and Bhuyan (2013) said that jute is mainly produced in three countries in southern Asia, namely India, Bangladesh and Nepal. Over 90 percent of global jute is grown in India and Bangladesh. Jute is a vital sector in the agricultural, industrial and commercial context of both countries. It provides livelihoods and food security for the weaker part of society. About 4 million farmers and 2.60 lakh workers in India and 1.1 million families, another 0.3 million people in Bangladesh are engaged in the jute sector for a living (Changdee *et al.,* 2008; Islam and Alauddin, 2012).

### 2.2.4 General characteristics of *Corchorus capsularis*

Khatun and Sobhan (1985) conducted an experiment at the Bangladesh Jute Research Institute, Dhaka, on improved cultivars of *Corchorus capsularis L.* to study the variability and correlation of the characters for the yield potential. Wide ranges of variability for seven characters were observed in the studied materials. Correlation studies have shown a strong association between fiber yield and plant height, base diameter, bark weight and bar weight independently in all cultivars and also between fiber and seed yield, except CC-45 (Sahoo *et al.*, 2003). The partial correlation analysis indicated that the fiber yield depended more on the plant height in C1 and the base diameter in D-154, C2, C5 and CC-45, but in the case of the fiber yield C3, C4, C6, CVL-1 and CVE-3 depended on both characters. The multiple correlation revealed that the combined effect of plant height and base diameter maintained a highly significant correlation ($r = 0.71 - 0.98$) with the fiber yield in all cultivars.

## 2.3 Economic significance of jute

### 2.3.1 Economic importance of jute in the world

Thigpen and Akiyama (1986) conducted an experiment about prospects for the world jute industry. Due to its economic importance, the globe jute industry until the mid-1990s is being analysed to identity trends which may follow the confusion of the jute market caused by the 1984/85 fiber supply crisis. This importance derives from the analysis of the knowledge obtained from the surveys on the applying of jute in numerous countries and from the results of the simulation of a recently constructed econometric model of the planet economy of jute. Jute fiber is principally used as a textile staple for the manufacture of packaging products, carpets, industrial fabrics and wire. The rapid loss of the markets for sack and jute bags because of the movement of huge quantities in industrial countries and developing cereal exporters within the 70s is style of over. Losing the textile markets where synthetic products have several advantages in terms of technical performance or costs (Anderson *et al.*, 1980).

### 2.3.2 Economic importance of jute in Bangladesh

Islam and Ali (2017) conducted an experiment about economic importance of jute and its production, achievement and diversification. Jute was found cultivated in Bangladesh almost exclusively as a rain-fed crop with none irrigation or system. Jute status as a cash crop in Bangladesh was not entirely satisfactory. Numerous people in Bangladesh relay on all matters associated with jute collection. Lack of adequate government policy on jute, lack of jute production, random closings of jute factories, lack of modernization of the cultivation system and production units, mismanagement and neglect, come by demand for jute on the market throughout the world, using balternative sources for jute, etc. In Bangladesh, problems were encountered within the event of jute fiber. Adequate government policy could solve the problems in Bangladesh's jute sector.

Bangladesh produces the foremost effective quality jute within the planet. Within the past it’s contributed strongly to the economy of Bangladesh, therefore it’s been considered the "golden fiber" of Bangladesh. But with the growing use of synthetic fiber, the jute industry has lost its glory. Bangladesh is that the second country within the world for jute production. The increased demand for natural fibres creates a market opportunity for jute. Especially within the geotextile, automotive, infrastructure and packaging sectors, there’s a bright future prospect for jute. With the most effective economic opportunity plus environmental benefits, the jute industry will expand and build more job opportunities. Already in Bangladesh, nearly 25 million people are directly and indirectly related to the jute industry. Therefore, there is a social influence of jute for the people associated with it (Jahan, 2019). The social sustainability of the farmer depends on the successful cultivation of jute. Local entrepreneurs also gain social access by becoming independent and secure.

### 2.4 Abiotic stresses

### 2.4.1 Abiotic stress

Athar and Ashraf (2009) conducted an experiment about the approaches for crop development along side salinity and drought stress . Abiotic stresses are of several types. Singhal *et al.,* 2015 conducted an experiment about plant abiotic stress including a potential approach to exploit promoters as asubstitute of getting rid from the increasing burden. Plants are sessile by evolution to adapt and tolerate various stresses. They need evolved with every alternative strategy to house severe abiotic stress through an interesting degree of developmental plasticity. Understanding the mechanism of the genes answerable for adapting plants to the environment will help predict the scenarios, expanding the genetic aspect of abiotic stress regulatory genes to guard and extrapolate the amount of tolerance or vulnerability conferred in natural ecosystems. Completing the agronomic need for greater abiotic stress tolerance, studying the response to abiotic stress from plants can help obtain information on plant biology which will be practically applied to unlock secrets to boost productivity of plants to feed the growing human population (He *et al.*, 2018).

### 2.4.2 Influences of drought stress on crops

Basu *et al.* (2016) conducted an experiment about plant reworking to drought stress . Plants adapted to drought strain within the environment are foreseen through variety mechanisms, staring from transient responses to low soil moisture and plants selected by man are expected to supply products like wheat, vegetables or fruitlet in positive surroundings by great levels of water and fertilizers produce an affordable product in response to inputs. Cultivated plants selected for his or her economic performance must survive drought stress through mechanisms that maintain the harvest.

### 2.4.3 Effect of salinity stress on crops

Parihar *et al*. (2014) directed a research about the result of salt strain on plants and its acceptance. Stresses caused by environment is a crucial field of scientific attention since it limits the productivity of plants and crops. This example was further aggravated by anthropogenic activities (Sriram and Tyaji, 2004) For this reason, researchers have a lot of scientific work to boost crop productivity under environmental stress to address the growing demand for food. In step with an FAO estimate, more than 6% of the lands throughout the world are stricken by salinity. Salt stress is a crucial important limit for the productivity of plants and crops (Yokoi *et al.*, 2002).

#### 2.4.4 Effect of metals/metalloids on crops

Gill (2014) conducted an experiment about HMs stress in plants. HMs like Cd, Cu, Pb, Cr and Hg are the utmost environmental pollutants. Trace metals often induces the synthesis and accumulation of the identical defence-related secondary metabolites. This known discovery still awaits an evidence of the common characteristics of both patterns of stress. ROS generation could be a common occurrence in both heavy metal treatments (Mithofer *et al.*, 2004).

#### 2.4.5 Influences of of water logging strains on crops

Ashraf (2012) conducted an experiment about waterlogging stress in plants and found thet this strain cause severe damage to plant growth. Furthermore, disturbances in physiological mechanisms can influence carbohydrate stores and translocations. Indeed, flood tolerant and sensitive plant species will be discriminated against supported their efficient use of carbohydrates. Akhtar and Nazir (2013) conducted an experiment about effect of waterlogging and drought stress in plants. Crisis of water is that the most serious problem for agriculture throughout the world. When the water is present in quantities greater than its optimal requirement, it means to the water register. In soils impregnated with water, water fills within the pores of the soil, therefore the oxygen concentration within the soil decreases. O deficiency reduces the expansion and survival of plants that grow in floods and infrequently induce stoma closure mainly in C3 plants.

### 2.5 Jute (*Chorchorus capsularies*) reactions and forbearance to abiotic pressures

#### 2.5.1 Jute (*Chorchorus capsularies*) for bearance to drought stress

Significance decrease in the transpirationrates, chlorophyll amounts and total photosynthetic occurs in plants subjected to water deficiency. Plants accumulated higher amount of proline and soluble sugars that submitted to 40% FC than the controls ones. By developing the osmo-regulators objects, increasing roots and decreasing leaf size tossa jute seedlings make tolerance against water deficiency

(Sharmin *et al.*, 2011; Yakoub *et al.*, 2016) A specific form of water deficiency stress condition was named as dehydration stress.

### 2.5.2 Jute (*Chorchorus capsularies*) for bearance to salt stress

Yang *et al.* (2017) directed a research on transcriptome sequence of two jute species under salinity stress condition. They drive high-through put transcriptome sequence of 24 *C. capsularis* and *C. olitorius* under salt stress condition. After the persistent salt stress, jute seedling will adapt with salt stress by accelerating ROS scavenging, altering signal transduction, lipid metabolism and cell wall metabolism, enhancing nucleotide metabolism and altering cytoskeleton in roots. Ali *et al.* (2003) conducted an investigation on plant biomass production and some biochemical parameters of two soyabean genotypes, such as soyabean 95-1 and Ertou no 2 with the effect of four different level of salinity of four different levels i,e control, 3.0, 4.5, 6.5 dS/m. It was found that plant biomass production and plant height had a great reduction with the salinity induction. Shazia *et al.* (2001) observed the effect of Indole-3-Acetic Acid as foliar application.

### 2.5.3 Jute (*Chorchorus capsularies*) for bearance to metal/metalloids stress

Saleem *et al.* (2020) conducted an experiment in pots and used four different varieties of jute and found *C. capsularis* has high resistance against different metallic salts. However, growth and yield of jute species reduced significantly due to Cd strins.

### 2.5.4 Jute (*Chorchorus capsularies*) forbearance to waterlogging stress

Bashar *et al.* (2019) conducted an experiment to study about strategies in plants under flooding stress. Flooding causes waterlogging or submergence stress that severely hinders plant growth and development.  Plant tries to survive under such unfavorable conditions by adapting with different mechanisms (Changdee *et al.*, 2008)

## 2.6 Jute responses (*Chorchorus capsularies*) forbearance to oxidative stress

Islamit *et al.* (2014) investigated the phytotoxicity of chromium (Cr) and arsenic (As) in jute plants. The phytotoxicity is observed in- plants development, chlorophyll innards, total photosynthesis, oxidative strain, antioxidant enzymes work in jute species. Under greenhouse condition two different varieties of jute such as, O-9897 (Cr-sensitive) and O-795 (Cr-tolerant) are produced at different amount of As and Cr in pots. Cr or As stress significantly decreases plants growth, rate of photosynthesis, chlorophyll florescence, chlorophyll concentration and occurs oxidative damage with comparing to control, then increased MDA and $H_2O_2$ contents (Hassan *et al.,* 2016).

# CHAPTER-3
# MATERIALS AND METHODS

This chapter demonstrates an ephemeral explanation regarding the period of experimentations, locations, meteorological conditions, seed or planting resources, actions, investigational strategy and arrangement, harvest rising process, fertilizer application, intercultural processes, data gathering and statistical investigation of the experiment.

## 3.1 Locations

**Experiment-1:** The lab experiment to study the germination percentage under different abiotic stresses was conducted at the central laboratory of Sher-e -Bangla agricultural university during the period from April 2019 to June 2019.

**Experiment-2:** The field experiment were conducted at the experimental shed of crop science laboratory at the Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka during the period from April 2019 to June 2019 to study the morpho-physiological responses under different abiotic stresses

## 3.2 Properties of Soil

The soil of experiment-1 belonged to the Modhupur tract. The soil was collected from medium high land and well prepared under the shed house. Properly fertilized sandy loam soil was used. The pH value of the soil was 5.6. The lab experiment was conducted in sandy loam soil with same pH.

## 3.3 Meteorolgical situation of the study site

The area of experiment-1 was under the subtropical climate and was characterized by high temperature, high humidity and heavy precipitation with occasional gusty winds during the period from April to June.

## 3.4 Materials

### 3.4.1 Plant materials

Only one Jute variety Shabuj pat (CVL-1) was used in both experiments. This variety was released in 1977. Plant height is more than 2m, seeds are dark colored and flower color is yellow. Fruits are round and fiber is white in color. Crop Duration is 120-130 days with a fibre yield of 5.16t/ha.

### 3.4.2 Plastic pots

In experiment-2 empty plastic pots with 18 inch depth and 14 inch diameter with 12 liter capacity were used. Twelve kilograms well prepared sun-dried soils along with organic manures and fertilizers were put in each pot. After that, pots were prepared for seed sowing.

### 3.4.3 Earthen pots

In experiment-1 small earthen pots were used. Sun dried sandy soil after washing was put in each pot. After that germination test was performed.

## 3.5 Treatments

**Experiment-1** consisted of following six treatments:

1. Control
2. Mild stress, $S_1$: 50 mM NaCl
3. Severe stress, $S_1$: 75 mM NaCl
4. Salt stress, $S_{3:}$ 100 mM NaCl
5. Cadmium stress, $Cd_1$: 0.50mM $CdCl_2$
6. Cadmium stress, $Cd_2$ :1 mM $CdCl_2$

**Experiment-2** consisted of nine treatments as follows:

1. Control (C)
2. Mild salt stress, $S_1$: 200 mM NaCl
3. Severe Salt stress, $S_2$: 400 mM NaCl
4. Drought stress, $D_1$: 10 days of water deficit condition till 25DAS
5. Drought stress, $D_2$: 15 days of water deficit condition till 30 DAS
6. Cadmium stress, $Cd_1$: 2 mM $CdCl_2$
7. Cadmium stress, $Cd_2$: 4 mM $CdCl_2$
8. $WL_1$: 5 days of waterlogged condition at 15 and 30 DAS
9. $WL_2$: 20 days of continuous waterlogged condition till 35 DAS

At 15 DAS all the stress treatments were applied on seedlings. Morphological data were started taken 5 days after treatments imposed and biochemical assessment was done after 10 days of stress treatments.

### 3.6 Experimental design

Both the experimentations were performed in a Randomized Completely Block Design (RCBD). Each experiment was conducted at least tree times.

In experiment-1 there were 18 pots for measuring germination parameters and $2^{nd}$ set with 27 pots for measuring different parameter.

### 3.7 Seed collection

Seeds of deshi jute cv. CVL-1 were collected from Bangladesh Jute Research Institute (BJRI), Manik Mia Avenue, Dhaka 1207. Seeds from same lot were used in both experiments.

### 3.8 Pot preparation

In experiment-1, sand was washed with water thoroughly and then sun dried. After that earthen pots were filled with clean and dry sand.

In experiment-2 the collected soil was sun dried, crushed and sieved. The soil, organic manure and manures were assorted well before insertion the soils in the pots. Each pot was filled up with 12 kg soil. Then the pots were placed at the Crop Science Laboratory of Sher-e-Bangla Agricultural University.

### 3.9 Fertilizer application

In the experiment-2, organic manure and the fertilizer doses are given as follows:

**Table 3.1** Manure dose during pot preparation

| **Manures** | **Dose (Kg/ha)** | **g/pots** |
|---|---|---|
| Organic manure | 5000 | 3 |
| Urea | 20 | 2.5 |
| Triple super phosphate | 20 | 3.2 |
| Muriate of potash | 20 | 1.0 |

### 3.10 Seed sowing technique

In experiment-1, 20 seeds were placed in each earthen pot. In experiment-2 seeds were broadcasted maintaining the seed rate 1.5g/pot.

### 3.11 Intercultural operations

#### 3.11.1 Thinning

After sowing seeds were kept in regular observation. In experiment-2 thinning was done to maintain the desired plant population and spacing. First thinning was done at 8 DAS and second one at 14 DAS and keeping 12 seedlings per pot.

#### 3.11.2 Weeding

Some unwanted plant observed in pots which were uprooted manually.

#### 3.11.3 Irrigation

In experiment-1, 140 ml of relevant treatments were poured in each earthen pot. In experiment -2 treatments were started to given as control, salt stress (200 and 400 mM NaCl), Cadmium stress (2 and 4 mM $CdCl_2$) to impose stress condition at 15 DAS seedlings. Similarly, 2$^{nd}$, 3rd, 4th, 5th treatments were given at 20, 25, 30 and 35 DAS, respectively. In waterlogging treatments, waterlogged condition was started 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 till 35 DAS. In drought stresses after maintaining 10 and 15 days of water deficit condition first irrigation was given at 25 DAS in first drought stress and 30 DAS in second drought stress respectively. In each pots 1L water were given.

### 3.11.4 Plant protection measure

The fungicide Ridomild gold was supplemented in seedling conditions two times with a seven days interval in order to prevent the root rot disease. Another fungicide, malathion was applied in seedlings to control leaf curling disease.

## 3.12 General observation of the experimental pots

During the intercultural processes, continuous monitoring of experimental pots was performed regularly to find out the apparent differences among the treatments as well as to prevent the plant from various types of insects, pests and diseases.

## 3.13 Collection of data

In Experiment- I, the number of germinated seeds was recorded till 4th days after sowing and at 10 DAS the survivability percentage (SI) of seedlings was also counted. At 15 DAS growth parameters were taken.

**Procedure of taking germination parameter**

### 3.13.1 Germination percentage (GP%)

The germination percentages of seeds were determined by the following equation (AOSA, 1990; Redondo Gomez et al., 2007; Cokkizgin and Cokkizgin, 2010; Tanveer et al., 2010). Seed germination was considered when there was radical porpagets through the seed coat (Redondo Gomez et al., 2007). Five days after germination, the germination percentage (GP) was obtained by dividing the number of germinated seeds in any pot by the total number of seeds, multiplied by 100.

GP (%) = Total number of germinated seeds /total number of seeds sown for germination x 100.

### 3.13.2 Mean germination time (MGT)

The mean germination time (MGT) was calculated to assess the rate of germination (Ellis and Roberts, 1981). MGT was calculated by (Nichols and Heydecker, 1968) as follows :

MTG = $\sum$(ni x di)/N. where ni is the number of seeds germinated at day i, di is the incubation period in days, and N is the total number of germinated seeds. (Brenchely and Probert, 1998; Redondo-Gomez et al., 2007).

### 3.13.3. Root-shoot ratio

Root-shoot proportion was counted by using the following formula:

Root-shoot ratio = Root length (cm) /Shoot length (cm)

**3.13.4. Vigor index**

Vigor index was calculated by the folllwing method (Abdul-Baki and Anderson, 1973).

Vigor index = Germination percentage× Seedling length

**3.13.5 Survivability%**

At 10DAS total number of survived seedlings was counted from each pot then percentage was calculated.

**3.13.6 Vigor value**

Vigor value was calculated by using the following formula:

[(a /1) + (b /2) + (c /3) + (d /4) + (e /5)]

Here a, b, c, d and e were the number of seeds germinated at $1^{st}$, $2^{nd}$, $3^{rd}$, 4th and $5^{th}$ day of germination test respectively.

**3.13.7 Co-efficient of velocity germination**

Coefficient of velocity of germination (CVG) was evaluated according to Maguire (1962) as follows:

$100(A_1+A_2+A_3+A_4) / (A_1T_1+A_2T_2+A_3T_3+A_4T_4)$

Here, A= Number of seed germinated ($A_1$-$A_4$), T= Day of germination ($T_1$-$T_4$)

**3.13.8 Timson germination index (TGI)**

Timson (1965) proposed an index of germination based on the progressive total of daily cumulative germination over 10 d (hence SlO) with supple-mentary totals over other periods being quoted as desired (e.g. ∑5 for 5 d, ∑ 20 for 20 d, and so on).

TGI= ΣG/T, where G is the percentage of seed germinated per day, and T is the germination period.

**3.13.9 Mean germination rate (MGR)**

Mean germination rate was calculated by using following formula

CV/100 = 1/T; where T is mean germination time and CV: coefficient of velocity.

At $4^{th}$ day of germination number of normal seedlings, abnormal seedlings and were counted.

In Experiment-II growth and morphological parameters were collected at 21, 28 and 35 DAS where biochemical parameters were collected at 26 and 36 DAS.

Data were collected on following sequences:

**3.14.1 Crop growth parameters**:

1. Plant height
2. Above ground fresh weight plant-$^{1}$
3. Above ground dry matter weight plant-$^{1}$
4. Stem diameter

**3.14.2 Physiological parameters**:

1. SPAD value of leaf
2. Relative water content (RWC)
3. Electrolyte leakage

**3.14.3 Oxidative stress indicators**

- Lipid peroxidation
- $H_2O_2$ content
- Activities of antioxidant enzyme (CAT)

**3.15 Procedure of sampling for growth study during the harvest progress period**

**3.15.1 Plant height**

Plant height was measursed three times at 21, 28 and 35DAS with the aid of a scale.

**3.15.2 Fresh weight plant-$^{1}$**

The uprooted plants were weight up in a balance and averaged them to have fresh weight plant-1.

**3.15.3 Dry weight plant-$^{1}$**

Three sample plants after weighing for fresh weight was dried them in an electric oven maintaining 60 °C for 72 hours. Then the plans were weighed in an electric balance and averaged them to have dry weight plant-1. The data were collected after completion of treatment duration.

**3.15.4 Stem diameter**

Three sample plants from each pot were taken to measure the diameter of stem of 35 DAS of seedlings by using the measuring tape. The average diameter of three stems was quantified as the diameter of stem of plant of each pot.

### 3.16 Procedure of sampling physiological parameters

#### 3.16.1 SPAD value

Five leaves were selected randomly from every single pot. The LEAF (FT Green LLC, USA) as at LEAF value was used to measure the top middle and bottom of each leaflet. Then it was averaged and entire chlorophyll content was calculated by the conversion of at LEAF value into SPAD units and then total chl content was also measured.

#### 3.16.2 Relative water content

Relative water content (RWC) was measured according to Barrs and Weatherly (1962). Relative water content was calculated using the following formula:

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

#### 3.16.3 Measurement of electrolyte leakage of Jute leaves

In order to quantify the percentages of electrolyte leakages, 30 DAS of seedlings were used. Relative electrolyte leakage (%) was determined according to the Dionisio-Sese and Tobita (1998) method using the following equation:

$EL = EL_1/EL_2 \times 100$

### 3.17 Procedure of measuring oxidative stress indicators

#### 3.16.1 Measurement of lipid peroxidation

According to Heath and Packer (1968), the lipid peroxidation was quantified by calculating malondealdehyde (MDA) content with slight modification by Hasanuzzaman*et al.* (2012b).

#### 3.17.2. Determination of $H_2O_2$

For Hydrogen peroxide ($H_2O_2$) assessment, the method of Yu *et al.*, 2003 was followed.

#### 3.17.3. Enzyme extraction and assays

The enzyme extraction and catalase (CAT; EC: 1.11.1.6) action was assayed following the modified method of (Hasanuzzaman *et a*l., 2012b) by indentifying the reduction of absorbance at 240 nm for 1 min resulting from the decomposition of $H_2O_2$ with a UV-vis spectrophotometer.

### 3.18 Statistical analysis

The data acquired in the present study at different experimental stages were analyzed by using the software XLSTAT 2016 (AddinSoft, 2016) and mean values were obtained by Least Significant Difference (LSD) at 5% level of significance.

# CHAPTER-4

# RESULTS AND DISCUSSION

## Experiment I

## 4.1 Germination parameter

### 4.1.1 Germination (%)

Germination is a vital stage to begin life cycle of plant and this also the most sensitive to any kind of stress. The germination process involves water imbibition, enzyme initiation, hydrolysis of reserved factual, beginning of development, fracture of coating in seed and seedling. In the present study, germination percentages of *C.capsularis* seedlings were decreased markedly under different abiotic stresses like salinity and cadmium stress. Germination was high at control (90%) and low (50 mM) concentration (80%) of salt. Excessive saline concentration i.e. 75 and 100 mM permitted only 75 and 60 % germination, correspondingly. Cadmium had rigorous effects on germination of *C.capsularis* seedlings. Germination decreased significantly at all stages of Cd stress, reaching between 27% at 0.5 mM Cd to 33% at 1.0 mM Cd, comparing to the control. Seed germination depends on stored food material utilization of seed. Water absorption process in seed inhibited by salinity. This later prohibits the hydrolysis in seed stored food which eventually inturrupts and diclines germination of seed (Begum *et al.,* 2010). Purhpan and Rangasamy (2002) observed that seed germination is significantly pretentious by the upsurge in brine content. Salt stress from 50-150 mM NaCl provided progressive gradual reduction in germination percentage by the upsurge of concentration, comparing with the control (fig.1). This results support (Naik *et al.,* 2015) in deshi and tossa jute where salt stress inhibits the germination process. These results recognized different studies, indicating that halophytes are salt sensitive at germination phase (Khan *et al.*, 2002). Germination of seed under stress of Cd could be reduced because of excessive interruption of stored food in seed embryo. These results are agreed with previous report (Raziuddin *et al.*, 2011; Aydinalp and Marinova, 2009; Junyu *et al.*, 2008; Titov *et al.*, 1996), stated that Cd stress reduced germination of seed in different crops. In this study, with increasing Cd stress, the germination percentage of the seeds dropped dramatically. A parallel decrease in the germination of other seeds imperiled to Cd stress was reported by Pandit and Prasannakumar (1999) in sorghum (Hu *et.al.,* 2014) in wheat seeds.

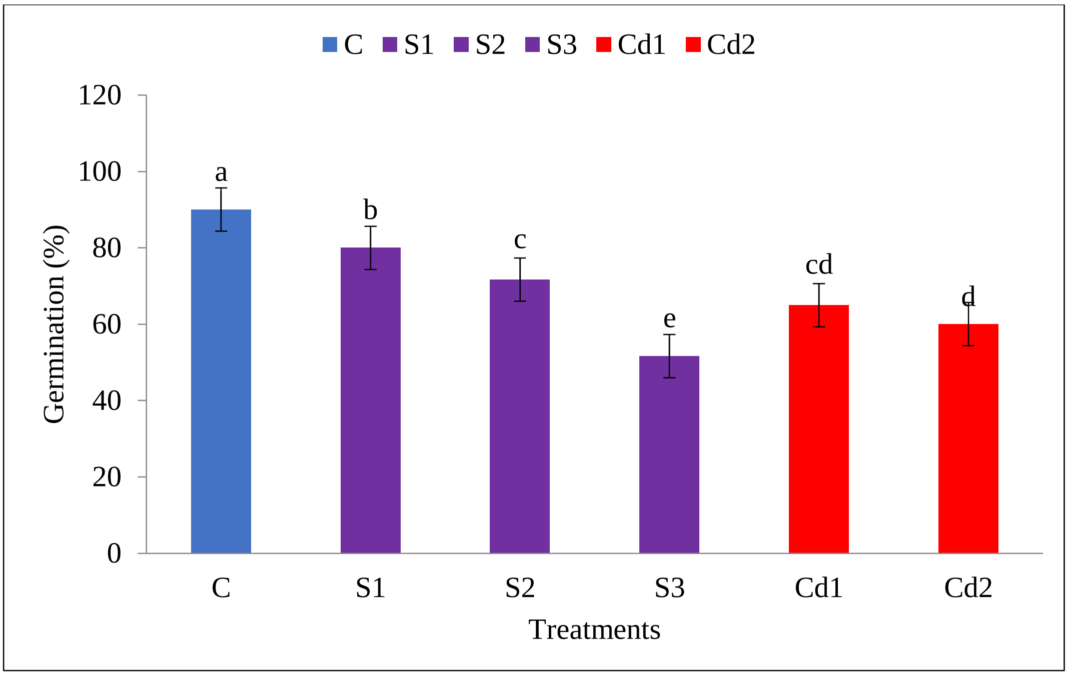


**Figure 1. Germination percentage of *C. capsularis* plants affects by different abiotic strains. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test. *S= Salt stress, *Cd=Cadmium, *C= Control**

### 4.1.2 Root shoot ratio

In this experiment, root shoot ratio was significantly decreased under salt stress. With the increase of salinity level root shoot ratio remain statistically similar. However, it was decreased by 13,16 and 19% at 50, 100 and 150 mM NaCl – induced salt stress over respective control. Otherwise, root shoot ratio decreased at moderate Cd stress.

Excessive applications of salts interrupt the capability of roots to remove water and extreme salts inside the plant themself is considered detrimental (Jaiswal *et al.*, 2014) for example uptake of nutrients. These effects decrease growth of plant, development and existence (Munns and Tester, 2008). Figure 2 comparing the salinity effects according to shoot/root ratio. Ghorbanpour *et al.* (2011) reported about the salinity stress on root shoot ratio of fenugreek seed. Stimulation of root and shoot length with increasing salt levels has been documented in fibre species, including *Hibiscus sabdariffa* (Moosavi *et al.*, 2013). Cd had adverse effects on length of root and shoot, which are excellent indicator for evaluating metal toxicity. (An, 2004; Correa *et al.*, 2006; Ahmad *et al.*, 2008; Jun-yu *et al.*, 2008; Ahmad *et al.*, 2011b). The results showed that Cd reduced the root and shoot length and ultimately reduced the root shoot ratio. It was observed in another crops like wheat by Ahmed *et al.*, 2012.

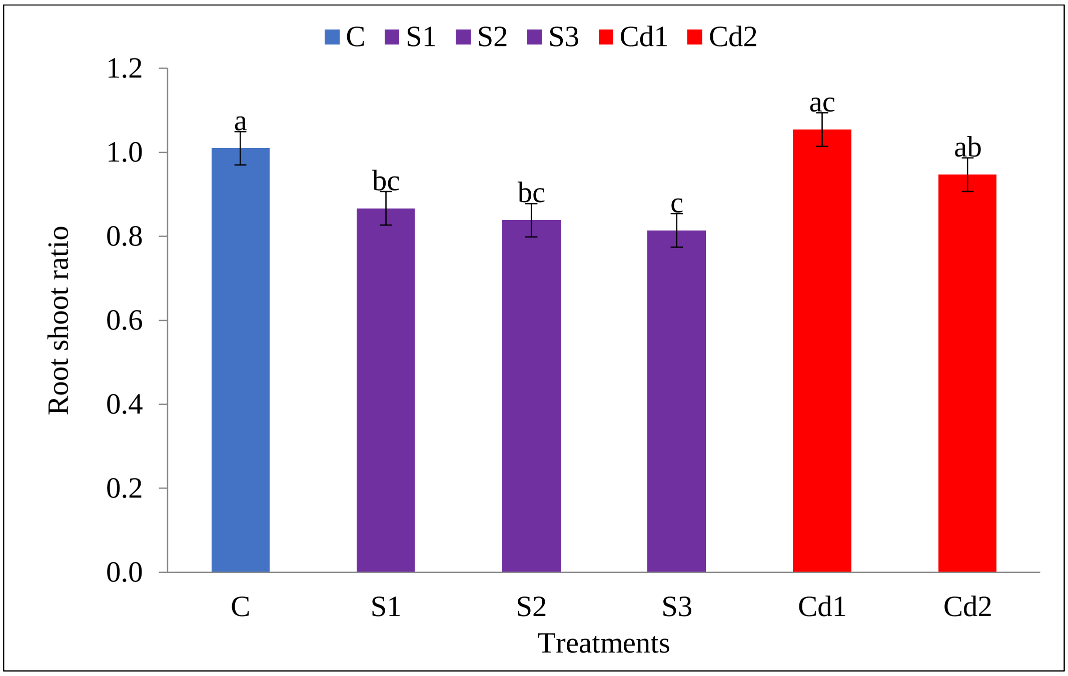


**Figure 2. Root shoot ratio of *C. capsularis* plants affected by different abiotic stresses. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.**

### 4.1.3 Vigor index

Vigor index reduced remarkably at different abiotic stresses like salinity and cadmium stress. Vigor index decreased under salt stress and with the increase of salinity level it reduced more significantly. Minimum Vigor index was recorded at 100 mM NaCl. Vigor index decreased by 11, 24 and 49% at 50, 75 and 100 mM NaCl – induced salt stress. Cd stress also reduced vigor index which was 34 and 39% at 0.5 and 1 mM $CdCl_2$. The harmful effects of salinity were again recognized by evaluating vigor index. With the decline in percentage of germination and seedling length in *C. capsularis* plant vigour index demonstrated a major dicrease pattern under salinity stress (Fig. 3). Hoque *et al.* (2014) also find out the same results in maize seed. Therfore, it is obvious that the germination (%), shoots and root distance demonstrate a constructive and noteworthy correlation with vigor index. Under Cd stress vigor index was markedly decreased with the increase of Cd concentration (fig. 3). These results are agreed with earlier report (Raziuddin *et al.*, 2011; Aydinalp and Marinova, 2009; Junyu *et al.*, 2008; Titov *et al.*, 1996; Keshavarzi *et al.*, 2011; Cokkizgin, 2012). Those reports stated that vigor index of several crops decreased with Cd stress.

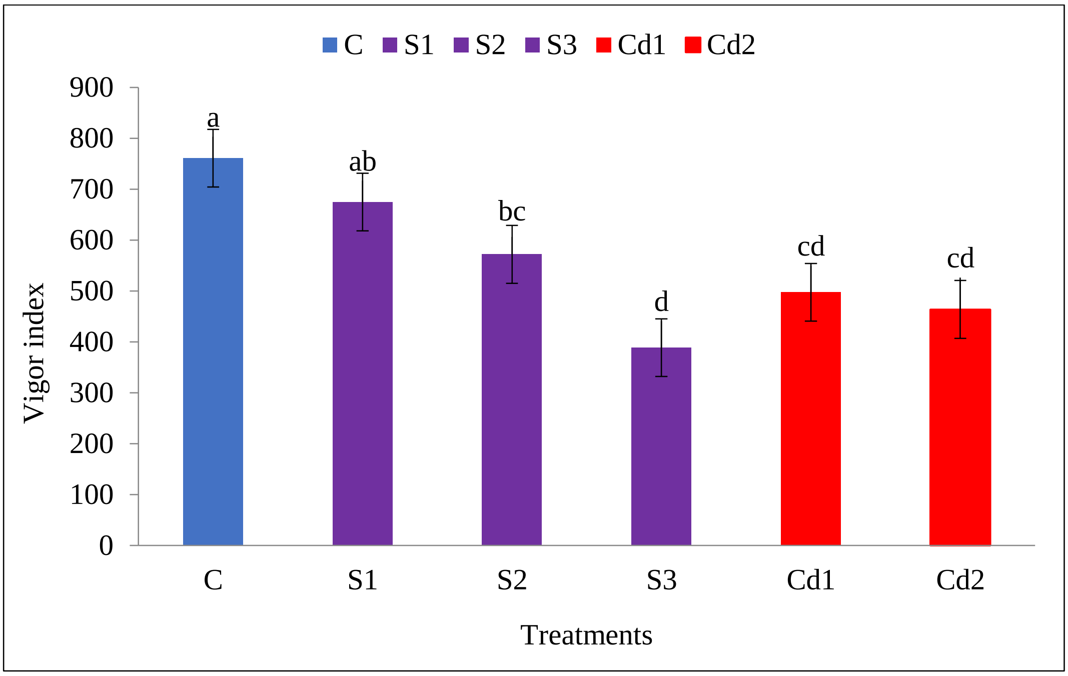


**Figure 3. Vigor index of *C. capsularis* plants affected by different abiotic stresses. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.**

**4.1.4 Survivability (%)**

Abiotic stress has detrimental effects on survivability of germinated seed where survivability of germinated seeds decreased with the increase of stress condition (Fig. 4). Upon exposure to salt stress survivability of seedlings decreased gradually with the increase of salinity level. Minimum percentage of survivability was recorded at 100mM NaCl condition. Survivability percentage was reported 7% and 15 % at 50 and 75 mM NaCl. Upon exposure to Cd stress survivability percentage was decreased by 7% and 14 % under 0.5 and 1mM $CdCl_2$ over respective control.

In this experiment, the survivability during germination time of the treated seeds declined with increasing dosages of treatments. The lowest laboratory germination prefers lowest survival seedling was recorded in 100mM NaCl whereas highest germination with highest survival seedlings was recorded in control. Cd due to its metal toxicity showed detrimental effect on survivability percentage.

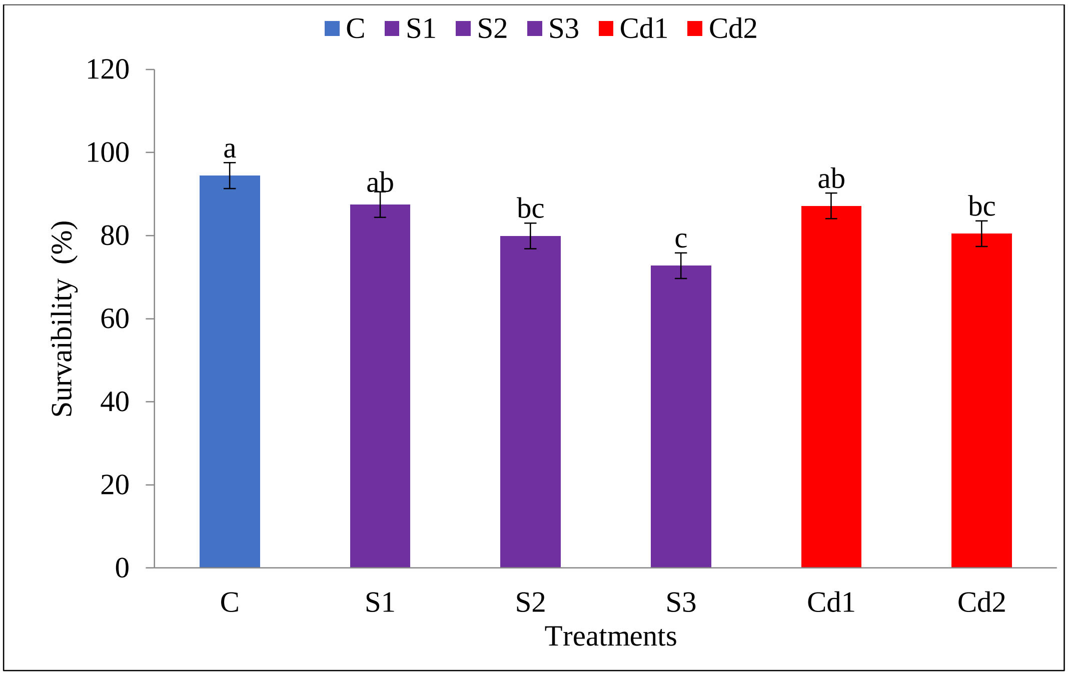


**Figure 4. Survivability % of *C. capsularis* plants affected by different abiotic stresses. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at p ≤ 0.05 applying LSD test.**

### 4.1.5 Vigor value

In this study, vigor value was markedly decreased in all doses of stress treatments (Fig 5). With the increase of salt stress, vigor value reduced by 33, 42 and 54% at 50, 75 and 100 mM NaCl and 40 and 55% at 0.5 and 1mM Cd stress, respectively corresponding to control.

The shoot and root growth was inhibited by salinity stress (Fig. 5). The extent of decrease was recorded highest under greatest salinity intensities. Similar result was observed (Jaleel *et al.*, 2007; Xiong and Zhu, 2002) in *Catharanthus roseus.* Cd stress has detrimental effect on vigor value of *C.capsularis* seed. Vigor value was reduced with the increase of dose dependent manner in Cd stress (fig.5).

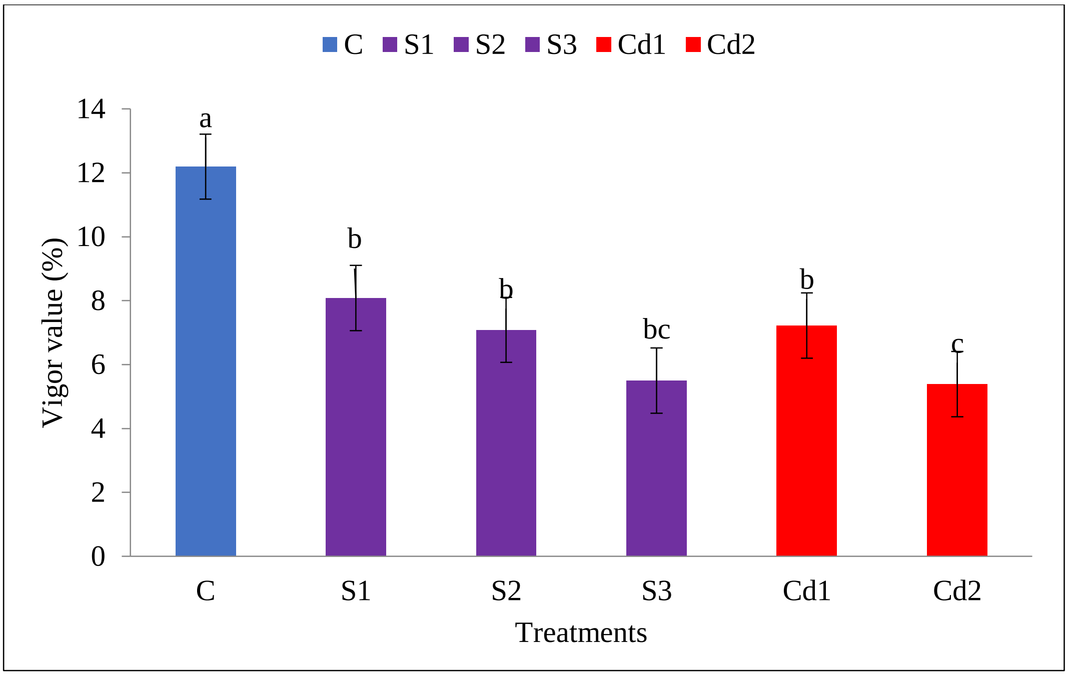


**Figure 5. Vigor value of *C.capsularis* plants affected by different abiotic stresses. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.**

### 4.1.6 Co efficient of velocity of germination

Upon exposure to different abiotic stresses Co efficient of velocity of germination (CVG) decreased sharply. With the increase of salt stress CVG reduced by 18, 22 and 24% at 50, 75 and 100 mM NaCl – induced salt stress and 13 and 24% at 0.5 and1 mM $CdCl_2$, respectively corresponding to control.

No noteworthy variances were detected amongst NaCl treatments with reverence to CVG. However, substantial reduction was noticed in CVG, based on NaCl osmotic potential compared to respective control shows the CVG at various NaCl osmotic potentials (fig.6). These results are also in agreement with those reported by Katembe *et al.*,1998. This supports the findings of Cokkijzin, 2012 in common bean. CVG decreased over respective control with the upsurge of Cd stress.

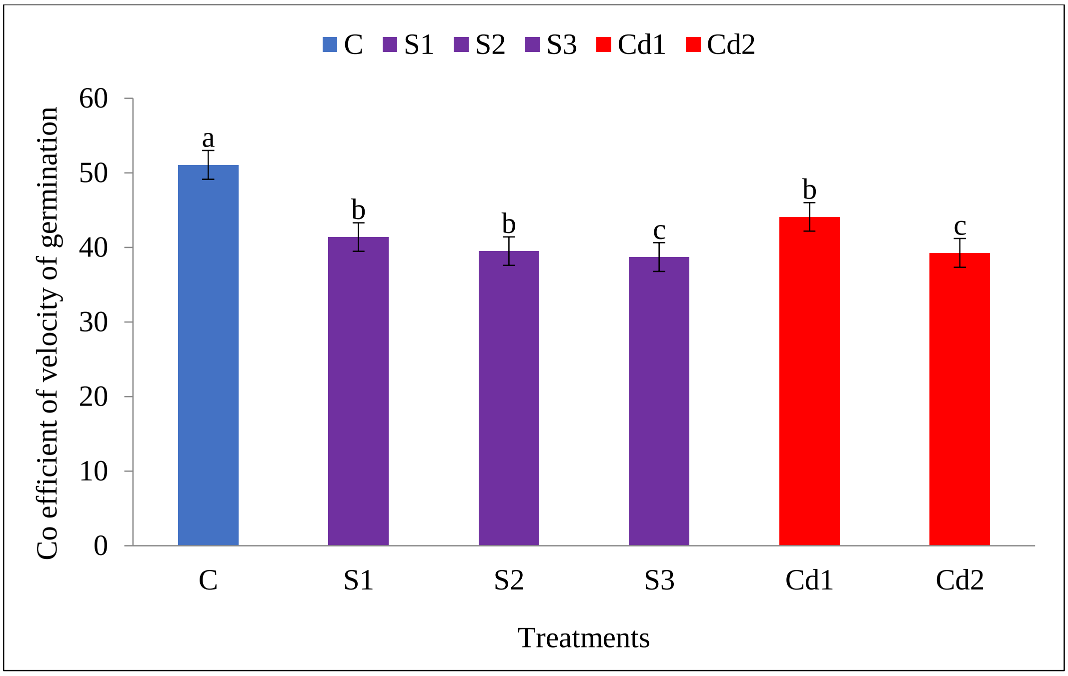


**Figure 6. Co efficient of velocity of germination of *C.capsularis* plants affected by different abiotic stresses. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.**

### 4.1.7 Timson germination index

In this study, a sharp decrease in TGI was noticed at any doses of treatments. With the increase of salt stress, TGI reduced by 18, 23 and 31% at 50, 75 and 100 mM NaCl – induced salt stress and 15 and 29% at moderate and severe Cd stress, respectively over control. Highest reduction of TGI was observed at 100 mM NaCl – induced salt stress.

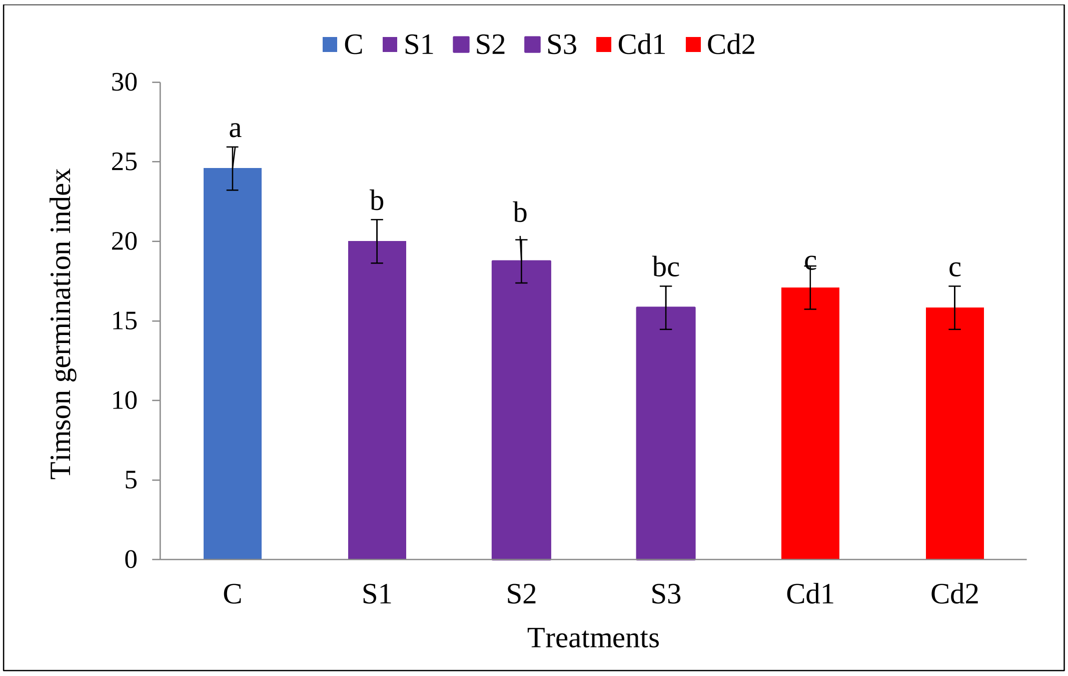


**Figure 7. Timson germination index (TGI) of *C. capsularis* plants affected by different abiotic stresses. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.**

### 4.1.8 Mean germination time

Mean germination time (MGT) of deshi jute significantly increased at all stress treatments. Due to salt stress both germination percentage and mean germination rate increased and mean germination time decreased. Upon exposure to salt stress MGT increased with the increased doses of salinity recorded by 22, 28 and 31% at 50, 75 and 100 mM NaCl. Cd stress also increased MGT by 15 and 29% at 0.5 and 1 mM $CdCl_2$.

Matthews and Khajeh Hosseini (2007) pointed out that MGT can be thought of as the mean of the lag period, for all the seeds in a sample, between the time that the seeds start to imbibe and the first sign of germination (radicle protrusion) or physiological germination (2 mm radicle). The germination period was hindered by salt stress. Increase of mean germination time (MGT) supports that germination is delayed (Hakim *et al.*, 2011). The MGT was also unfavorably pretentious by salinity stress and with the increase of salinity level MGT increased (fig.8). The similar trend was recorded by XU *et al.*, 2011. Actually, salt stress significantly affected germination percentage, (quantified by the MTG value).The results also agreed with the other findings of Hossein *et al.*, 2003) in soybean seeds ( Redondo Gomez *et al.*, 2008) in Limonium emarginatum (Hoque *et al.*, 2014) in maize (Cokkijzin, 2012) in common bean where MGT was higher in salt stress condition. The stress of Cd had extreme effects on mean germination time (MGT) which is showed in fig (8). Cd had Inhibitory effect on wheat seed germination and it was considered as prolonged MGT (Ahmed *et al.*, 2012). Other results prove that seedling growth also pretentious by Cd. Root, shoot and length of seedling are complex and indications for measuring HMs deadliness (An, 2004; Correa *et al.*, 2006; Ahmad *et al.*, 2008; Jun-yu *et al.*, 2008; Ahmad *et al.*, 2011).

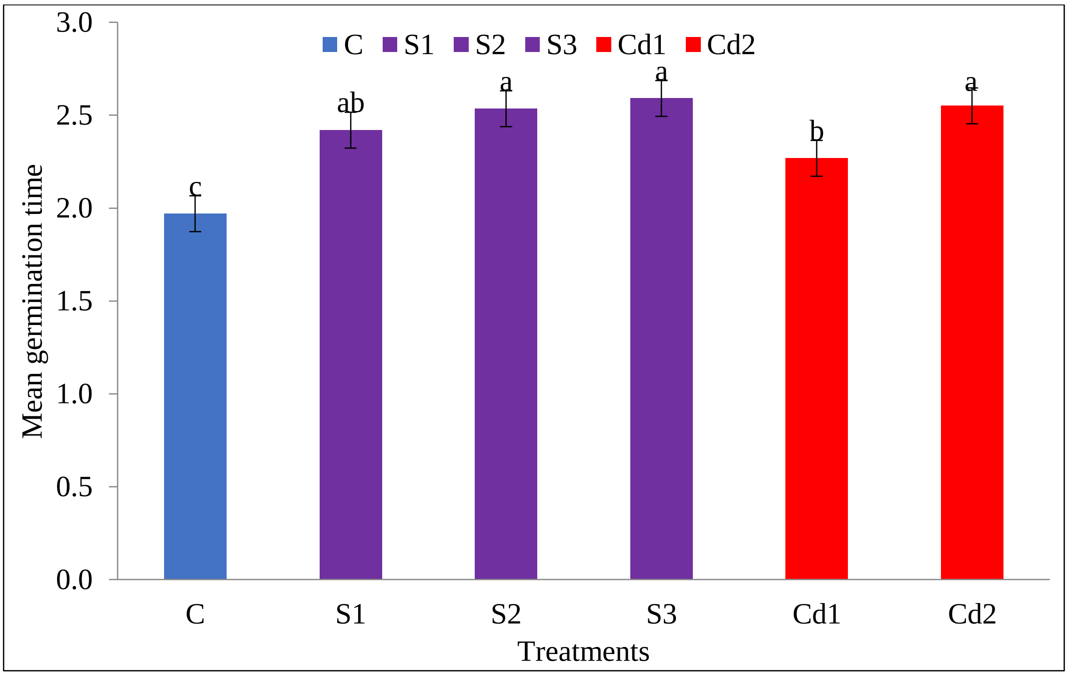


**Figure 8. Mean germination time (MGT) of *C. capsularis* plants affected by different abiotic stresses. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.**

### 4.1.9 Mean germination rate

In the present study, mean germination rate reduced markedly upon exposure to salt stress and cadmium stress. MGR decreased by 19, 23 and 25% at 50, 75 and 100mM NaCl. Cd stress decreased mean germination rate 13 and 23% at 0.5 and 1 mM $CdCl_2$. This results demonstrate reductions in germination of *C.capsularis* seeds subjected to NaCl concentrations 50,75 and 100 mM (fig.9). Similar results also observed in seeds of *C.olitorious* by Mguis *et al.*, 2014. This supports findings of Reduction in germination rate at salinity conditions was reported by other researchers (Murillo-Amador *et al.*, 2002 and Soltani *et al.*, 2006). Machado *et al.* (2004) have been observed osmotic and toxic effects of saltin germination process. This may prove adjustment of seed osmotic was exaggerated andthe stress favoured entering of another ion into the seeds. Furthermore, Smith and Comb (1991) endorsed lower content of humidity may increase salinity stress, and result in ending of metabolism or prohibited different phases in the metabolic sequence of germination. It seems that, decrease of germination percentage and germination rate is related to reduction in water absorption into the seeds at imbibition and seed turgescence stages (Mostafavi, 2011). MGR decreased as a result of prohibitory effect of Cd on germination of *C.capsularis* seed. Mean germination rate decreased by Cd stress with the increase of Cd concentration more severely over respective control.

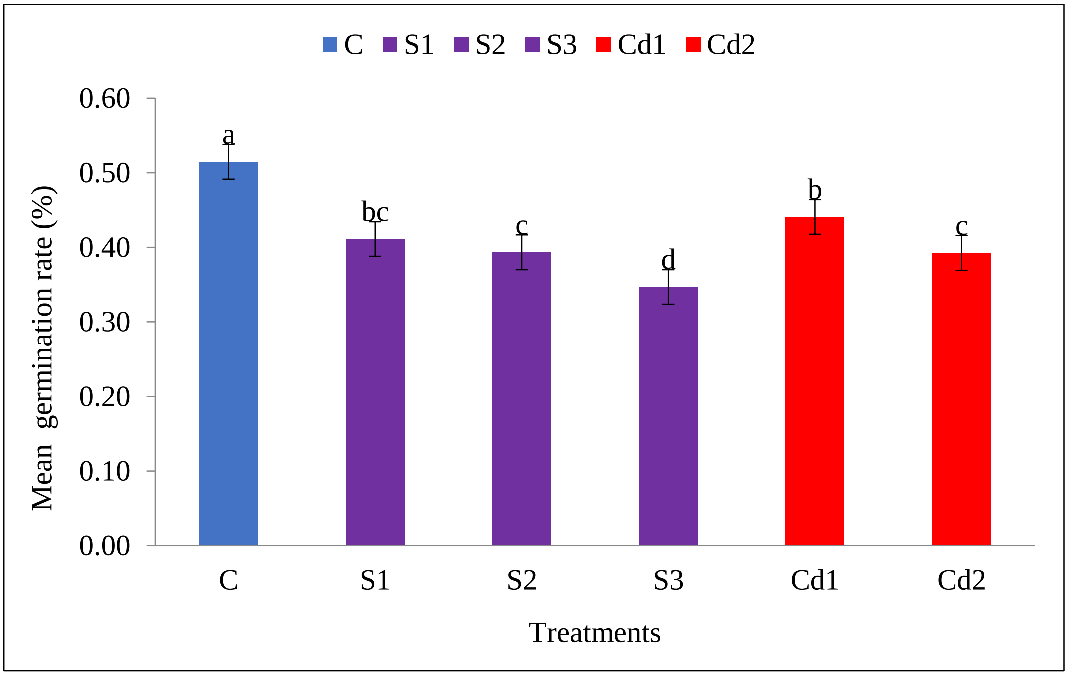


**Figure 9. Mean germination rate (MGR) of *C. capsularis* plants affected by different abiotic stresses. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.**

### 4.1.10 Normal seedling (%)

Reduction in normal seedling percentage was observed in both salt and Cd stress. However, remarkable reduction was observed in severe salt stress compared to other stresses. Minimum normal seedling was recorded at 100mM NaCl. Normal seedling

% was decreased by 17 and 34% at 50, 75 mM NaCl – induced salt stress and 18 and 43% at moderate and severe Cd stress.

The present study demonstrated that salt stress adversely affect the seedling establishment. The number of normal seedling was recorded higher in control while it was decreased with the severity of salinity increased. Minimum number of normal seedling was reported at 100 mM NaCl. Due to the inhibitory effect of Cd stress seedling establishment was hampered. Due to toxicity of Cd normal seedling establishment was reported declined under Cd stress.

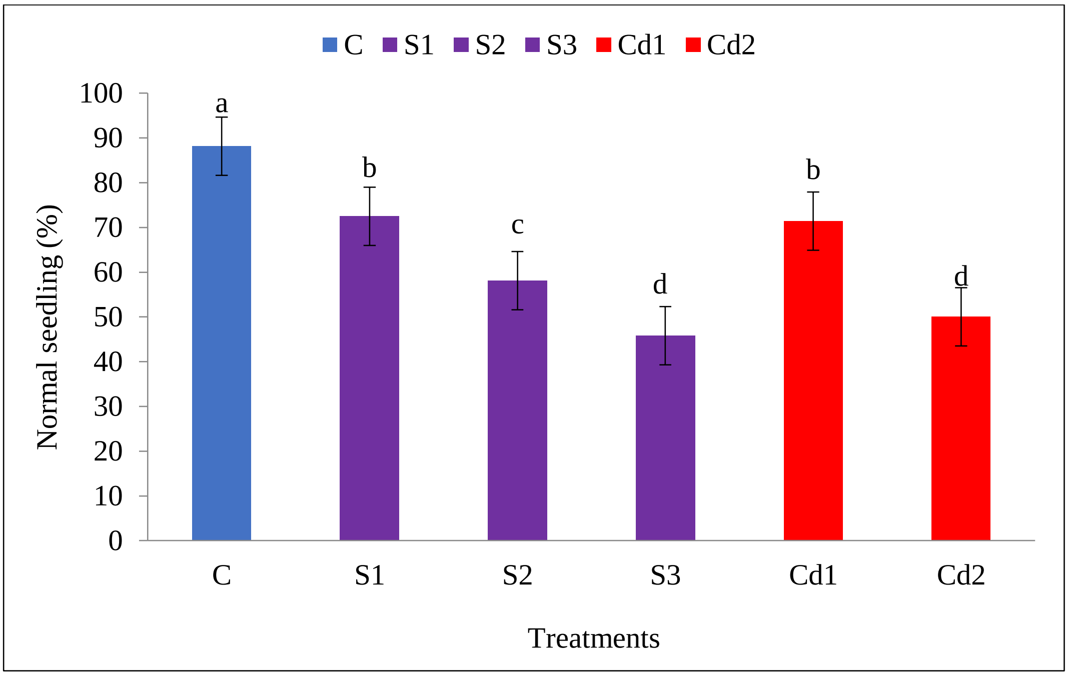


**Figure 10. Normal seedling (%) of *C. capsularis* plants affected by different abiotic stresses. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.**

### 4.1.11 Abnormal seedling (%)

Number of abnormal seedlings increased markedly under all stress treatments. However, higher number of abnormal seedlings values were noticed at 100mM NaCl while minimum number of abnormal seedlings at 0.5 mM $CdCl_2$. Abnormal seedling was recorded higher in other stress over control.

The present investigation represents salt stress badly affected germination process, emergence and production of *C.capsularis*. Salinity persuades numerous alterations in seeds and hampers germination procedure. The number of abnormal seedlings was increased with the increased level of salinity due to the detrimental effect of salinity on seedling establishment. Salinity causes significant reductions in percentage of germination and emergence of many vegetable crops, which in turn may lead to the reduction of crop yields (Yildirim *et al.*, 2002; Yildirim &Guvenc, 2006). Due to the toxic effect of Cd abnormal seedling percentage was reported higher under Cd stress.

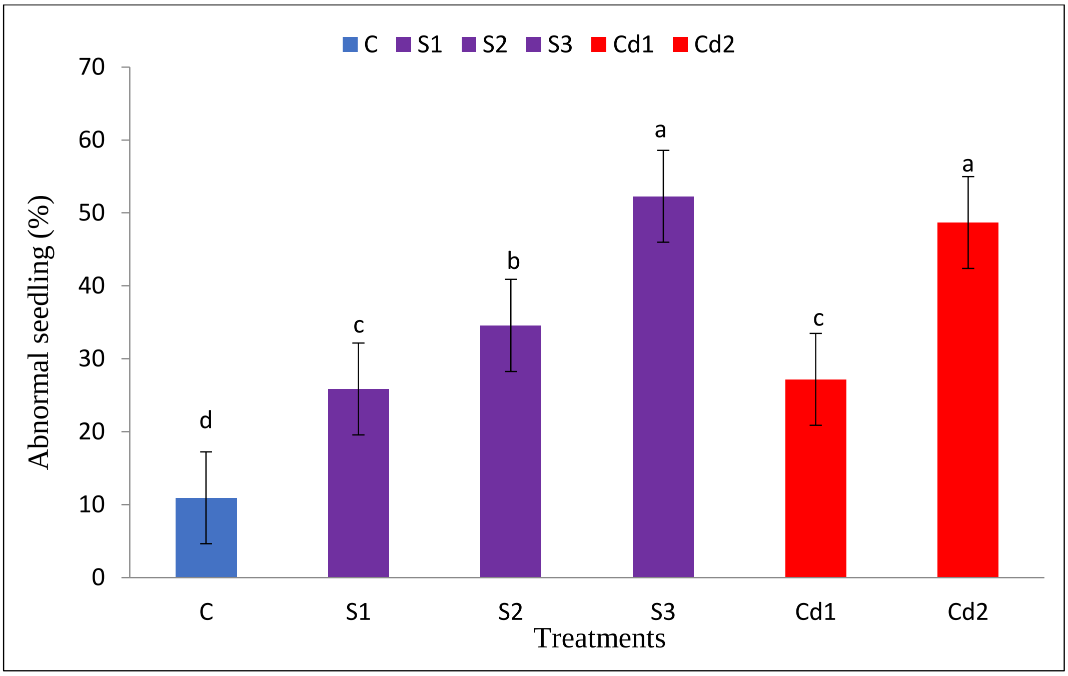


**Figure 11. Abnormal seedling % of *C. capsularis* plants affected by different abiotic stresses. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.**

**Experiment II**

### 4.2 Crop growth parameters

#### 4.2.1 Plant height

Plant height of *Corchorus capsularis* was decreased remarkably at any growth stage upon exposure to different abiotic stresses. At every case, the height of the plant was decreased with severity of stress. At 21 DAS, mild and severe salt stress caused 16%and 21% reduction in plant height over control. Similarly, moderate and severe drought stress reduced plant height by 21 and 22%, respectively. Therefore, there is no significant changes was observed in plant height under cadmium stress. However, waterlogging was very much crucial to decrease plant height which was 15 and 33% under short and long duration of waterlogging. At 28 DAS, it reduced significantly at severe salt stress which was 28 and 13% by moderate salt stress. Mild and severe drought stress caused 20 and 23% reduction comparing to control. plant height also decreased by 9 and 21% under moderate and severe Cd stress . Therefore, plant height was reduced by 5 and 11% upon exposure to short and long duration of waterlogging. At 35 DAS, plant height was reduced extensively under severe drought and severe salt stress which was 38% and 31%, respectively over control. *Corchorus capsularis* is moderately waterlogging tolerant at mature stage, and there was no noteworthy variation in plant stature at 35 DAS. Plant height was lessened by 14 and 26% under moderate and extreme cadmium stress .

Plant height was decreased upon exposure to different level of abiotic stresses in any growth stages. Osmotic stress from salinity hinders growth of plant by decreasing capacity of water removal and photosynthesis (Shabani *et al.*, 2013). Salt stress causes osmotic stress which noticed by osmotic potential, and increasing with the long-term stress which decreased plant height (fig.12) that supports the findings of Bhuyan *et al.,* 2018 who revealed that plant height of tossa jute cv. O-9897 reduced under salt stress. Likewise, salt stress has also been observed to decrease plant height in other crops like rice (Rahman *et al.,* 2016) and mungbean (Nahar *et al.,*2016). The reduction in plant height was associated with a decline in the cell enlargement and more leaf senescence under water stress (Bhatt *et al.,* 2005). Plant height was decreased markedly with the increase in drought stress intensity (fig.12) that agreed with the findings of (Yakoub *et al.,* 2016) in *C.olitorious* plant in which plant height was significantly inhibited when exposure to water deficit condition. Moreover, water stress has also been found to lessen plant height in different field crops like mungbean (Ahmad *et al.,* 2015), rice (Todaka *et al*., 2015), wheat (Yavas and Unay, 2016; Shan *et al.,* 2018), lentil (Gorim and Vandenberg, 2017), maize (Su *et al*., 2019) etc. Cd toxicity cause detrimental effect on plant height (Larson *et al.,*1998). Cd can interfire different metabolic processes in plant such as inhibit the proton pump, reduce root elongation, and destroy photosynthetic machinery (Najeeb and others, 2011). Under Cd stress, plant height decreased and with the severity of stress it reduced more promptly. Plant height also reported to decline under Cd stress found in other crops like rice by (Cai *et al.,* 2010), cotton (Farooq *et al.*, 2013). Reduction in plant height of *C. capsularis* plants under waterlogging or flooding stress was noticed in this study. This also reported in sesame plant by other scientists (Mensah *et al.*, 2006; Wei *et al.*, 2013; Saha *et al.*, 2016).

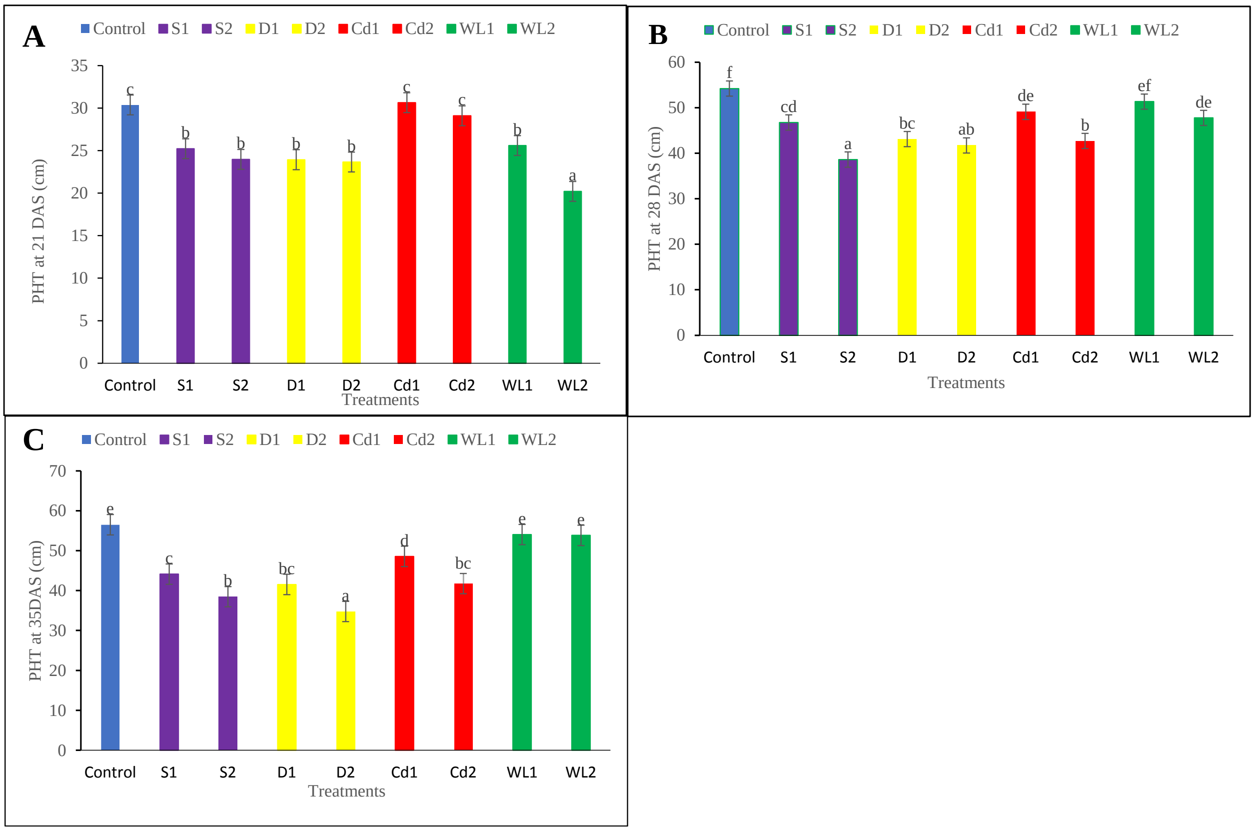


**Figure 12. Plant height of *C. capsularis* plants affected by different abiotic stresses. $S_1$, $S_2$, $D_1$, $D_2$, $Cd_1$, $Cd_2$, $WL_1$, $WL_2$ represents 200 mM NaCl, 400 mM NaCl, 10 day of water deficit, 15 day of water deficit, 2 mM $CdCl_2$, 4 mM $CdCl_2$, 5 day of water logging and 20 day 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.**

### 4.2.2 Above ground fresh weight plant$^{-1}$

Above ground fresh weight of *Corchorus capsularis* plant was reduced under different abiotic stresses. At 22 DAS, FW reduced sharply under severe drought stress which was 27 and 10% by moderate drought stress. FW also reduced significantly upon exposure to two doses of salt stress which was 22 and 15 % under severe and moderate salt stress. No significant changes were observed in FW under both cd stress and waterlogging stress. At 29 DAS, it was observed that FW reduced sharply under severe salt stress which was 49 and 27 % by moderate salt stress. FW also reduced significantly 36 and 30% under severe drought and moderate drought stress. FW also reduced by 22 and 19 % by moderate and severe cd stress respectively over control. There was less effect on above ground fresh weight plant$^{-1}$ under waterlogging condition.

Significant decrease was observed in above ground FW under salt stress and with the severity of stress it decreased (fig .14) it supports the findings of other observations like (Kao *et al.*, 2006) in glycine species, (Rahman *et al.*, 2016) observed in rice at salt stress .Above ground FW decreased markedly with the increase in drought stress intensity (fig.14). Moreover, in different studies drought stress reduced the above ground FW in some other crops like maize (Anjum *et al.*, 2017 and Hussain et al., 2019), rice (Saha *et al.*, 2019; Nasrin *et al.*, 2020), lentil (Sehgal *et al.*, 2017), wheat (Hasanuzzaman *et al.*, 2018) etc.Upon exposure to Cd stress above ground FW decresed markedly in this study(fig.14) this also found in other studies where exposure to increasing Cd concentrations reduced the above ground fresh weight in rapeseed seedlings (Filek *et al.*, 2008). Plants waterlogged for longer duration reduced FW is obtained in (fig.14). In tobacco (Yu and Rengel, 1999) and barley (Zhang *et al.*, 2007) similar results were demonstrated.

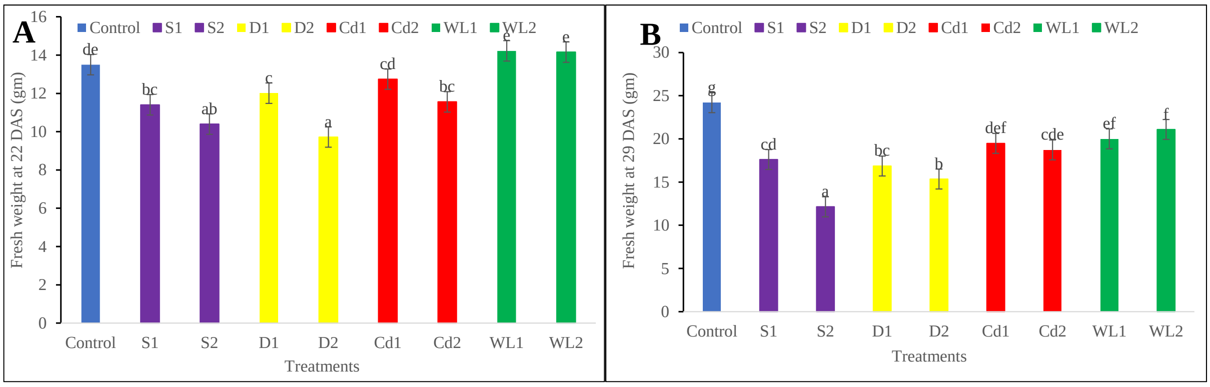


**Figure 13. Above ground fresh weight plant$^{-1}$ affected by different abiotic stresses. $S_1$, $S_2$, $D_1$, $D_2$, $Cd_1$, $Cd_2$, $WL_1$, $WL_2$ represents 200 mM NaCl, 400 mM NaCl, 10 day of water deficit, 15 day of water deficit, 2 mM $CdCl_2$, 4 mM $CdCl_2$, 5 day of water logging and 20 day 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.**

### 4.2.3 Above ground dry matter weight plant$^{-1}$

Plant DW was reduced with the severity of stress. At 22 DAS, DW reduced remarkably under waterlogging stress. There was significant decrease also observed under salt stress which was 22  and 20 % by moderate and severe salt stress. There was no considerable changes were found in FW under both Cd and drought stress. At 29 DAS, drought stress showed remarkable change in FW and it was reduced by 31 and 28% under severe and moderate drought stress corresponding to control. FW also reduced by 18 and 11% under severe and moderate Cd stress. Therefore, no considerable changes were found in FW of the plant at waterlogging treatment. Adverse effect on $CO_2$ assimilation declines the net photosynthesis rate, which finally result in reduction of nutrient uptake and plant growth (Seeman and Sharkey, 1986; Cha-Um and Kirdmanee, 2009). In this study, a remarkable reduction was accounted in above ground dry matter weight under salt stress in stressed plant compared to control (fig.15). Moreover, same results was investigated in other  crops like rice (Rahman *et al.*, 2016) maize.Upon exposure to different levels of drought stresses, plants exhibited reduction in above ground DW (Fig. 15). (Yakoub *et al.*, 2016) explained that drought stress reduced the above ground DW along with above ground FW in *C .olitorious*  plant. Similar findings were observed in rice, maize, wheat, lentil etc. (Anjum *et al.*, 2017; Sehgal *et al*., 2017; Hasanuzzaman *et al.*,  2018; Hussain *et al.*, 2019; Saha *et al*., 2019; Nasrin *et al.*, 2020). The dry weights of shoot were decreased with increased doses of $CdCl_2$ (fig.15). This result agreed with (Nizam *et al.*, 2006) who found that above ground DW of all these jute varieties BJC-7370, jutet CVE-3, kenaf HC-3, kenaf HC-95, and mesta Samu-93, were decreased under Cd stress respectively over control. Under waterlogging stress above ground DW decressed over respective control (fig.15). Overall, it also found in another crops like cowpea plant where waterlogging decreased above ground DW (Hong *et al.*, 1977).

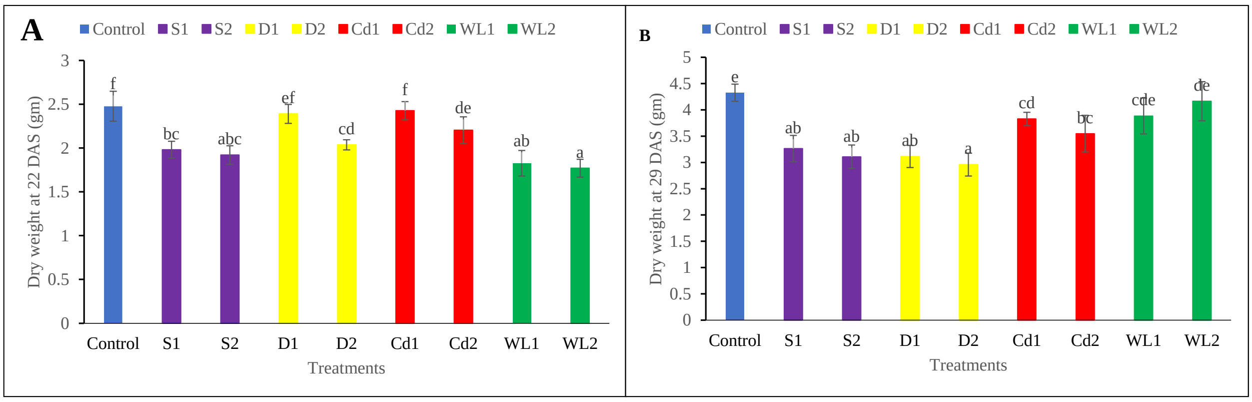


**Figure 14. Above ground dry weight plant$^{-1}$ affected by different abiotic stresses. $S_1$, $S_2$, $D_1$, $D_2$, $Cd_1$, $Cd_2$, $WL_1$, $WL_2$ represents 200 mM NaCl, 400 mM NaCl, 10 day of water deficit, 15 day of water deficit, 2 mM $CdCl_2$, 4 mM $CdCl_2$, 5 day of water logging and 20 day of 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 Stem diameter

Stem diameter of *C.capsularis* plant was reduced with increasing stress. It was found that drought stress reduced the stem diameter remarkably at any level. There was significant reduction was observed in stem diameter under severe drought stress which was 79 and 42% by moderate drought stress respectively over control. It was decreased by 58 and 41 % upon exposure to severe salt and moderate salt stress. Stem diameter also reduced under Cd stress and waterlogging stress. The results revealed that stem diameter was significantly decreased under salt stress which means salt concentration increase with the long-term treatment and resulted in lessening of stem diameter in *C.capsularis* plant  Stem diameter was reduced sharply upon exposure to drought stress (fig.16). This results also agreement with those of Yakoub *et al.*, 2016 who report a reduction in stem diameter in *C.olitorious* plant under water deficit  over control. Stem diameter also reduced under Cd stress in (fig.16) that agreed with the findings of Nikolić *et al.*, 2008 in hybrid poplar plants. Stem diameter also reduced under waterlogging treatment (fig.16).

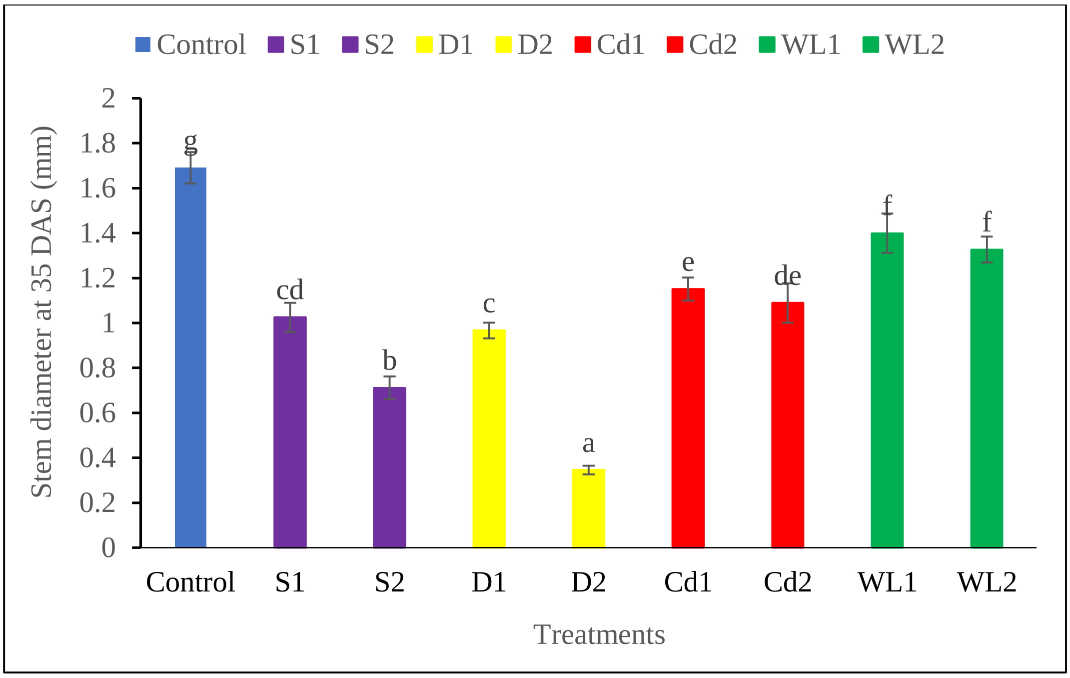


**Figure 15. Stem diameter affected by different abiotic stresses. $S_1$, $S_2$, $D_1$, $D_2$, $Cd_1$, $Cd_2$, $WL_1$, $WL_2$ represents 200 mM NaCl, 400 mM NaCl, 10 day of water deficit, 15 day of water deficit, 2 mM $CdCl_2$, 4 mM $CdCl_2$, 5 day of water logging and 20 day of waterlogging. Mean (±SD) 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.**

### 4.3 Physiological parameters

#### 4.3.1 SPAD value of leaf

SPAD value indicates the chlorophyll (chl) concentration of leaves. At 21 DAS, it was observed that SPAD reading was significantly decreased under longer duration of waterlogging treatment which was 24% over control. Upon exposure to salt stress it was reduced by 20 and 12 % under severe and moderate salt stress. There was less reduction was observed in SPAD reading under Cd stress. SPAD reading decrease 13 % and 8% by under moderate and severe drought stress. At 28 DAS, SPAD reading was sharply decreased under waterlogging treatment which was 15 and 21% under shorter and longer duration of waterlogging. SPAD reading also decrease by under severe salt and drought stress which is statistically similar 14 and 9% by moderate drought stress respectively. At 35 DAS, SPAD reading was declined under all stress wherse it was superior under severe drought stress which was 14% over control. Salinity-induced toxicity of ion and osmotic stress reduced chl content by increasing chlorophyllase and ROS (Saha*et al.,* 2010; Hasanuzzaman *et al.,* 2014). The results revealed that SPAD reading reduced upon acquaintance to salt stress which was observed in (fig.16) which supports the findings of Turan *et al. (*2009) and Cha-Um and Kirdmanee (2009) in two maize varieties. The reduction in chl under drought stress is primarily the result of impairment to chloroplasts triggered by ROS (Smirnoff, 1995). Drought stress showed the higher decline in chlorophyll content of leaf and with the duration of stress it reduced (fig.16) which is supported by the result of (Yakoub *et al.,* 2016) in *C. olitorius* seedling. This result concide with the findings of (Ommen *et al.,*1999) who reported that leaf chl content decreases as a result of drought stress. Manivannan *et al.* (2007) investigated that drought stress results a large decay in the chl-a content, the chl-b content, and the total chl content in all sunflower varieties. *C. capsularis* plants can be said hyperaccumulator of HMs (Saleem *et al.,* 2020*). C. olitorius* leaves have the multimodal protective role and mechanism of Cd intoxication (Saikat *et al.,* 2013). In this study Cd-persuaded reduction of chl content was not so significant. Moreover, in other ovservations reduction of chlorophyll content under Cd stress recorded in Indian mustard and mung bean (Šimonová *et al.,* 2007) and Phaseolus vulgaris (Zengin and Munzuroglu, 2005). SPAD reading showed plant leaves under water logging stress indicate lower value than the control plants (Fig. 16) which is supported by Tan *et al.,* 2008 in wheat plants.

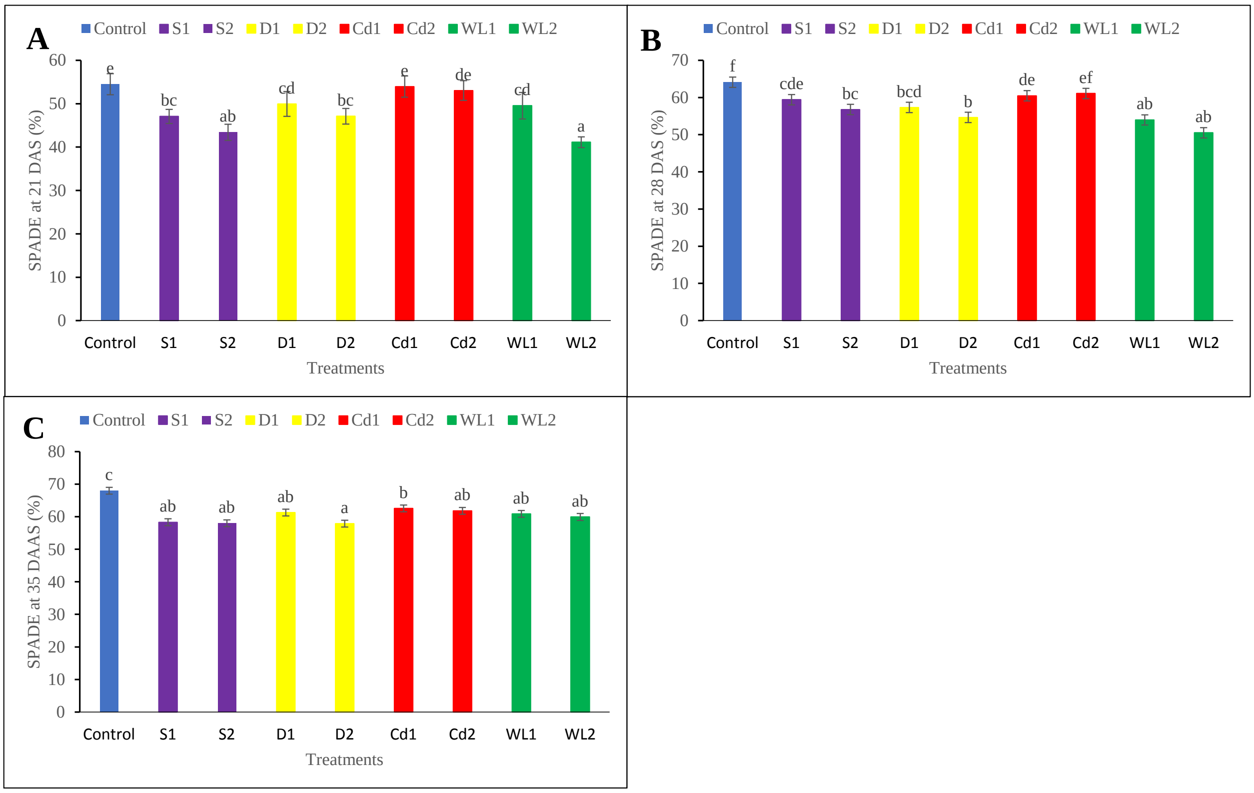


**Figure 16. SPAD value of C. capsularis plant was affected by different abiotic stresses. $S_1$, $S_2$, $D_1$, $D_2$, $Cd_1$, $Cd_2$, $WL_1$, $WL_2$ represents 200 mM NaCl, 400 mM NaCl, 10 day of water deficit, 15 day of water deficit, 2 mM $CdCl_2$, 4 mM $CdCl_2$, 5 day of water logging and 20 day 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.**

### 4.3.2 Relative water content of leaf

Relative water content (RWC) of *C. capsularis* plant was dramatically varied along with increase of different stresses. At 22 DAS, it was noticed that RWC of leaf was markdly reduced under drought stress which was 41 and 30% by severe and moderate drought stress, respectively. Upon exposure to salt stress, leaf RWC was reduced by 25 and 13 % under severe and moderate salt stress. RWC was also reduced by 20 and 18 % under severe and moderate Cd stress wherease it was increased under waterlogging stress. At 29 DAS, leaf RWC was sharply decreased under drought stress which was 20 and 40% by severe and moderate drought stress. It was also observed that leaf RWC was reduced by 26, 20 and 17, 16% under severe salt and Cd stress and moderate salt and Cd stress.

The relative water content reduced significantly with induction of salinity stress  and duration of brine stress . In the current investigation, RWC was decreased underneath salty stress  with the increase of salinity level the decrease of RWC also increased (fig 17). This result also agreement with those of Chaudhuri *et al.*, 1997 who reported a reduction in RWC in both *C. capsularis* and *C. olitorious* plant under short term salinity stress and the effect being greater with higher NaCl concentration and duration of stress. Drought stress showed the higher decline in RWC of leaf and with the duration of stress it reduced (fig.17) that agreed with the findings of Yakoub *et al.*, 2016 in *C. olitorious* plant and by Choudhury *et al.*, 1985 in both *C. capsularis* and *C. olitorius* plant. Bartholomew (2005) reported reduction in RWC with flooding, which promote decreased with the long-term flooding stress. RWC of leaf was not significantly decreased in this study under waterlogging stress which is statistically similar through the control plant.

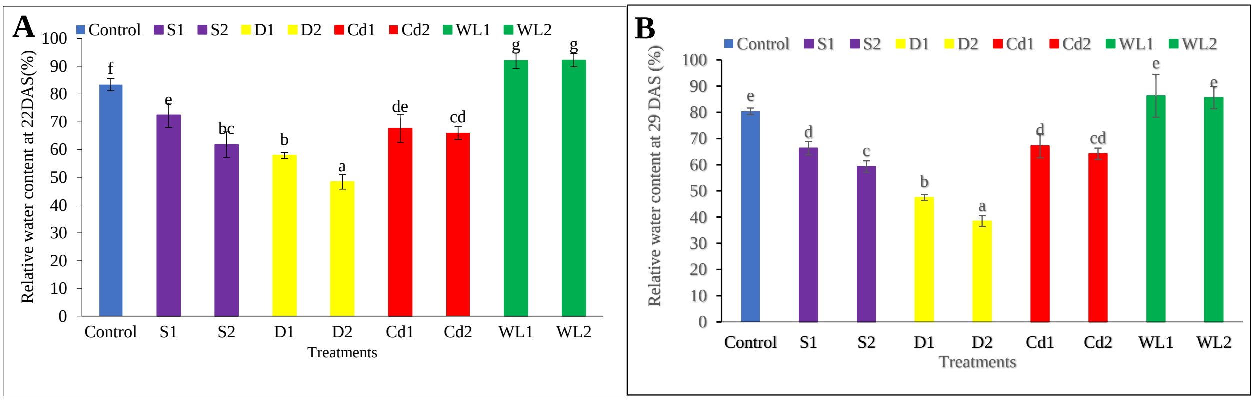


**Figure 17. Changes in leaf RWC of C. capsularis plant was affected by different abiotic stresses. $S_1$, $S_2$, $D_1$, $D_2$, $Cd_1$, $Cd_2$, $WL_1$, $WL_2$ represents 200 mM NaCl, 400 mM NaCl, 10 day of water deficit, 15 day of water deficit, 2 mM $CdCl_2$, 4 mM $CdCl_2$, 5 day of water logging and 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.**

### 4.3.3 Electrolyte leakage

Electrolyte leakage (EL) of *C.capsularis* plant was increased with the severity of stress. It was observed that highest EL value is found under severe drought and salt stress which was 140% over respective control (fig.18). Therefore, lowest  EL value is observed under waterlogging stress. Upon exposure to Cd stress El value is observed 22% And 20 % under severe and moderate Cd stress. In this study, the results showed that electrolyte leakage increased under salt stressed condition, which proportionally rose with increased level of salinity (fig.18). Similar observation was found in rice (Lutts *et al.*, 1996) and sugar beet (Ghoulam *et al.*, 2002) (khan *et al.*, 2013) in cucumber plant. Electrolyte leakage also enhanced severely under drought stress condition showed in (fig .18). This result also analogous with the findings of Chowdhury *et al.*, 1985, who perceived that the tissue penetrability (as designated by EL) too increased under water stressed condition, which proportionally increased with enlarging duration of water stress in both *C. capsularis* and *C.olitorious* plant upon exposure to Cd stress electrolyte leakage was augmented by the relentlessness of stress it increased. Saleem *et al.* (2019) perceived that metal (Cu) concentration in diverse varieties of jute considerably amplified the EL in the roots and leaves. Velasco *et al.* (2019) recorded that waterlogging amplified the EL in bean cultivars signifying an upsurge in the penetrability of cell tissues.

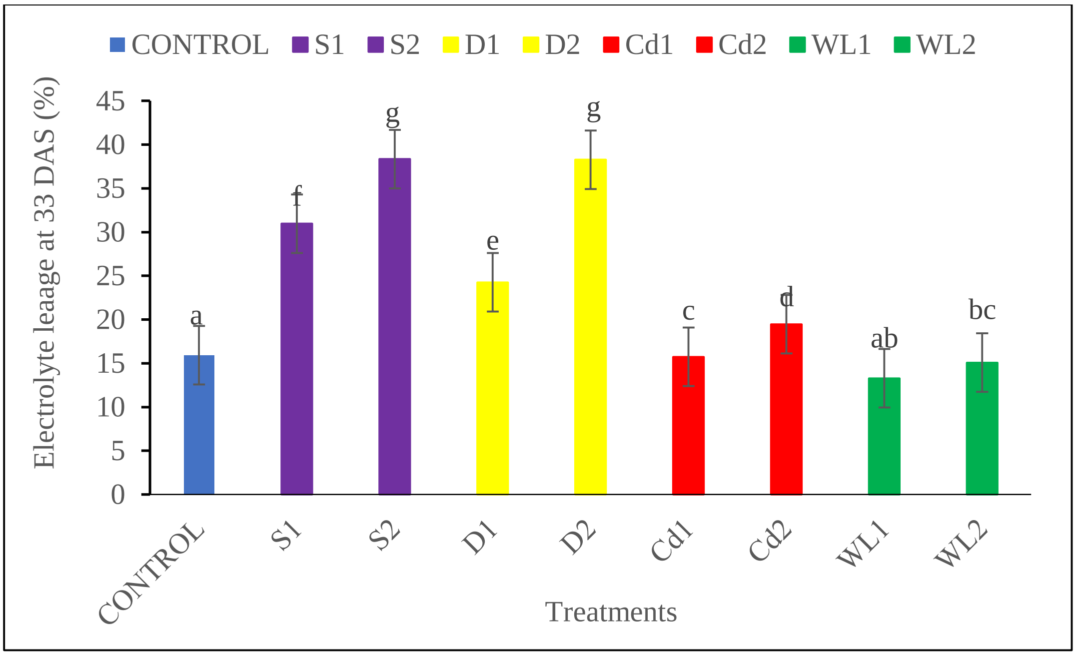


**Figure 18. Changes in electrolyte leakage of C. capsularis plant was affected by different abiotic stresses. $S_1$, $S_2$, $D_1$, $D_2$, $Cd_1$, $Cd_2$, $WL_1$, $WL_2$ represents 200 mM NaCl, 400 mM NaCl, 10 day of water deficit, 15 day of water deficit, 2 mM $CdCl_2$, 4 mM $CdCl_2$, 5 day of water logging 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.**

### 4.4 Oxidative stress indicators

#### 4.4.1 Lipid peroxidation (MDA content)

To determine the amount of oxidative stress, lipid peroxidation is considered as an important index and it is found that content of MDA increases with the oxidative stress triggered by abiotic stress including waterlogging (Hasanuzzaman *et al.*, 2012). Increased severity of each stress resulted with the raise of lipid peroxidation in tissues of leaf, calculated as the malondialdehyde (MDA). At 26 DAS, highest lipid peroxidation was observed under drought stress which was 153 and 98% under severe and moderate drought stress. Upon exposure to salt stress, it was increased by 102 and 65 % under severe and moderate salt stress. With the increase of Cd stress it increased by 69 and 35 % under severe and moderate Cd stress over respective control. No significant increase was observed under waterlogging stress. At 36 DAS, higher lipid peroxidation was observed under drought stress which was 104 and 85 % under severe and moderate drought stress. Upon exposure to salt stress, it was increased by 64 and 16% under severe and moderate salt stress. Lipid peroxidation also increased under Cd stress over control.

Malondialdehyde concentration is a renowned index for identifying the degree of oxidative stress. It can affect plant cells through reducing fluidity of membrane, enhancing leakiness in membrane, and destroying proteins, ion channels, and enzymes in membrane (Gerg *et al.*, 2009). In the current investigation, malondialdehyde concentration was greater than before markedly upon exposure to salt stress (fig.18) that observed by Hasanuzzaman *et al.*, 2011 in other crops where content of malondialdehyde was improved by 69 and 129% at 100 and 200 mM NaCl ,correspondingly in rapseed seedlings using control seedlings as a standard upon exposure to salt stress. The drought stress changes state of lipid peroxidation and consequent fluctuations in the membrane permeability and outcomes exhibited that the level of MDA amplified underneath water stress (fig.19) that supports the data of Chowdhury *et al.*, (1985). Lipid peroxidation levels in tissues of leaf increased with the Cd concentration which measured as MDA content. Therefore, it is generally accepted that the MDA level can cause the oxidative damage of membrane system induced by Cd stress (Li *et al.*, 2013). It was seen that MDA content increase with theincrease of Cd stress (fig.19) this results also concide with (Deng *et al.*, 2017) in kenaf seedlings. Moreover, in other studies like in rapseed seedlings by Hasanuzzaman *et al.*, (2011), malondialdehyde content amplified with the increase of Cd stress. Malondialdehyde concentration decreased markedly under waterlogging stress (fig.19). Likewise, in other crops MDA content decreased under waterlogging stress observed by (Xu *et al.*, 2012; Saha *et al.*, 2016 and Wei *et al.*, 2013) in sesame seedlings (Zhang *et al.*, 2007 and Yin *et al.*, 2009).

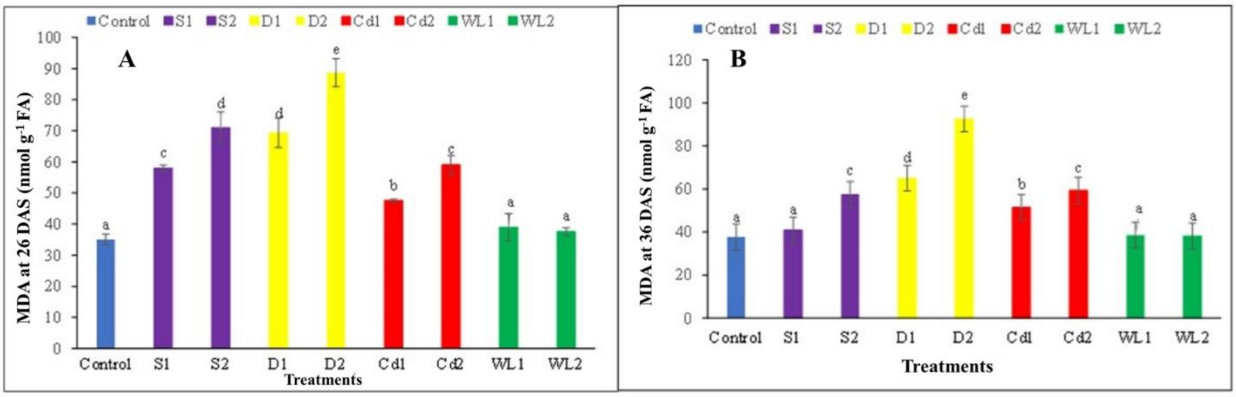


**Figure 19. MDA content of C. capsularis plant was affected by different abiotic stresses. $S_1$, $S_2$, $D_1$, $D_2$, $Cd_1$, $Cd_2$, $WL_1$, $WL_2$ represents 200 mM NaCl, 400 mM NaCl, 10 day of water deficit, 15 day of water deficit, 2 mM $CdCl_2$, 4 mM $CdCl_2$, 5 day of water logging and 20 day 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.**

#### 4.4.2 $H_2O_2$ content

$H_2O_2$ is significant for permeating membranes and consequently, it is not stored in the cell. The level of $H_2O_2$ was amplified noticeably with the upsurge of severity of stress. At 26 DAS, $H_2O_2$ was increased sharply under drought stress which was 68 and 36% by severe and moderate drought stress. Upon exposure to Cd stress it was increased by 50 and 24%, respectively under severe and moderate Cd stress. Salt stress increases $H_2O_2$ level 36 and 18 % at severe and moderate salt stress. No significant increase was observed under waterlogging stress over respective control. At 36 DAS, higher $H_2O_2$ level was observed under severe and moderate drought stress. It was increased by 105 and 59% under sever and moderate salt stress while 85 and 20 % by severe and moderate Cd stress.

At higher concentration, $H_2O_2$ leads to the occurrence of oxidative stress and oxidation of their thiol groups may produce inactivate enzymes (Quan *et al.*, 2008). Accumulation of $H_2O_2$ increased with the salt stress and supports the result observed by Hasanuzzaman *et al.*, 2011 which state that salt stress arisen the availability of $H_2O_2$ in rapseed seedlings. Upon exposure to water stress results intensification of the $H_2O_2$ level in (fig. 20) that supports Chowdhury *et al.*, (1985), who found that upon exposure to water stress results amplification of the $H_2O_2$ level inside *C.capsularis* and *C.olitorious* seedlings pretentious the inclusive membrane veracity. Upon exposure to Cd stress the $H_2O_2$ accumulation significantly increased shown in fig. 20. This results concide by the research outcomes of Hasanuzzaman *et al., (*2011) in the rapeseed seedlings. Increased production of $H_2O_2$ under waterlogging stress was recrded in wheat (Zheng *et al.*, 2009), pigeon pea (Sairam *et al.*, 2009) and Onion (Yiu *et al.*, 2009).

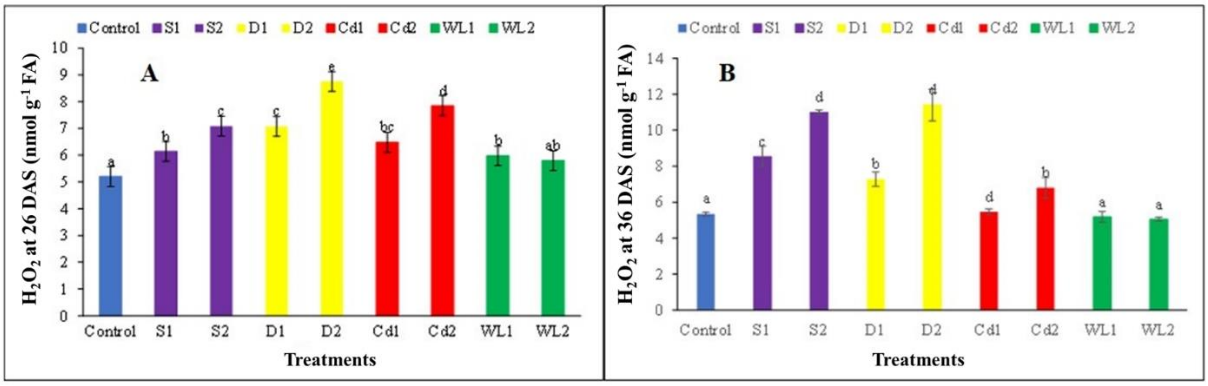


**Figure 20. $H_2O_2$ of C. capsularis plant was affected by different abiotic stresses. $S_1$, $S_2$, $D_1$, $D_2$, $Cd_1$, $Cd_2$, $WL_1$, $WL_2$ represents 200 mM NaCl, 400 mM NaCl, 10 day of water deficit, 15 day of water deficit, 2 mM $CdCl_2$, 4 mM $CdCl_2$, 5 day of water logging and 20 day of 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.**

### 4.4.3 CAT activity

In the present investigation, CAT movement was significantly reduced underneath drought stress while CAT activity was recorded higher under waterlogging stress over respective control. Upon exposure to salt stress it abridged by 24 and 22% under modest and extreme salinity stress . Cd stress reduced the CAT activity 30 and 20 % at severe and moderate Cd stress.

In this study, CAT activity was decreased sharply with the severity of stresses except waterlogging stress. CAT activity declined with salt stress which results in elevated levels of $H_2O_2$, and (fig. 21) these results agree with the (Hasanuzzaman *et al.*, 2011) in rapseed seedlings. It was noticed in this study that CAT action was reduced under drought stress. This results sopports with the findings of Chowdhury *et al.*, (1985) who reported that the catalase activity deteriorated by water stress in *C. capsularis* and *C. olitorious* seedlings favouring $H_2O_2$ accumulation. When exposed to Cd stress, CAT activities were significantly decreased (fig. 21) that supports with the findings of (Hasanuzzaman *et al.*, 2012) in other crops like rapseed seedlings. Therefore, *C. capsularis* is moderately waterlogging tolerant plant. CAT activity was increased with the duration of waterlogging treatment which was observed in (Fig. 21). Moreover, in different studies waterlogging induced reduction of CAT activity has also been reported in barley (Zhang *et al.*, 2007), wheat (Tan *et al.*, 2008), onion (Yiu *et al.*, 2009) and sesame (Xu *et al.*, 2012).

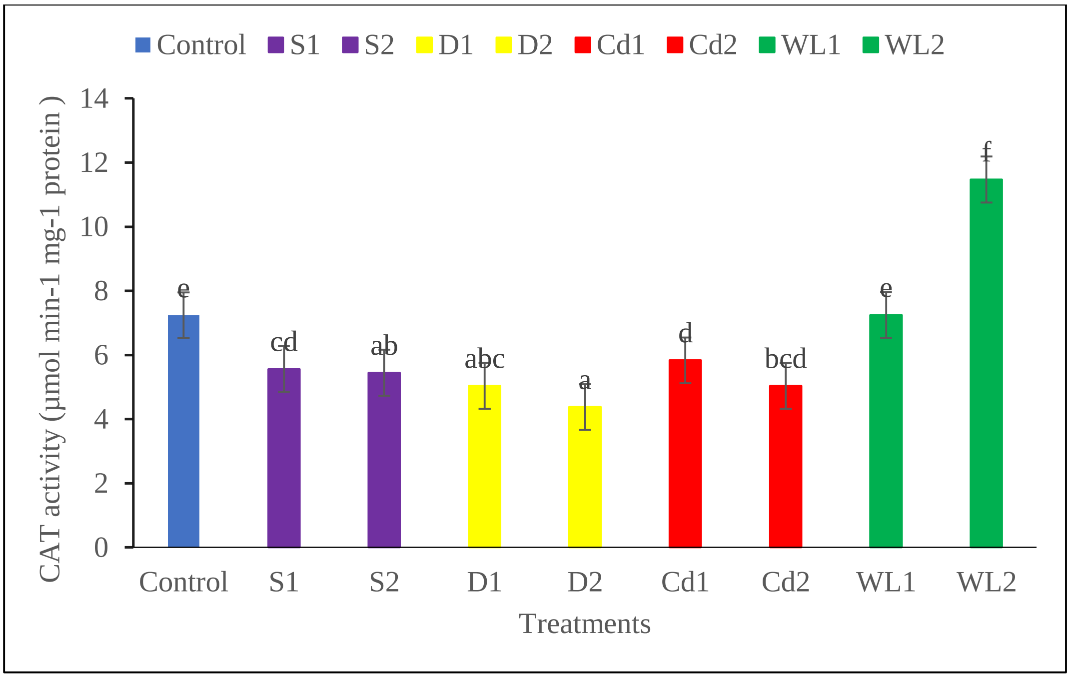


**Figure 21. Activities of catalase enzyme of C. capsularis plant was affected by different abiotic stresses. $S_1$, $S_2$, $D_1$, $D_2$, $Cd_1$, $Cd_2$, $WL_1$, $WL_2$ represents 200 mM NaCl, 400 mM NaCl, 10 day of water deficit, 15 day of water deficit, 2 mM $CdCl_2$, 4 mM $CdCl_2$, 5 day of water logging 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.**

# CHAPTER-5
## SUMMARY AND CONCLUSION

There were two experiments conducted at different locations and periods to investigate the morphophysiological responses deshi jute (*Corchorus capsularis*) under different abiotic stresses. Experiment-I was conducted in laboratory to study the germination percentage under different abiotic stress at the central laboratory of Sher-e -Bangla agricultural university during April 2019 to June 2019 where studied the different germination parameter like total germination percantage (TG) Mean germination test (MGT), Root-shoot ratio, Vigor index, Survivability percantage (10 days after germination), Vigor value, Co-efficient of velocity germination, Timson germination index (TGI), Mean germination rate (MGR), normal seedlings, abnormal seedlings and dead seeds ( 4th days of germination). A field experiment was conducted on morpho-physiological responses under different abiotic stress at the experimental shed of crop science laboratory at the Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh, from April 2019 to June 2019. The experiments were replicated three times and conducted in randomized completely block design. Experiment -1 consists of six treatments i.e., Control, Salt stress (50 mM ,75 mM and 100 mM NaCl) Cadmium stress (0.50mM and 1 mM $CdCl_2$). Earthen pots were used to facilitate the germination of seedlings where seeds were sown in sandy soil to investigate the germination percentage under different stress. Experiment -2 consists of nine treatments viz., Control, Salt stress (200 mM and 400 mM NaCl) Mild drought stress (10 days of water deficit condition till 25DAS) Severe drought stress (15 days of water deficit condition till 30 DAS) Cadmium stress (2mM and 4mM $CdCl_2$) waterlogging stress (5 days of waterlogged condition at 15 and 30 DAS and 20 days of continuous waterlogged condition till 35 DAS). Treatments were imposed on seedlings at 15 days after sowing (DAS). Morphological data were taken 5 days after treatments at 20 DAS and biochemical assessment was done after 10 days of stress treatments at 30 DAS. Different data of morphology (Plant height, above ground fresh weight plant-$^{1}$, above ground dry matter weight plant-$^{1}$, stem diameter) physiology (SPAD value of leaf, relative water content of leaf, Electrolyte leakage) and biochemical (Lipid peroxidation, $H_2O_2$ content, CAT) were needed to show the morpho- physiological responses of *C.capsularis* under different abiotic stresses.

In this study, germination percentage was sharply reduced under salt stress and cadmium stress. Lowest germination was recorded at 100 mM NaCl. With the increase of doses of salt level germination percentage was declined gradually. Germination percentage was reduced 80 and 75 % at 50 and 75 mM NaCl. Due to the inhibitory effect of cadmium showed detrimental effect on germination percentage. Germination decreased significantly at all of the Cd stresses, ranging between 27% at 0.5 mM Cd to 33% at 1.0 mM Cd as comparing with the control. Root-shoot ratio was observed higher at o.5 mM $CdCl_2$ although it was declined at 1mM $CdCl_2$ concentration. Root-shoot ratio was decreased significantly under salt stress where the extent of reduction was statistically similar. However, it was decreased by 13, 16 and 19% at 50, 100, 150 mM NaCl – induced salt stress over respective control. Vigor index was reduced sharply under salt stress and Cd stress. Minimum vigor index was recorded at 100 mM NaCl. Vigor index decreased by 11, 24 and 49% at 50, 75 and 100 mM NaCl – induced salt stress. Cadmium having toxic effect reduced seedling vigor ability. Cd stress also reduced vigor index 34 and 39% at 0.5 and 1 mM $CdCl_2$.

Survivability percentage was observed higher at 50 mM NaCl and declined gradually with the increase of doses of salinity which was 15 and 22 % at 75 and 100 mM NaCl. Survivability percentage was comparatively higher under Cd stress than the salt stress. Survivability percentage was decreased by 7 and 14 % under 0.5 and 1mM $Cdcl_2$ over respective control. Minimum Vigor value was observed at 100 mM NaCl and 1mM $CdCl_{2.}$ Moreover, vigor value reduced by 33, 42 and 54% at 50, 75 and 100 mM NaCl and 40 and 55% at 0.5 and 1mM Cd stress, respectively corresponding to control. Coefficient of velocity of germination was observed statistically similar at all level of salinity where it was observed slightly higher at 0.50 mM $CdCl_2$ concentration. CVG reduced by 18, 22 and 24% at 50, 75 and 100 mM NaCl – induced salt stress and 13 and 24% at 0.5 and 1 mM $CdCl_2$, respectively corresponding to control.

Timson germination index was reduced noticeably under salt stress and cadmium stress. The reduction was statistically similar under all stress where higher reduction was observed at 100 mM NaCl. Mean germination time was increased under salt stress and cadmium stress. Germination was delayed by salinity and cadmium due to its toxic effect which ultimately increased the mean germination time of seedlings. MGT increased gradually with the increased doses of salinity recorded by 22, 28 and 31% at 50, 75 and 100 mM NaCl. Cd stress also increased MGT by15 and 29% at 0.5

and 1 mM $CdCl_2$. Mean germination rate decreased under different stresses due to the inhibitory effect salt and cadmium on the germination which ultimately decreased mean germination rate. MGR decreased by 19, 23 and 25% at 50, 75 and 100mM NaCl. Cd stress decreased mean germination rate 13 and 23% at 0.5 and 1 mM $CdCl_2$.

In this study normal seedling percentage was recorded lower at 100 mM NaCl. Normal seedling percentage was decreased by 17 and 34% at 50, 75 mM NaCl – induced salt stress and 18 and 43% at 0.5 and 1mM $CdCl_2$ over control. Abnormal seedling percentage was increased under all stress condition. Higher abnormal seedling percentage was recorded at 100 mM NaCl. Abnormal seedling was increased with the increase of salinity level at 50 and 75 mM NaCl. Due to the inhibitory effect of Cd abnormal seedling was recorded higher under Cd stress.

Plant height was reduced markedly at any growth stages under all abiotic stresses. Highest reduction was observed at severe salt stress condition at 21, 28 and 35 DAS. Plant height also reduced at other stress condition. Plant height reduced under severe salt stress (21, 28 and 31%) and moderate salt stress (16, 13 and 21%) severe drought stress (22, 23 and 38%) moderate drought stress (21, 20 and 26%) and severe Cd stress (18, 21 and 26%) and moderate Cd stress (6, 9 and 14%) at 21, 28 and 35 DAS. Waterlogging stress showed no significant effect on plant height. Above ground FW was decreased sharply under drought stress compare to other stresses. Salt stress also showed significant effect on above ground FW where it reduced under severe salt stress (22 and 49%) moderate salt stress (15 and 27%) at 22 and 29 DAS. Drought stress also showed effect on above ground FW which is reduced by (27 and 36%) under moderate drought stress and (10 and 30%) under severe drought stress at 22 and 29 DAS. Cadmium and waterlogging stress showed also negative effect on above ground FW. Above ground DW reduced severely under salt stress compared to other stresses. Drought stress also reduced above ground DW (17 and 31%) and (15 and 28%) by severe and moderate drought stress. Cadmium and waterlogging stress showed less significant effect on above ground DW. Stem diameter was sharply reduced under drought dress compared to other stresses. Salt stress also reduced stem diameter (41 and 58%) by moderate salt stress and severe salt stress. Cadmium and waterlogging stress showed less effect than drought and salt stress on stem diameter.

SPAD value reduced under all stresses. Salt and drought stress showed significant reduction in SPAD reading than other stresses at 21, 28 and 35 DAS. Cadmium and waterlogging stress showed less effect on SPAD reading. RWC of leaf was reduced noticeably under drought stress (41 and 50%) by severe drought (30 and 40%) by moderate drought stress at 22 and 29 DAS compared to other stresses. Salt stress also showed significant reduction in RWC of leaf (25 and 26%) and (13 and 17%) by severe and moderate salt stress at 22 and 29 DAS. Cadmium and waterlogging stress showed less significant effect on RWC of leaf. Highest electrolyte leakage was recorded under severe salt and drought stress which was 140% over respective control. Electrolyte leakage was found lower at cadmium and waterlogging stress than the other stress.

Plants exposed to different abiotic stresses resulted in a marked increment in the MDA content and $H_2O_2$ content but extreme stress from drought and salt stress compare to the another stresses. MDA and $H_2O_2$ content were higher at drought and salt stress compared to other stresses. MDA content was increased by 153 and 104 % under severe drought and 98 and 85 % under moderate drought stress at 26 and 36 DAS. $H_2O_2$ also increased 68 and 113 % by severe drought and 36% by moderate drought stress at 26 and 36 DAS. Salt stress increased the MDA content (102 and 64 % ) at severe salt stress and(65 and 16%) at moderate salt stress at 26 and 36 DAS. MDA and $H_2O_2$ content were lower at waterlogging stress than the other stresses. Cadmium stress also increased the MDA and $H_2O_2$ level but lower than the drought and salt stress. Drought stress also found to decrease catalase activity severely than the other stress while waterlogging increased the catalase activity. Catalase activity was also reduced under salt stress and cadmium stress. Consideraton on the above fact, it could be resolved that deshi pat (*C. capsularis*) showed significant responses under all abiotic stresses like salinity, drought, cadmium, waterlogging stress. With prolonged stress time all the stresses exhibited different impacts on the responses of morphological, physiological and antioxidant defense system. From this study, it can be concluded that *C.capsularis* is very much sensitive to drought stress and salt stress. However, it was observed that *C.capsularis* is moderate sensitive to Cd stress whether it showed tolerant response to waterlogging stress. Moreover, future studies are needed regarding molecular mechanisms of stress tolerance by *C. capsularis* in the field level.

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# APPENDICES

**Appendix I: Mean square values and degree of freedom (DF) of germination, root shoot ratio, vigor index, survivability, vigor value of *C.capsularis* under salt stress and cadmium stress.**

| Source | DF | Mean square values | | | | |
|---|---|---|---|---|---|---|
| | | Germination (%) | Root shoot ratio | Vigor index | Survivability (%) | Vigor value |
| Treatments | 5 | 578.056 | 0.029 | 57739.537 | 171.003 | 18.629 |
| Error | 12 | 19.444 | 0.005 | 5640.486 | 22.738 | 6.429 |

**Appendix II: Mean square values and degree of freedom (DF) of Coefficient velocity of germination, Timson germination index, Mean germination time, Mean germination rate, normal seedling (%), Abnormal seedling (%) *C.capsularis* under salt stress and cadmium stress.**

| Source | DF | Mean square values | | | | | |
|---|---|---|---|---|---|---|---|
| | | Coefficient of velocity of germination | Timson germination index | Mean germination time | Mean germination rate | Normal seedling (%) | Abnormal seedling (%) |
| Treatments | 5 | 66.424 | 33.212 | 0.168 | 0.010 | 762.471 | 714.724 |
| Error | 12 | 6.429 | 2.951 | 0.017 | 0.000 | 18.957 | 81.32 |

**Appendix III: Mean square values and degree of freedom (DF) of plant height, at 21,28 and 35 DAS *of C.capsularis* under salt stress, drought stress, cadmium stress, waterlogging stress**

| Source | DF | Mean square values | | |
|---|---|---|---|---|
| | | Plant height<br>21 (DAS) | Plant height<br>28 (DAS) | Plant height<br>35 (DAS) |
| Treatments | 8 | 36.764 | 75.418 | 475.265 |
| Error | 18 | 2.659 | 4.687 | 4.410 |

**Appendix IV: Mean square values and degree of freedom (DF) of above ground FW and above ground DW of *C.capsularis* at 22 and 29 DAS under salt stress, drought stress, cadmium stress, waterlogging stress**

| Source | DF | Mean square values | | | |
|---|---|---|---|---|---|
| | | Above ground FW (22DAS) | Above ground FW<br>(29DAS) | Above ground DW<br>(22DAS) | Above ground DW<br>(29DAS) |
| Treatments | 8 | 7.645 | 36.016 | 0.216 | 0.742 |
| Error | 18 | O.658 | 1.285 | 0.014 | 0.069 |

**Appendix V: Mean square values and degree of freedom (DF) of SPAD value of leaf of *C.capsularis* at 21, 28 and 35 DAS under salt stress, drought stress, cadmium stress, waterlogging stress**

| Source | DF | Mean square values | | |
|---|---|---|---|---|
| | | SPAD (21 DAS) | SPAD (28 DAS) | SPAD (35 DAS) |
| Treatments | 8 | 64.6667 | 51.985 | 29.581 |
| Error | 18 | 5.062 | 4.185 | 7.174 |

**Appendix VI: Mean square values and degree of freedom (DF) of RWC at 22 and 29 DAS, stem diameter, electrolyte leakage of *C.capsularis* at 21, 28 and 35 DAS under salt stress, drought stress, cadmium stress, waterlogging stress**

| Source | DF | Mean square values | | | |
|---|---|---|---|---|---|
| | | RWC (22DAS) | RWC (29 DAS) | Stem diameter | Electrolyte leakage |
| Treatments | 8 | 694.516 | 807.394 | 0.462 | 368.0180 |
| Error | 18 | 40.629 | 14.330 | 0.004 | 2.280 |

**Appendix VII: Mean square values and degree of freedom (DF) of CAT, MDA and $H_2O_2$ of *C.capsularis* at 26 and 36 DAS under salt stress, drought stress, cadmium stress, waterlogging stress**

| Source | DF | Mean square values | | | | |
|---|---|---|---|---|---|---|
| | | CAT | MDA (26DAS) | MDA (36DAS) | $H_2O_2$ (26 DAS) | $H_2O_2$ (DAS) |
| Treatments | 5 | 135.159 | 984.407 | 965.865 | 3.670 | 18.375 |
| Error | 12 | 1.429 | 11.313 | 5.438 | 0.195 | 0.194 |