ROLE OF CALCIUM TO MITIGATE SALT STRESS IN CHILLI

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CERTIFICATE

This is to certify that the thesis entitled ***"ROLE OF CALCIUM TO MITIGATE SALT STRESS IN CHILLI"*** *submitted to the Department of Horticulture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE in HORTICULTURE****, embodies the result of a piece of authentic research work carried out by* ***MD. RIAZUL ISLAM,*** *Registration No.* ***13-05328*** *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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ABBREVIATIONS AND ACCORONYMS

AEZ	Agro-ecological Zone
Agric.	Agricultural
ANOVA	Analysis of Variance
BARI	Bangladesh Agricultural Research Institute
Biol.	Biology
CV	Co-efficient of variance
DAT	Days After Transplanting
et al.	And others
Ex	Experiment
g	Gram
Hort.	Horticulture
LSD	Least Significance difference
RCBD	Completly Randomized Blocked Design
Res.	Research
SAU	Sher-e-Bangla Agricultural University
Sci.	Science
Spp.	Technology
Technol.	Species
Viz.	Namely
MSTAT	Michigan State University Statistical Package for Data Analysis

DEDICATED

TO

MY BELOVED PARENTS

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The Author

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ABSTRACT

The experiment was conducted at the Horticulture Farm of Sher-e-Bangla Agricultural University, Dhaka from October 2018 to April 2019 to mitigate salt stress in chilli. Seedlings of 30 days of BARI Morich-3 were used as planting material. The two factors experiment was laid out in Randomized Complete Block Design with three replications. Factor A: Four levels of sodium chloride (Na^+) salt viz. (i) S_0: Control, (ii) S_1: 4 dSm^{-1}, (iii) S_2: 8 dSm^{-1} and (iv) S_3: 12 dSm^{-1}; Factor B: Three levels of calcium nitrate (Ca^{2+}) as mitigating agent (i) M_0: Control, (ii) M_1: 6 mM and (iii) M_2: 12 mM Ca^{2+}. The results of this experiment showed that, the salt stress reduced the morphological parameters and yield (kg) of chilli with the increased level of salinity. The shortest plant height (35.28 cm), number of branches per plant (8.22), number of leaves per plant (18.33), individual fruit weight (1.29 g) and yield per plant (23.11 g) was recorded at S_3 whereas the highest value (68.66g) was recorded from S_0. The results also showed that Ca^{2+} significantly increased the growth contributing characters as well as yield of chilli in both saline and non-saline conditions. For combined effect, the tallest plant (54.26 cm) was found from S_0M_1 at 75 DAT and highest number of fruits per plant (44.33) counted from S_0M_2, highest weight of individual fruit (2.22 g) and the highest yield per plant (99.33 g) was recorded from S_0M_2; whereas the lowest yield per plant was recorded (17.33 g) from S_3M_0. This result suggests that, exogenous Ca^{2+} can effectively mitigate the detrimental effect of salt stress in chilli.

CHAPTER 1

INTRODUCTION

Chilli (*Capsicum frutescens*) is one of the most important spice crops in Bangladesh. It is well known for its hot and burning flavor used as spices or minor ingredients in various dishes, spice blends and sauces. Chemical analysis of chilli has shown that red chilli fruit contains 15.9% protein, 31.6% carbohydrate, 50 mg/100g vitamin C and small quantities of vitamin A, B and E with minerals like molybdenum, manganese, folate, potassium, thiamin. Chilli contains seven times more vitamin C than orange. Chilli has been included in ayurvedic medicines and used as tonic to remove many diseases.

Depending on yield and consumers preference a number of chilli genotypes are being cultivated throughout the country. The actual area under chilli cultivation in Bangladesh is not available due to its seasonal nature of cultivation.

However, various abiotic environmental stresses such as drought, high or low temperature, salinity, flooding, metal toxicity, etc. which pose serious threat to chilli production. The coastal areas of Bangladesh have minimum cropping intensity due to dry season salinity of soil and water. Salinity is a major environmental constraint limiting yield of crop plants in many semi-arid and arid regions. High concentrations of salts in soil are responsible for the decrease in the yield of chilli. Salinity disturbs the physiology of plants by changing the metabolism of plants (Garg *et al.*, 2009). It is well known that the basal or foliar application of Ca^{2+}, Mg^{2+}, K^{+}, proline, salicylic acid can mitigate the adverse effects of salinity.

Among them, Calcium (Ca^{2+}) Nitrate can be exogenously applied for improving the soil chemical properties leading to the enhancement of crop productivity in the saline soil. Ca^{2+} restricts the entry of Na^{+} into the plant cells under salt stress reported (Hossain, 2010; Lazof and Bernstein, 2012). Calcium is a slowly moving element and it is passively transported by the xylem through the transpiration streams from leaves and fruits. It is reported that chilli fruit surface has no stomata thus cuticular transpiration is the only way of water movement from fruit to atmosphere.

Some earlier reports showed that exogenous Ca^{2+} can ameliorate the detrimental effects of salt stress in different chilli species. Ca^{2+} has obtained particular attention because of inducing protective effects on plants under NaCl salinity. To overcome the current need of chilli, the chilli production needs to be increased and saline area must be considered for chilli cultivation through proper use of Ca^{2+} to obtain the following objectives-

1. To find out the effect of salt stress on growth, yield and yield contributing characters of chilli

2. To study the role of Ca^{2+} to mitigate the detrimental effect of salt stress in chilli

CHAPTER 2

REVIEW OF LITERATURE

Chilli is an important spices crop of many countries of the world as well as in Bangladesh. It is adapted to a wide range of climates ranging from tropics to within a few degrees of the Artic Circle. However, in spite of its broad adaptation, production is concentrated facing in a diverse biotic factor and abiotic stress conditions. But very few researches are work available related to growth, yield and development of chilli due to stress especially salt and also the mitigation of salt stress. The research work so far done in Bangladesh is not adequate and conclusive. However, some of the important and informative works and research findings related to the salt stress and also the mitigation of salt stress in vegetable crops as well as chilli, so far been done at home and abroad, have been reviewed in this chapter under the following heads:

2.1 Effect of salinity on morphological characters of plant

Ali (2004) conducted a research on Salt tolerance in eighteen advanced rice genotypes was studied under an artificially salinized (EC= 8.5 dSm^{-1}) soil conditions after 90 days of transplanting. The results showed that the yield per plant, and number of productive tillers, panicle length and number of primary branches per panicle of all the genotypes were reduced by salinity.

Babu and Thirumurugan (2001) conducted a pot experiment to study the effect of salt priming on growth and development of sesame under induced salinity condition. Salinity was induced by addition of 35, 70 and 140 mM NaCl solution to create three levels of salinity and observed that plant height decreased with the increased salinity level. Similar results were also observed by many researchers in sesame by Ragiba (2000).

BINA (2008) studied the screening of wheat varieties for growth and yield attributes contributing to salinity tolerance and reported that wheat varieties of high yielding and tolerant group recorded a higher value of number of effective tillers plant^{-1}.

Chakraborti and Basu (2001) conducted a pot experiment to study the effect of salinity (0, 6 and 9 dSm^{-1}) on growth and development of sesame under induced salinity condition and observed that number of leaves decreased with the increased salinity level. Moreover, Javaid *et al.* (2002) investigated the salinity effect (0, 20, 50 and 75 mM NaCl) on plant height, stem diameter, TDM, leaf number and leaf area in four *Brassica* species and reported that salinity affected the morphological characters of the studied plants and leaf number as well as leaf area decreased with increased salinity levels.

Çiçek and Çakirlar (2002) observed salt stress caused a significant decrease in shoot length, fresh and dry weights of shoot and leaf area of both cultivars with the increase of stress treatments. However, high concentration of proline (100 mM) was not so much effective as compared to low concentration i.e. 50 mM.

Ewase (2013) said that, a pot experiment was carried out to study the effect of salinity stress on plants growth of Coriander (*Coriandrum sativum* L.) by the selection method. For this purpose, four treatments of different concentrations of NaCl were used, namely, 0, 1000, 2000, 3000 and 4000 ppm of NaCl. The following parameters of: plant length, number of leaves, roots number and length were recorded. The Obtained results showed that all growth parameters were reduced by increasing the NaCl concentration. Coriander plants were found to resist salinity up to the concentration of 3000 ppm NaCl only.

Islam (2004) conducted a pot experiment to study the effect of salinity (3, 6, 9, 12 and 15 dSm^{-1}) on growth and development of rice under induced salinity condition and observed that number of leaves decreased with the increased salinity level. Similar result was also observed by Rashid (2005) in rice.

Jafari (2009) studied the interactive effects of salinity, calcium and potassium on physio-morphological traits of sorghum (*Sorghum biclolor* L.) in a green-house experiment. Treatments included 4 levels of NaCl (0, 80, 160, and 240 mM NaCl), 2 levels of $CaCl_2$ (0 and 20 mM), and 2 levels of KCl (0 and 20 mM). Salinity substantially reduced the plant growth as reflected by a decrease in the plant height, shoot and root weight.

Javaid *et al.* (2002) investigated the salinity effect (0, 20, 50 and 75 mM NaCl) on plant height in four rice variety and reported that salinity affects the morphological characters of the studied plants and plant height decreased with increased salinity levels. Similar results were also reported by Thakral *et al.* (2001) in twenty-nine Ethiopian mustard (*Brassica carinata*) and by Uddin *et al.* (2005) in *B. campestris*.

Liu *et al.* (2008) reported significant reduction in the dry biomass of halophyte *Suaeda salsa* when exposed to different concentration of NaCl under different water regimes.

Memon *et al.* (2007) conducted a pot experiment on silty clay loam soil at Sindh Agriculture University, in Tando Jam, Pakistan. Sarokartuho variety of Sorghum (*Sorghum bicolor* L.) was continuously irrigated with fresh (control) and marginally to slightly saline EC 2, 3, 4 and 5 (dSm^{-1}) waters. Increasing water salinity progressively decreased plant height and fodder yield (fresh and dry weight) per plant.

Milne (2012) studied on the effects of 30 and 60 mM NaCl on Lettuce (*Lactuca sativa* L.), grown in soilless culture, with additions of 0, 1, 2 and 4 mM Si was evaluated. Height, leaf number, weight, chlorophyll content and elemental analysis of plants were examined.

Mortazainezhad *et al.* (2006) had observed that tiller number decreased with increasing salinity levels imposed at all growth stages in rice. Soil salinity affects the growth of rice plant. But the degree of deleterious effect may vary on the growth stages of plant. During germination rice is tolerant, but it becomes very sensitive during the early seedling stage. Similar result was also reported by many workers in rice (LingHe *et al.*, 2000; Burman *et al.*, 2002; Islam, 2004; Rashid, 2005; Karim, 2007). LingHe *et al.* (2000) further reported that decreased tiller number was the major causes of yield loss.

Munns (2002) observed that when salt concentration increases inside the plant, the salt starts to accumulate inside the older leaves and eventually they die. If these older leaves die at a rate greater than that at which new leaves generate, it reduces the capacity of plants to supply the carbohydrate requirements of younger leaves leading to reduction in their growth rate (Munns *et al.*, 2006).

Munns (2005); Munns and Tester (2003) reported that salt-induced osmotic stress is the major reason of growth reduction at initial stage of salt stress, while at later stages accumulation of Na^+ occurs in the leaves and reduces plant growth.

Nawaz *et al.* (2010) reported that applications of salt in the growth medium caused reduction in shoot length of sorghum cultivars. Under saline conditions 50 mM proline was more effective to reduce the effect of NaCl than 100 mM proline in both cultivars. Proline level 50 mM showed 26.58% and 11.78% increased shoot length as compared to NaCl stresses plants.

Netondo *et al.* (2004) conducted an experiment where sorghum plants were grown in sand culture under controlled greenhouse conditions. The NaCl concentrations in complete nutrient solution were 0 (control), 50, 100, 150, 200, and 250 mM. Salinity significantly reduced leaf area by about 86% for both varieties of sorghum and these decreases were similar for the two sorghum varieties.

Parida and Das (2005) observed salt stress affects some major processes such as root/shoot dry weight and Na^+/K^+ ratio in root and shoots.

Saberi *et al.* (2011) conducted a pot experiment where two forage sorghum varieties (Speed feed and KFS_4) were grown under salinity levels of 0, 5, 10 and 15 dSm^{-1}. Leaf area of plants were also reduced in response to salinity and decreasing soil water availability, while the suppressive effect was magnified under the combined effect of the two factors. Salinity and water stress significantly affected the total leaf area of ratoon crop. The maximum total leaf area was obtained in the control treatment but with increasing salinity and infrequent irrigation, this parameter was found to decrease. Maximum leaf area of 1167 mm^2 $plant^{-1}$ was attained in plants with normal irrigation, without water stress. Under effects of salinity 5, 10 and 15 dSm^{-1} the leaf area was reduced by 7, 12 and 17%, respectively.

Saberi *et al.* (2011) conducted an experiment where two forage sorghum (*Sorghum bicolor* L. Moench) varieties (Speed feed and KFS_4) were grown under salinity levels of 0, 5, 10 and 15 dSm^{-1}. Maximum number of leaves was produced in non-saline soil (13.5 leaves $plant^{-1}$) with normal irrigation (12.4 leaves $plant^{-1}$). Low soil water and high salinity reduced the number of leaves as well as the number of tillers produced.

Saberi *et al.* (2011) studied the response of two forage sorghum varieties, Speed feed and KFS_4 to salinity and irrigation frequency. Two varieties were grown under salinity levels of 0, 5, 10 and 15dSm^{-1}. Salinity, irrigation frequency and variety significantly affected the number of ratoon tillers. The number of tillers declined with increase in salinity and with less frequent irrigation. Speed feed variety produced a higher number of tillers than KFS_4.

Parti *et al.* (2002) conducted an experiment where salinity levels of 4, 8 and 12 dSm^{-1} were obtained from adding chloride and sulphate salts of sodium, calcium and magnesium. All salinity treatments affected plant growth considerably.

Sixto *et al.* (2005) stated that depending on increasing salinity levels, decrease in vegetative growth parameters has been observed in plants. Decrease in root, stem and shoot developments, fresh & dry stem and root weights; leaf area and number and yield have been observed in plants subject to salinity stress. They also found that number of branch decreased with increased salinity in all the studied Brassica species. This phase may be recognized by the appearance of some specific symptoms of plant damage in the leaves such as color change, tip burn, marginal necrosis and succulence (Munns and Tester, 2008).

Uddin *et al.* (2005) studied salt tolerance on nine *Brassica juncea* varieties along with one *Brassica carinata* variety.

2.2 Effect of salinity on Physiological Attributes of plant

A higher level, salinity limits the concentration of K^+ and Ca^{2+} in the leaves and roots of *Brassica napus* (canola) cultivars (Ulfat *et al.*, 2007; Ashraf and Ali, 2008).

A significant decline in the net photosynthesis is an immediate effect of stomatal closure coupled with photorespiration in plants exposed to high salinity stress. This short-term response to salinity exposure lasts for 24-48 hours and completely ceases photosynthesis (Parida *et al.,* 2005).

A significant decrease in the stomatal density of tomato plants was recorded when treated with 70 mM of NaCl in a sand culture experiment (Romero-Aranda *et al.,* 2001).

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

Ali (2004) conducted a research on Salt tolerance in eighteen advanced rice genotypes was studied under an artificially salinized (EC=8.5 dSm^{-1}) soil conditions after 90 days of transplanting. The results showed that the chlorophyll concentration was reduced by salinity. Although ROS have roles as signaling molecules, active generation of which can be initiated by abiotic stresses (Desikan *et al.*, 2005).

Azooz et al., (2004) studied the salt tolerance of 3 sorghum (*Sorghum bicolor*) cultivars (Dorado, Hagen Shandawil and Giza 113) and their responses to shoot spraying with 25 ppm IAA. The differences in the tolerance of the sorghum cultivars were associated with large differences in K^+ rather than in Na^+, which was found to be similar in the whole plant. The youngest leaf was able to maintain a higher K^+ content than the oldest leaf. Consequently, the K^+/Na^+ ratios were higher in the most salt tolerant cultivar Dorado than in the other sorghum cultivars, and in the youngest than in the oldest leaf.

Bavei et al., (2011) studied the tolerance of sorghum varieties in terms of fresh weight, ion accumulations, proline content and peroxidase activity was analyzed in this study. Three sorghum varieties, Payam, Kimia, and Jambo, differing in salt tolerance, were grown in a greenhouse-hydroponic culture with a complete nutrition solution to which 0, 50, 100, 150 and 200 mM NaCl was added. Plant roots and leaves were harvested at 15 and 30 days after treatment and subjected to analysis. Clear decline in K^+ and Ca^{2+} concentrations and increase in Na^+ and proline contents were observed in the root and leaf tissues at each NaCl concentration in all varieties during the NaCl treatment.

Cicek and Cakirlar (2002) observed the effect of salinity on physiological attributes of maize cultivars. They found that salinity caused a marked decrease in relative water contents of maize plants. They further concluded that amount of proline, Na^+ and Na^+/K^+ ratio increased under salt stress condition.

GSH are well known antioxidants, and higher concentrations would infer superior antioxidative defence (Tausz *et al.*, 2004), and would therefore logically result in a decrease in ROS concentrations brought about by salinity stress (Foyer *et al.*, 2005).

Haghighiet (2012) conducted a study to evaluate the effectiveness of salinity on on seed germination and growth characteristics of chilli seeds A laboratory experiment was performed on completely random-ized design with two levels of salinity (25 and 50 mM NaCl) A laboratory experiment was performed on completely randomized design with two levels of salinity (25 and 50 mM NaCl) and 2 concentration of Si (1 and 2 mM) with 4 replications. Germination percentage, germination rate, seedling shoot and root length, fresh and dry weight of seedling and mean germination time was measured. Seed germination of *Lycopersicon esculentum* L. was significantly affected by salinity levels, Si and their interaction. Germination characteristics of chilli seeds decreased drastically increasing by NaCl concentrations. However, 1 mM Si had positive effects on seed germination characteristics and improved germination percentage, germination rate and mean germination time. Si alleviated the harmful effect of salinity stress on chilli seed germination at almost all germination characteristics.

Hamayun (2010) reported that, the adverse effects of NaCl induced salt stress on growth attributes and endogenous levels of gibberellins (GA), abscisic acid (ABA), jasmonic acid (JA) and salicylic acid (SA) soybean cv. Hwangkeumkong was showed. Chlorophyll content was significantly decreased in response 70 mM and 140 mM concentrations of NaCl.

In barley, short term exposure to high salinity leads to an immediate and significant drop in stomatal conductance, due to osmotic stress and local synthesis of ABA (Fricke *et al.*, 2004 and 2006).

In view of these reports, it is quite clear that salt stress limits the accumulation of essential nutrients such as K^+, Mg^{2+} and Ca^{2+} while increases the concentration of Na^+ in most of crop species thereby resulting in reduced growth and yield. This argument is further supported by a number of studies in which it was found that exogenous application of salt-induced deficient nutrient such as Ca, K or N can mitigate the adverse effects of salinity on growth of many crops e.g., wheat, sunflower and beans etc. (Shabala *et al*,. 2003)

Increases in GSH concentrations are known to be associated with salinity stress (Leyva *et al.*, 2011).

Ionic imbalance occurs in the cells due to excessive accumulation of Na^+ and Cl^- and reduces the uptake of other mineral nutrients, such as K^+, Ca^{2+} and Mn^{2+} (Karimi *et al.*, 2005).

It has also been reported that high salt concentration either causes an increase in the N-contents and high protein content in some glycophytic plants (Jones and Mac Millan, 1987) or increase in soluble proteins (Shaddad *et al.,* 2005).

Lacerda (2001) studied the effects of high NaCl concentration on plant growth, on inorganic solute transfer to shoot and on the accumulation and distribution of inorganic solutes (Cl^-, Na^+, K^+, Ca^{2+} and Mg^{2+}) were evaluated to better understand the mechanism of salt tolerance in two sorghum cultivars (salt tolerant; ST87-11- ST88-03-ST89-02-ST90-01-ST91-03-ST91-B-Ca92B and salt sensitive: 51-Ca84-B1-Ca87-B2-BCa89). NaCl at 0 and 100 mM were added in 25 mM increments. At 0, 4 and 8 days after the beginning of NaCl treatment, samples were collected to evaluate the root dry matter yield and to determine ion contents in the shoot. The Na^+ and Cl^- transfer rates to the shoot during the experimental period of (0-4) days increased an average of about five times with increasing saline treatment in both cultivars. The sensitive cultivar showed higher Na^+ plus Cl^- transfer rates to the shoot, especially in the beginning of the stress application and greater accumulation of these ions in the leaves.

Memon *et al.* (2007) experimented on sarokartuho variety of sorghum that was continuously irrigated with fresh (control) and marginally to slightly saline EC 2, 3, 4 and 5 (dSm^{-1}) waters in a pot experiment. Saline water treated plants contained more Na^+, less K^+ and showed lower leaf K^+/Na^+ ratio.

Moreover, numerous studies have revealed that salt stress can reduce K^+, Ca^{2+} and N accumulation in different crop plants, e.g. wheat (Raja *et al. 2006*) sunflower, radish, cabbage (Jamil *et al.*, 2007) and canola (Ulfat *et al.*, 2007).

Mostafa (2004) observed that at low and moderate salinity levels, sugars and consequently the total carbohydrates are decreased. Soluble protein is generally decreased in response to salinity (Parida *et al.,* 2002 and Abed-Latef, 2005).

Netondo *et al.* (2004) conducted an experiment where sorghum plants were grown in sand culture under controlled greenhouse conditions. The NaCl concentrations in complete nutrient solution were 0 (control), 50, 100, 150, 200, and 250 mM. Chlorophyll a and b, net assimilation, stomatal conductance, and transpiration rate decreased significantly with the increase in salinity, and these decreases were similar for the two sorghum varieties.

Netondo *et al.* (2004) conducted an experiment where sorghum plants were grown in sand culture under controlled greenhouse conditions. Leaf growth, gas exchange, and chlorophyll fluorescence of two sorghum (*Sorghum bicolor L. Moench*) varieties, Serena and Seredo, were measured in response to increasing NaCl concentration. The NaCl concentrations in complete nutrient solution were 0 (control), 50, 100, 150, 200, and 250 mM. The results indicate that salinity affected photosynthesis per unit leaf area indirectly through stomatal closure, and to a smaller extent through direct interference with the photosynthetic apparatus. In addition, salinity decreases whole plant photosynthesis by restricting leaf area expansion. This effect starts from low levels of salinity, in contrast to that of net photosynthesis per unit leaf area, which occurs at higher levels of NaCl concentration. It has been stated that depending on increasing salinity levels decrease in chlorophyll amount have been observed in plants subject to salinity stress.

Netondo *et al.* (2004) studied two Kenyan sorghum varieties, Serena and Seredo, were grown in a greenhouse in quartz sand supplied with a complete nutrient solution to which 0 (control), 50, 100, 150, 200, and 250 mM NaCl was added. Results showed that roots and stems accumulated substantial amounts of sodium, saturating at 150 mM external NaCl. Accumulation of K^+ and Ca^{2+} in the roots, stems, and leaves was strongly inhibited by salinity. Leaves continuously accumulated sodium, which was preferentially deposited in the sheaths. Mature leaves contained more Ca^{2+} and Mg^{2+} than young ones. The two sorghum varieties appear to sequester Na^+ predominantly in roots, stems, leaf sheaths, and older leaf blades.

Osmotic stress (drought problem), ion imbalances, particularly with Ca and K, and the direct toxic effects of ions on the metabolic process are the most important and widely studied physiological impairments caused by salt stress (Zhu, 2001; Munns, 2002; Munns *et al,*. 2006). Salt stress, like many abiotic stress factors, also induces oxidative damage to plant cells catalyzed by reactive oxygen species (Mittler, 2002; Demidchik *et al,*. 2003; Azevedo-Neto *et al.,* 2006).

Patel *et al.* (2010) reported that, Salinity induced a significant increase in Na^+, Cl^- and proline concentrations, while reduced the accumulation of K^+ and Ca^{2+} in leaves of all the cultivars of cowpea.

Plant stresses, including salinity stress, are known to disturb cellular homeostasis, enhancing the production of ROS (Dat *et al.*, 2000). Additionally, osmotic stress, one of the foremost stresses associated with high salinity levels, has shown to cause the production of ROS (Xiong and Zhu, 2002).

Salinity adversely affects reproductive development by inhibiting microsporogenesis and stamen filament elongation, enhancing programmed cell death in some tissue types, ovule abortion and senescence of fertilized embryos. In Arabidopsis, 200 mM NaCl stress causes as high as 90% ovule abortion (Sun *et al.*, 2004).

Salinity arrests the cell cycle transiently by reducing the expression and activity of cyclins and cyclin-dependent kinases that results in fewer cells in the meristem, thus limiting growth (West *et al.* 2004).

Salinity stress is known to result in an excess production of reactive oxygen species (ROS), oxidative damage and a change in concentrations of antioxidants (Bor *et al.*, 2003; Sekmen *et al,* 2007; Gao *et al,* 2008). Consequently, ROS are good cellular indicators of stress (Mittler, 2002).

This is interesting when considering that, contrary to conventional thought, active plant growth cessation, as opposed to stress limiting growth, has been linked to surviving adverse environmental conditions (Harberd *et al.*, 2009), including salinity stress (Magome *et al.,* 2008).

Zuccarini (2008) studied the effect of Si on *Phaseolus vulgaris* L. under two level of salinity (30 and 60 mM). His results showed that salinity decreased stomatal conductance and net photosynthetic rate.

2.3 Effect of salinity on yield and yield contributing characters of plant

A field experiment was conducted by Leena and Kiran(2003) in Vadodara, Gujarat, India to test the effect of salt stress on *Sorghum bicolor*. Though there was a reduction in the chlorophyll content of the plants subjected to salt stress, the fresh and dry weights of the plants were reduced only at the earlier stages. Abdullah *et al*. (2001) conducted an experiment for finding out the effect salinity stress on seed set of IR 28 rice under different salinity levels and found that panicle length was significantly decreased due to salinity stress.

Again, Hossain (2002) conducted a pot experiment with three salinity levels (0, 6 and 9 dSm^{-1}) and observed that harvest index decreased with increase of salinity level in rice. Similarly, Islam (2004) reported that harvest index decreased with the increase of salinity level in rice.

Again, Hossain (2006) worked with rice to know the effect of different levels of salinity (0, 6, 9, and 15 dSm^{-1}) on yield attributes and dry matter partitioning and reported that harvest index decreased with increased salinity levels. Similar result was also reported by Rana (2007) in rice.

Ali *et al.* (2003) conducted an experiment to know the effect of four levels of salinity (0, 3.0, 4.5 and 6.5 dSm^{-1}) on plant biomass production, leaf area and yield attributes of two soybean varieties viz. Ertou and S-95-1. They found that salinity induced a marked reduction in yield attributes like siliqua plant-1 and 1000-seed weight and seed yield.

Ali *and Islam* (2005) conducted a pot experiment with three salinity levels (0, 6 and 9 dSm^{-1}) and observed that 1000-seed weight decreased with increased salinity level in sesame.

Amini and Ehsanpour (2006) reported decrease in chlorophyll content in chilli cultivar due to salt stress.

Chakraborti and Basu (2001) studied salt tolerance ability in 9 sesame varieties under saline condition and reported that capsules plant^{-1}, seeds capsule^{-1} and seed yield decreased under saline condition in all studied varieties of sesame.

Debnath (2003) and Rahman (2003) worked with mustard to know the effect of different levels of salinity (0, 5, 7, 10 and 15 dSm^{-1}) on yield attributes and dry matter partitioning and reported that harvest index decreased with increased salinity levels.

Gain *et al.* (2004) conducted pot experiment to study the effect of different levels of salinity (0, 7.81, 15.62, 23.43 and 31.25 dSm^{-1}) on growth and yield attributes of rice and reported that total dry mass decreased gradually with increased levels of salinity. Campos *et al.* (1999) grew rice varieties in nutrient solution with NaCl and reported that total dry mass (TDM) was decreased with increasing salinity. Similar result was also reported by many workers in rice (Cristo *et al.,* 2001; Hossain, 2002; Islam, 2004; Hossain, 2006; KyuSeeong *et al*., 2007).

Gain *et al.* (2004) reported that elevated salinity levels significantly decreased the total and filled grains panicle^{-1} and 1000-grain weight which resulted decrease grain yield. Similarly, Similar result was also reported by Rashid (2005) in rice. Again, Hossain (2002) carried out an experiment with rice to know the effect of salinity (0, 3, 6 and 9 dSm^{-1}) on growth, yield attributes and yields, and reported that grain yield decreased with increasing salinity levels.

Gain *et al.* (2004) studied the effect of salinity (0, 7.81, 15.62, 23.43 and 31.25 dSm^{-1}) on yield attributes and yield in rice and reported that number of spikelet panicle^{-1} decreased with increasing salinity levels but the decrement was less in salt tolerant varieties than salt susceptible varieties This statement was supported many workers (Islam, 2004; Hossain, 2006). Chakraborti and Basu (2001) studied salt tolerance ability in 9 sesame varieties under saline condition and reported that capsule per plant, seeds per capsule and seed yield decreased under saline condition in all studied varieties of sesame.

Gain *et al.* (2004) studied the effect of salinity (0, 7.81, 15.62, 23.43 and 31.25 dSm^{-1}) on yield attributes and yield in rice and reported that 1000-grain decreased with increasing salinity levels but the decrement was less in salt tolerant varieties than salt susceptible varieties. Sen (2002) conducted a pot experiment with three salinity levels (3, 6 and 9 dSm-1) and observed that 1000- grain weight decreased with increased salinity level in rice. Similar result was also reported by Abudullah *et al.* (2001) in rice who observed that 1000-grain weight decreased with increased salinity levels. Again, Ali and Awan (2004) opined that generally, salinity caused a marked reduction in yield and yield components in rice except a few highly tolerant varieties.

Hajer *et al.* (2006) conducted two different experiment separately on chilli under saline condition and reported that fruit yield decreased with increased salinity separately.

Hajer *et al.* (2006) reported that the effect of NaCl salinity stress on the growth of chilli plants was reflected in lower fresh and as well as dry weights.

Karim (2007) conducted an experiment to investigate the effect of different salinity levels (0, 6, 9 and 12 dSm^{-1}) and reported that all parameters including panicle length decreased with increased salinity levels. Panicle length was adversely affected by soil salinity levels as reported by most of the researchers (Hossain, 2002; Islam, 2004; Natarajan *et al.*, 2005; Rana, 2007).

Karim (2007) conducted an experiment to investigate the effect of different salinity levels (0, 6, 9 and 12 dSm^{-1}) and reported that all parameters including grain yield decreased with increased salinity levels. The yield was decreased due to production of decreased number of effective tillers $hill^{-1}$, decreased number of grains $panicle^{-1}$and 1000-seed weight. Similar result was also reported by many researchers (Hossain, 2002; Sen, 2002; Rashid, 2005; Hossain, 2006).

Netondo *et al.* (2004) conducted an experiment to determine how salinity affects growth, water relations, and accumulation of cations of nutritional importance in various organs of grain sorghum. Two Kenyan sorghum varieties, Serena and Seredo, were grown in a greenhouse in quartz sand supplied with a complete nutrient solution to which 0 (control), 50, 100, 150, 200, and 250 mM NaCl was added. The 250 mM NaCl treatment significantly reduced the relative shoot growth rates, measured 25 days after the start of salt application, by 75 and 73%, respectively, Serena and Seredo, and stem dry weight.

Parti *et al*. (2002) conducted an experiment where salinity levels of 4, 8 and 12 dSm^{-1} were obtained from adding chloride and sulphate salts of sodium, calcium and magnesium. All salinity treatments affected plant growth considerably. The dry matter weight was maximum at 4 dSm^{-1} and beyond this level, a constant decreased with increased salinity in TDM, plant height and siliqua $plant^{-1}$ was observed.

Rafat and Rafiq (2009) reported that, total chlorophyll content in chilli plant proportionally decreased with the increase in salinity levels up to 0.4% sea salt solution (EC 5.4 dSm^{-1}).

Rana (2007) carried out a pot experiment with 5 levels of salinity (0, 3, 6, 9 and 12 dS/m) of three rice varieties viz., BRRl dhan 42, STM-1 and STM-2 and reported that plant height, number of tillers $hill^{-1}$, TDM $hill^{-1}$, leaf area $hill^{-1}$, root dry weight $hill^{-1}$ and yield contributing characters and yield decreased significantly with increase in salinity levels. Among the advanced rice lines BRRIdhan-42 showed more tolerance for all studied parameters compared to STM-1 and STM- 2.

Rivelli *et al.* (2002) conducted an experiment during 1997 and 1998 in Metaponto (MT), Italy, to evaluate the growth and yield response of paper sorghum (*Sorghum bicolor* x *S. dochna*) to irrigation with saline water (EC 0.9, 5, 5 plus leaching requirement, and 10 dSm^{-1}). A significant increase in soil salt concentration (EC) was measured at the end of the growing cycle. Winter rain and the application of leaching requirement were not sufficient for soil reclamation. A significant decrease in yield was observed due to saline stress. In 1997, the total epigeous dry matter decreased from 3900 g/m_2 in the control to 2450 g/m_2 of the most saline treatment. In 1998, sorghum sown in the same spot as the previous year produced lower yield than in 1997 due to higher salt accumulation in the soil. The soil threshold limit for salinity was 3.5 dS/m; where for each unitary decrease of soil EC a relative yield decrease of 9.4% was measured. Paper sorghum can be classified between moderately tolerant and moderately sensitive crops.

The responses of forage sorghum (*Sorghum bicolor* L. Moench) varieties to salinity and irrigation frequency were studied from December 2007 to December 2009 by Saberi *et al.* (2011). Increased salinity significantly reduced forage dry yield from 44.09 gm plant^{-1} in the control to 32.76 g plant^{-1} at salinity with 15 dSm^{-1}. For every one unit increase in salinity, the forage yield decreased by 5.2 units and for every one unit increase in water stress (irrigation frequency), the forage yield decreased by 3.6 units. The variety Speed feed had higher total dry mass than KFS4 under well-watered conditions but KFS4 performed better than Speed feed under water stress. For both varieties, infrequent watering reduced dry matter and biomass accumulation, but increased water use efficiency (WUE) (6.88).

Thimmaiah (2002) grew sorghum (*Sorghum bicolor*) under different levels of salinity (1, 2, 4, 6, 8 and 12 dSm^{-1}) in irrigation water and investigated for yield and yield components and biochemical composition. Seed and straw yield, seed weight per ear, N, P, K and Ca content, protein content and total amylolytic enzyme activity differed significantly due to salinity. However, these parameters were, more or less, at par with each other in the range of 2 to 8 dSm^{-1}. The 1000-seed weight, Mg^{2+} content and invertase [beta-fructofuranosidase] enzyme 14 activity were unaffected by salinity. 1000-seed weight, yield and yield components decreased significantly at 12 dSm^{-1} salinity.

Uddin *et al.* (2005) conducted an experiment to study salt tolerance of *B. napus* and *B. campestris* varieties under saline conditions (1.2-11.5 dSm^{-1}) and observed that siliqua number and seeds $siliqua^{-1}$ decreased with increased salinity.

2.4 Role of Ca^{2+} to mitigate the saline toxicity

Anbu and Sivasankaramoorthy (2014) worked on a pot culture was carried out with *Oryza sativa* L. vari-Co-39, to investigate the effects of supplementary calcium chloride on plants grown at NaCl (50mM) concentration. Treatments were: (1) Control: nutrient solution alone (C); (2) nutrient solution plus 50mM sodium chloride (NaCl); (3) nutrient solution plus 10mM calcium chloride ($CaCl_2$); (4) nutrient solution plus 15mM calcium chloride ($CaCl_2$); (5) nutrient solution and 50 mM NaCl plus supplementary 10 mM $CaCl_2$ (NaCl + $CaCl_2$); and (6) 50 mM NaCl plus additional mixture of 15 mM $CaCl_2$ in nutrient solution (NaCl + $CaCl_2$). The plants grown under salt stress produced low dry weight and relative water content than those grown in standard nutrient solution and in $CaCl_2$ alone. Supplemental calcium chloride added to nutrient solution containing salt significantly improved growth and relative water content. Membrane permeability increased with high NaCl application and these increases in root membrane permeability were decreased with supplementary Ca. The concentration of chloride (Cl) increases highly for all treatments. Sodium (Na) concentration in plant tissues increased in both shoots and roots at high NaCl treatment. Application of supplementary Ca lowered Na concentration. Concentrations of Ca. K and N were at deficient ranges in the plants grown at high NaCl levels and these deficiencies were corrected by supplementary Ca. The ameliorating effect of Ca on growth and physiological variables could reduce the negative effect of salinity of *Oryza sativa* L. plants.

Calcium is an essential plant nutrient and has a role in metabolic activities, like stabilization of membranes, signal transduction through second messenger, and control of enzyme activity in Cassia angustifolia (Arshi *et al.* 2006). Ca^{2+} can help to remediate the adverse effect of salinity on plants. It helps in maintaining membrane integrity and ion-transport regulation and is essential for K^+/Na^+ and Ca^{2+}/Na^+ selectivity in *Cornus stolonifera* (Renault, 2005). Elevated Ca^{2+} concentration in nutrient solution mitigates the adverse effects of NaCl by inhibiting Na^+ uptake (Kaya *and Higgs*, 2002) and reducing membrane leakage.

Tuna *et al. (*2007) reported that K^+ concentrations reduced by salinity, can be restored to adequate levels by an additional supply of calcium, as it protects cell membranes from adverse effect of Na^+ and minimizes the leakage of cytosolic potassium. Calcium plays a vital role in the regulation of ionic relations in plants and in improving the soil physical conditions (Qadir *et al.,* 2001).

Calcium nutrition plays an important role in the maintenance of a high growth rate under saline conditions. Several reports show a significant role of Ca in improving the salt tolerance of plants. In studies on the soybean and cucumber, an additional supply of Ca to salt-stressed plants improved the salt tolerance of plants by reducing Na uptake and transport (Dabuxilatu and Ikeda, 2005).

According to Husain *et al.* (2004), the major role of Ca in increasing the salt tolerance of plants is related to its inhibitory effect on the xylem loading of Na and thus decreases in shoot Na concentration.

Chaum *et al.* (2012) reported that exogenous calcium in the root zone may absorb by root tissues, transfer to whole plant and function as salt defense mechanisms including calcium signaling in the abscisic acid (ABA) regulation system and calcium sensing in stomatal closure when plant subjected to salt stress.

Gobinathan et al., (2009) *Pennisetum* plants were grown with NaCl and $CaCl_2$ in order to study the effect of $CaCl_2$ on NaCl induced oxidative stress in terms of osmolyte concentration, proline (PRO)-metabolizing enzymes. The plants were treated with solutions of 100 mM NaCl, 100 mM NaCl with 5 mM $CaCl_2$

and 5 mM $CaCl_2$ alone. Groundwater was used for irrigation of control plants. Plants were uprooted randomly on 40 days after sowing (DAS). NaCl-stressed plants showed increased glycine betaine (GB) and PRO contents, decreased proline oxidase (PROX) activity and increased glutamyl kinase (GK) activity when compared to control. Addition of $CaCl_2$ to NaCl-stressed plants lowered the PRO concentration by increasing the level of PROX and decreasing the gama-GK activities. Calcium ions increased the GB contents. $CaCl_2$ appears to confer greater osmoprotection by the additive role with NaCl in GB accumulation.

Hameda and Ahmed (2013) a greenhouse experiment was carried out to study the response of presoaked chilli seeds (*Lycopersicon esculentum* Mill. var. *Cerasiforme*) in freshly prepared ascorbic acid (50 ppm ASC) or distilled water (control) for 12 h at natural environmental conditions, to reduce the effect of salinity stress. Generally, the chilli seeds germination occurred after 3 days, while, the germination rate (%) were faster after soaking the seeds in ascorbic acid (ASC) compared with control (soaked in distilled water). NaCl salt-stress treatments caused a reduction in all growth parameters (fresh and dry weights of plant, leaf area and number per plant) compared control, particularly at high NaCl level (8000 ppm) more reduced. In the meantime, ascorbic acid had reduced the effect NaCl salinity stress on all growth parameters. Photosynthetic pigments (chlorophyll a & b and carotenoids) and chloroplast efficiency were increasing with salinity stress, but the response was more pronounced at 8000 ppm NaCl whether alone or combined with ascorbic acid. Also, salinity stress treatments tended to increase all of the total available carbohydrates (Monosaccharide, Disaccharides & polysaccharides), nitrogenous components (protein, amino acids & proline), antioxidase, (catalase, peroxidase & superoxide dismutases) enzymes activities and inorganic mineral elements (Na^+, K^+, N^{3+}, P^{3+}, Ca^{2+}, Mg^{2+} & Cl^-) but after soaked the seeds in ascorbic acid (+ASC), these components tended to increased more. Application of NaCl salinity-stress on chilli plant induced the synthesis of nitrogenous components (protein, amino acids, proline), whereas, the chilli seeds soaked before planting in ascorbic acid (ASC) which leads to remarkably increasing more for all nitrogenous components, anti-oxidase, carbohydrates and inorganic mineral elements content.

Howladar and Rady (2012) studied the effect of coating the seeds with calcium paste before sowing, on plant growth, yield, the contents of some antioxidants and the activities of carbonic anhydrase and nitrate reductase in the *Pisum sativum* L. leaves under the influence of NaCl stress. NaCl stress reduced plant growth, photosynthetic pigment levels, ascorbic acid and calcium contents, and the activities of carbonic anhydrase and nitrate reductase. In contrast, proline and sodium contents were increased. These results are negatively reflected in the yield components. However, seed coating with calcium paste reduced the toxic effects of NaCl on plant growth and yield by increasing leaf pigments, ascorbic acid, proline contents and enzymatic activities. This study clearly highlights the effects of calcium paste as a seed coat in mitigating the phytotoxicity of NaCl stress in pea plants.

It has been mentioned in many reports that the proline was mostly accumulated when plant growth was ceased (Joly *et al.,* 2000). The function of this osmoprotectant is presumed to be protective, with a role in scavenging free radicals (Mansour, 2000). Minimization of reactive oxygen (RoS) as a result of inhibition of photosynthesis and maximization of their removal (scavenging) is likely to be an important response to high salinity, among other stresses (Zhu, 2001). When plants are subjected to stress, the amount of ROS in the cells increases which bring oxidative stress to crops (Xiong and Zhu, 2002).

Khan (2013) reported that salinity reduced the growth of wheat plants. When K and N were applied as foliar spray on the wheat plant, it reduced the effect of salinity and increased the plant growth such as plant height, leaf number plant fresh and dry weight and physiological attributes such as chlorophyll content of wheat plants. Similarly, grains yield is also decreased by salinity but foliar application of K and N mitigated the salinity effect on grains yield.

Lolaei *et al.* (2012) stated the effects of salinity and supplied calcium chloride on growth and leaf ions concentration of chilli (*Lycopersicon esculentum* L.) were investigated in Gorgan, Iran. A factorial experiment was conducted based on RCBD with four NaCl levels (0, 50, 100, and 150 mM) and four $CaCl^2$ levels (0, 100, 200 and 300 mg L^{-1}). Data of growth, yield and leaf's Ca, K, and Na content were subjected to analyze of variance. The results showed that fruit yield decreased under salinity stress. Increasing Ca^{2+} concentration in the nutrient solution increased the fruit yield. Leaf Ca^{2+}, K^+, and N content decreased under salinity stress. Chilli in its response to nutrient solution, salinized with sodium chloride and calcium chloride. The results obtained from this experiment show that salinity stress caused a significant reduction in plant growth, leaf number and fruit weight.

Manivannan *et al.* (2007) worked on the ameliorating effect of calcium chloride on sodium chloride-stressed plants of *Vigna radiata* L. Wilczek. Plants were treated with solutions of 100 mM NaCl, 100 mM NaCl with 5 mM $CaCl_2$, or 5 mM $CaCl_2$. Groundwater was used for irrigation as the control. Plants were harvested randomly 30 and 50 days after sowing. NaCl and $CaCl_2$ stressed plants showed reduced growth as indicated by decreased root length, stem length, total leaf area and dry weight. Proline and glycinebetaine content and the activity of the antioxidant enzymes superoxide dismutase, ascorbate peroxidase and catalase were increased under treatment with NaCl alone and $CaCl_2$ alone. When $CaCl_2$ was combined with NaCl, $CaCl_2$ altered the overall plant metabolism to ameliorate the deleterious effects of NaCl stress and increased the vegetative growth of the plants.

Sivasankaramoorthy (2013) observed a great increase of K^+/Na^+ and Ca^{2+}/Na^+ ratios was observed when $CaCl_2$ was applied alone. The K^+ and Ca^{2+} contents decreased under NaCl stress; but NaCl + $CaCl_2$ treatment reduced the extent of decrease caused by NaCl (Arshi *et al.*, 2010). High Na^+ concentration in root zone inhibits uptake and transport of Ca^{2+} and thus subsequently, salt stressed plants have lower Ca^{2+}/Na^+ ratios (Ashraf and Akhtar, 2004).

Song *et al.* (2006) reported that high levels of external Ca are essential for the maintenance of high root uptake and shoot accumulation of Ca and K on saline soils and thus for avoiding salinity damage in plants as shown in rice plants.

Soualem *et al.* (2014) studied the effect of calcium sulfate ($CaSO_4$) supply under salt stress was studied in two populations of Atriplex halimus from two locations (coastal western Algeria (Oran) and continental semi-arid zone (Djelfa)) contrasted for salinity gradients. The plants were grown in pots and subjected to salt stress (0, 300 or 500 mM NaCl) with a supply of (5 or 10mM) of $CaSO_4$. Growth, mineral, proline and soluble sugars contents were measured. The results showed a reduction in growth with increasing NaCl concentration. The impact of salinity was more pronounced on the inland population than the coastal one. The leaves Na^+ content increased with increasing salt stress and led to reduced plant growth. In response to the intensity of salt stress and $CaSO_4$ supply, plants accumulated more soluble sugars, proline and K^+.

Tzortzakis (2010) reported that, Salinity either of soil or of irrigation water causes disturbance in plant growth and nutrient balance and reduces crop yields. The effects of NaCl salinity and/or calcium or potassium level on the plant growth and severity of gray mold (Botrytis cinerea (De Bary Whetzel) were investigated in endive (*Cichorium endivia* L., cv. Green Curled) grown with the nutrient film technique under greenhouse conditions during early spring. Plants were supplied with nutrient solutions containing 40 mmol L^{-1} of sodium chloride (NaCl) and/or 10 mmol L^{-1} potassium sulphate (K_2SO_4).

Additionally, plants treated with foliar spray of 15 mmol L^{-1} calcium nitrate [$(CaNO_3)_2$] or distilled water. Salinity or K and Ca enrichment mainly affected the upper part of endive plants and reduced leaf area. However, when salinity combined with either K or Ca enrichment, the negative impact of salinity on plant growth was reversed. Salinized and/or K and Ca enriched, plants did not differ in plant biomass, leaf/root ratio, leaf fresh weight, leaf number, and root length. Salinity did not have any impacts on photosynthetic rate, stomatal conductance, and intercellular CO_2 concentration. Indeed, photosynthetic rate and stomatal conductance increased with Ca foliar application and decreased with K while the opposite effects were observed for the intercellular CO_2 concentration. Total nutrient uptake was reduced 2 fold in salt treated plants compared to controls. No symptoms of tip-burn or blackheart were recorded throughout the experimental study. Endive grown in the nutrient film technique had tolerance to NaCl salinity, and this method could be used to exploit saline water in soilless culture. These findings also suggest that a proper management of the salt concentration of the nutrient solution plus external elemental enrichment may provide an efficient tool to improve the quality of leafy vegetables with little effect on yield.

CHAPTER 3

MATERIALS AND METHODS

The experiment was conducted to find out the role of calcium to mitigate salt stress in chilli. The materials and methods include for this experiment are a short description of the experimental site, climatic and soil condition, materials used for the experiment, design of the experiment, data collection and analysis procedure. The details materials and methods for this experiment have been presented in this chapter under the following headings-

3.1 Experimental period

The experiment was conducted during the period from October 2018 to March 2019.

3.2 Experimental site

The experiment was conducted in the experimental field of Horticulture Farm of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. The location of the site is $23^{0}74^{/}$N latitude and $88^{0}35^{/}$E longitude with an elevation of 8.2 meter from sea level.

3.3 Characteristics of soil

The soil which was used in the pot for the experiment belongs to the Tejgaon series under the Agroecological Zone, Madhupur Tract (AEZ 28) and the general soil type is Shallow Red Brown Terrace soil. The soil was having a texture of silty clay with pH and organic matter 6.1 and 1.13, respectively. The results showed that the soil composed of 27% sand, 43% silt and 30% clay, which have been presented in Appendix I.

3.4 Climatic condition

The climatic condition of experimental site is subtropical and characterized by three distinct seasons, the post-monsoon from November to February and the pre-monsoon period or hot season from March to April and the monsoon period from May to October. The monthly average temperature, humidity and rainfall during crop growing period were collected from Weather Yard, Bangladesh Meteorological Department, and presented in Appendix II.

3.5 Treatments of experiment

The research work was conducted with two sets of treatment consisting of four levels of salinity and three levels of mitigating component, Ca^{2+}. The factors with their levels are as follows:

Factor A: Different levels of salinity

1. S_0 = 0 dSm^{-1} (Control)
2. S_1 = 4 dSm^{-1}
3. S_2 = 8 dSm^{-1}
4. S_3 = 12 dSm^{-1}

Factor B: Different levels of mitigating component, Ca^{2+}

1. M_0 = 0 mM (Control)
2. M_1 = 6 mM Ca^{2+}
3. M_2 = 12 mM Ca^{2+}

There were 12 (3×4) treatments combination such as S_0M_0, S_0M_1, S_0M_2, S_1M_0 S_1M_1, S_1M_2, S_2M_0, S_2M_1, S_2M_2, S_3M_0, S_3M_1 and S_3M_2.

3.6 Experimental design and layout

The two factors experiment was laid out in a Completely Randomized Block Design (RCBD) with three replications. There were 36 pots in total. The size of each pot is 35 cm x 30 cm.

3.6.1 Preparation of the pot

The experimental pots were first filled at 08 October, 2018. A ratio of 1:3 well rotten cow dung and soil were mixed and pots were filled 15 days before transplanting the seedlings. Silt loam soils were used for pot preparation. Weeds and all stubbles were completely removed from the soil. Potted soil was brought into desirable fine tilth by proper hand mixing. The stubble and weeds were removed from the soil. The final pot preparation was done on 15 December, 2018. The soil was treated with insecticides (Cinocarb 3G @ 4 kg/ha) at the time of final pot preparation to protect young plants from the attack of soil inhibiting insects such as cutworm and mole cricket.

3.7 Seed collection

BARI Morich-3 was used as plating materials for this experiment. It is a high yielding variety (8-10 t/ha), rich in Vitamin A and C. The seeds of BARI Morich-3 was collected from Bangladesh Agricultural Research Institute.

3.8 Raising of seedlings

The seedlings of BARI Morich-3 were raised at the Horticulture Farm, SAU, Dhaka, under special care in a (3×1) cm^2 size seed bed. The soil of the seed bed was well ploughed with a spade and prepared into loose friable dried masses and to obtain good tilth to provide a favorable condition for the vigorous growth of young seedlings. Weeds, stubbles and dead roots of the previous crop were removed. The seedbed was dried in the sun to destroy the soil insect and protect the young seedlings from the attack of damping off disease. To control damping off disease Cupravit fungicide were applied. Decomposed cowdung was applied @ 10t/ha for the preparation of seedbed for seedling raising. Five grams of seeds from each variety were sown in seedbed on October 20, 2018. After sowing, the seeds were covered with the finished light soil. At the end of germination shading was provided with polythene sheet over the seedbed to protect the young seedlings from scorching sunshine and heavy rainfall. Light watering, weeding was done as and when necessary to provide seedlings with ideal condition for growth.

3.9 Application of manure and fertilizers

The sources of N_2, P_2O_5, K_2O as Urea, TSP and MP were applied, respectively. The entire amounts of TSP and MP were applied during the final tob preparation. Urea was applied in three equal installments at 15, 30 and 45 days after transplanting of seedling. The rate of fertilizer used for the experiment was the following amount presented as tabular form recommended by BARI (2014).

Table 1. Dose of fertilizers during pot preparation

Manures and Fertilizers	Dose/ha	Application (%)			
		Basal	25 DAT	50 DAT	75 DAT
Cowdung	5 tons	100	--	--	--
Nitrogen	0-32 kg	--	33.33	33.33	33.33
P_2O_5 (as TSP)	0-19 kg	100	--	--	--
K_2O (as MP)	o-32 kg	100	--	--	--

3.10 Transplanting of seedlings

Healthy and uniform two seedlings were transplanted in the experimental pots on 23 November, 2018. The seedlings were uprooted carefully from the seed bed to avoid damage to the seedlings roots. To minimize the damage to the roots of seedlings, the seed beds were watered one hour before uprooting the seedlings. Transplanting was done in the afternoon and the seedlings were watered immediately after transplanting. The young transplanted seedlings were shaded by polythene sheet during day to protect them from scorching sunshine up to 7 days until they were set in the soil. After plant establishment, only the healthy one was kept in the pot for data collection.

3.11 Application of NaCl and Ca^{2+}

As per the treatment the required amount of NaCl was applied in the pot during application of water. The tray was used in the bottom of each pot to collect the water and different nutrient. The Ca^{2+} also applied through foliar spray on the plants according to treatment combination. Both NaCl and Ca^{2+} were applied at 45, 60 and 75 days after transplanting.

3.12 Intercultural operation

Various intercultural operations such as irrigation, weeding, stacking, pest and disease control etc. were accomplished for better growth and development of the chilli seedlings.

3.12.1 Irrigation

Light watering was provided with water cane immediately after transplanting the seedlings and this technique of irrigation was used as every day at early morning and sometimes also in evening throughout the growing period. But the frequency of irrigation became less in harvesting stage. Irrigation in those days when treatment was applied was done at evening as salt was applied with irrigation water. The amount of irrigation water was limited up to that quantity which does not leached out through the bottom. As such the salinity status was maintained in the desired level.

3.12.2 Weeding

The hand weeding was done 10, 25 and 40 DAT chilli seedlings to keep the plots free from weeds.

3.12.3 Staking

When the plants were well established, staking was given to each plant by bamboo sticks for support to keep them erect.

3.12.4 Pulverizing

After continuous application of the treatment with irrigation water, a compact condion was formed in the pot soil. To make sure the proper air circulation in soil, it was carefully loosen several times during research period.

3.13 Pest and disease control

Melathion 57 EC was applied @2 ml L^{-1} of water against the insect pests like cutworm, leaf hopper, fruit borer and others. The insecticide application was made fortnightly after transplanting and was stopped before second week of first harvest. *Furadan 10G* was also applied during pot preparation as soil insecticide. During foggy weather precautionary measure against disease attack of chilli was taken by spraying Diathane M-45 fortnightly @2 gm L^{-1} of water at the early vegetative stage. Ridomil gold was also applied @ 2 gmL^{-1} of water against blight disease of chilli.

3.14 Harvesting

Harvesting of all the chilli was not possible on a certain or particular date because the fruits initiation as well as ripening in different plants were not uniform. Fruits were harvested at 14 days interval and the final harvesting was done when they were attained slightly reddish color. Harvesting was started from February, 2019 and was continued up to March, 2019.

3.15 Data collection

Experimental data were recorded from 40 days after transplanting and continued until harvest. The following data were recorded during the experimental period.

3.15.1 Plant height

Plant height was taken to the length between the base of the plant and the shoot tip. The plant height was recorded at 30, 45, 60 and 75 days after transplanting (DAT).

3.15.2 Number of branches per plant

The total number of branches plant^{-1} was counted from each plant at 30 DAT, 45 DAT, 60 DAT and 75 DAT. There is no option to make average value from collected value due to only one plant was maintained per pot.

3.15.3 Number of leaves per plant

Number of leaves per plant was counted at 40, 50, 60 and 75 DAT. The number of leaves plant^{-1} was counted from each plant excluding the small leaves.

3.15.4 Leaf Area plant^{-1} (cm^2)

Leaf area was measured by multiply the leaf length and breadth. Mature leaves were measured all the time and were expressed in cm^2.

3.15.5 Number of flowers per plant

The number of flowers per plant was counted and recorded.

3.15.6 Number of fruits per plant

Total number of fruits per plant was counted from the plant of each of unit pot and number of fruits produced per plant were calculated.

3.15.7 Length of fruit (cm)

The length of fruit was measured with a slide calipers from the neck of the fruit to the bottom from each pot and there average was taken and expressed in cm.

3.15.8 Diameter of fruit (cm)

Diameter of fruit was measured at middle portion of several fruits from each plant with a slide calipers. Their average was taken and expressed in cm.

3.15.9 Wt. of individual fruit

Among the total number of fruits during the period from first to final harvest, fruit was considered for determining the individual fruit weight by the following formula:

$$\text{Weight of individual fruit} = \frac{\text{Total weight of fruits}}{\text{Total number of fruits}}\ \text{g}$$

3.15.10 Fruit yield per plant (g)

Fruit yield of chilli per plant was recorded as the whole fruit per plant harvested in different time and was expressed in gram.

3.16 Statistical analysis

The data obtained for different characters were statistically analyzed by using MSTAT-C computer package program to find out the significance of the difference for salt stress and calcium nitrate on yield and yield contributing characters of chilli. The mean values of all the recorded characters were evaluated and analysis of variance was performed by the 'F' (variance ratio) test. The significance of the difference among the treatment combinations of means was estimated by Least Significant Difference at 5% level of probability (Gomez and Gomez, 1984).

CHAPTER 4
RESULTS AND DISCUSSION

The results of the study on the effect of salinity levels and calcium on morphological, yield and yield contributing characters with physiological and biochemical properties of chilli have been presented and possible interpretations have been made in this chapter.

4.1 Plant height (cm)

Plant height of chilli varied significantly for different levels of salt stress at 30, 45, 60 and 75 days after transplanting (DAT) under the present trial (Figure 1 and Appendix III). At 30, 45, 60 and 75 DAT, the tallest plant (21.45 cm, 44.58 cm, 50.21 cm and 52.36 cm respectively) was recorded from S_0 (control), whereas the shortest plant (20.15 cm, 28.77 cm, 33.20 cm and 35.28 cm respectively) was recorded from S_3 (12 dSm^{-1}) which was closely followed (20.46 cm, 34.00 cm, 38.46 cm and 44.28 cm respectively) by S_2 (8 dSm^{-1}) for same DAT.

Statistically significant variation was recorded for different levels of calcium on plant height of chilli at 30, 45, 60, and 75 DAT (Figure 2 and Appendix III). Data revealed that at 30, 45, 60, and 75 DAT, the tallest plant (20.89 cm, 38.69 cm, 42.65 cm and 47.32 cm respectively) was found from M_1 (6 mM Ca^{2+}), which was statistically identical (20.82 cm, 36.42 cm, 41.88 cm and 45.28 cm respectively) to M_2 (12 mM Ca^{2+}), while the shortest plant height (20.44 cm, 34.63 cm, 41.41 cm and 43.05 cm respectively) was recorded from M_0 (control) for same DAT.

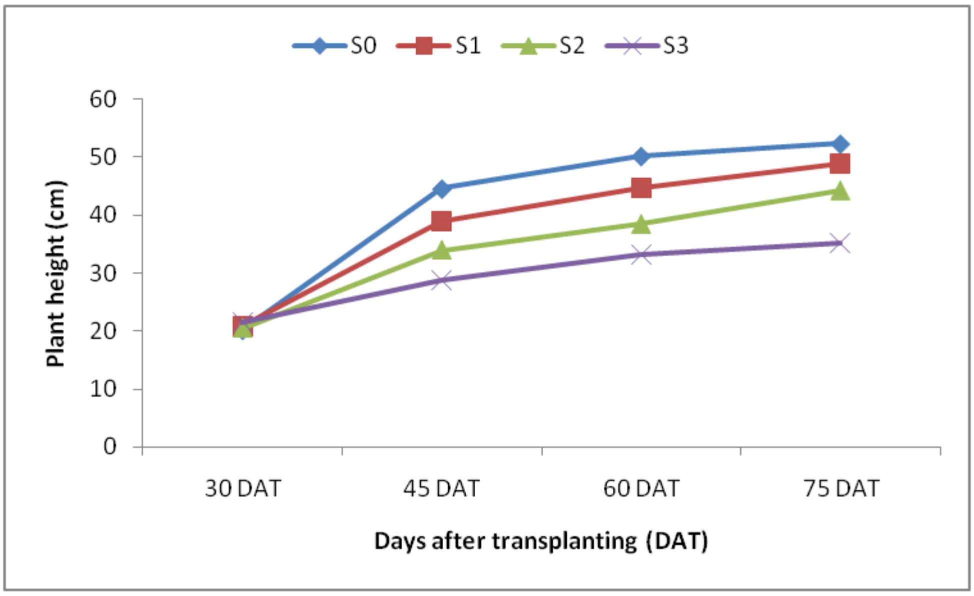

Fig. 1: Plant height of chilli influenced by different salinity levels at different days after transplanting.

(S_0 = 0 dSm^{-1}, S_1 = 4 dSm^{-1}, S_2 = 8 dSm^{1}, S_3 = 12 dSm^{-1})

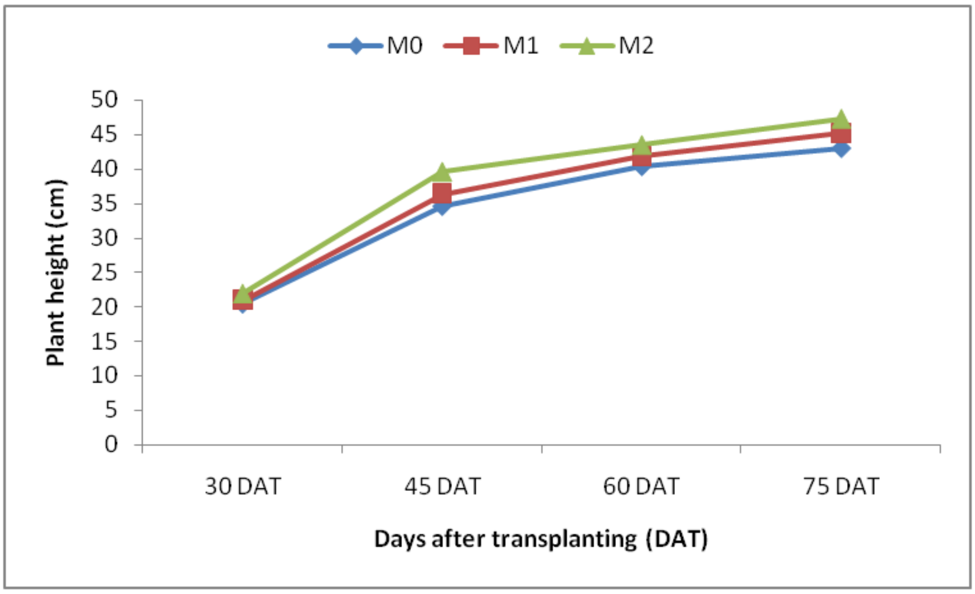

Fig. 2: Plant height of chilli as influenced by different Ca^{2+} levels at different days after transplanting.

(M_0 = 0 mM (Control) , M_1 = 6 mM Ca^{2+}, M_2 = 12 mM Ca^{2+})

Table 2. Combined effect of salt stress and calcium (Ca^{2+}) on plant height at different days after transplanting (DAT) of chilli

Treatments	Plant height (cm) at			
	30 DAT	45 DAT	60 DAT	75 DAT
S_0M_0	21.36	41.00 b	48.90 bc	51.33 b
S_0M_1	21.52	46.42 a	52.00 a	54.26 a
S_0M_2	20.22	46.33 a	49.72 b	51.48 b
S_1M_0	20.74	38.00 c	44.48 de	46.87 e
S_1M_1	20.44	40.88 b	45.00 d	51.00 b
S_1M_2	21.22	38.00 c	44.67 d	49.00 d
S_2M_0	19.67	32.00 d	36.92 g	41.33 g
S_2M_1	21.12	37.67 c	39.33 f	46.74 e
S_2M_2	20.60	32.33 d	39.12 f	44.76 f
S_3M_0	19.67	27.50 g	31.33 i	32.67 j
S_3M_1	21.48	29.80 e	34.27 h	37.29 h
S_3M_2	20.25	29.00 ef	34.00 h	35.87 i
$LSD_{0.05}$	NS	1.044	1.107	1.507
CV (%)	4.271	8.244	10.036	10.752

In a column, means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly as per 0.05 level of probability

S_0 = 0 dSm^{-1}, S_1 = 4 dSm^{-1}, S_2 = 8 dSm^{-1}, S_3 = 12 dSm^{-1}

M_0 = 0 mM Ca^{2+} (Control), M_1 = 6 mM Ca^{2+}, M_2 = 12 mM Ca^{2+}

Combined effect of different levels of salt stress and calcium showed significant differences on plant height of chilli at 30, 45, 60 and 75 DAT (Table 2 and Appendix III). At 30, 45, 60 and 75 DAT, the tallest plant (21.52 cm, 46.42 cm, 52.00 cm and 54.26 cm) was found from S_0M_1 (0 dSm^{-1}+ 6 mM Ca^{2+}) treatment combination, while the shortest plant (19.67 cm, 27.50 cm, 31.33 cm and 32.67 cm) was found from S_3M_0 (12 dSm^{-1}+ Control) treatment combination.

4.2 Number of branches per plant

Different levels of salt stress varied significantly in terms of number of branches per plant of chilli for at 30, 45, 60 and 75 days after transplanting (DAT) under the present study (Figure 3 and Appendix IV). At 30, 45, 60 and 75 DAT the maximum number of branches per plant (8.33, 9.33, 9 .33 and 1 0 .11 respectively) was noted from S_0 which was closely followed (7.56, 8.45, 8.50 and 9.45) respectively from S_1. On the other hand, the minimum number (5.67, 7.11, 7.33 and 8.22 respectively) was recorded from S_3 which was followed (6.78, 7.78, 7.78 and 9.00 respectively) from S_2 (Figure 3).

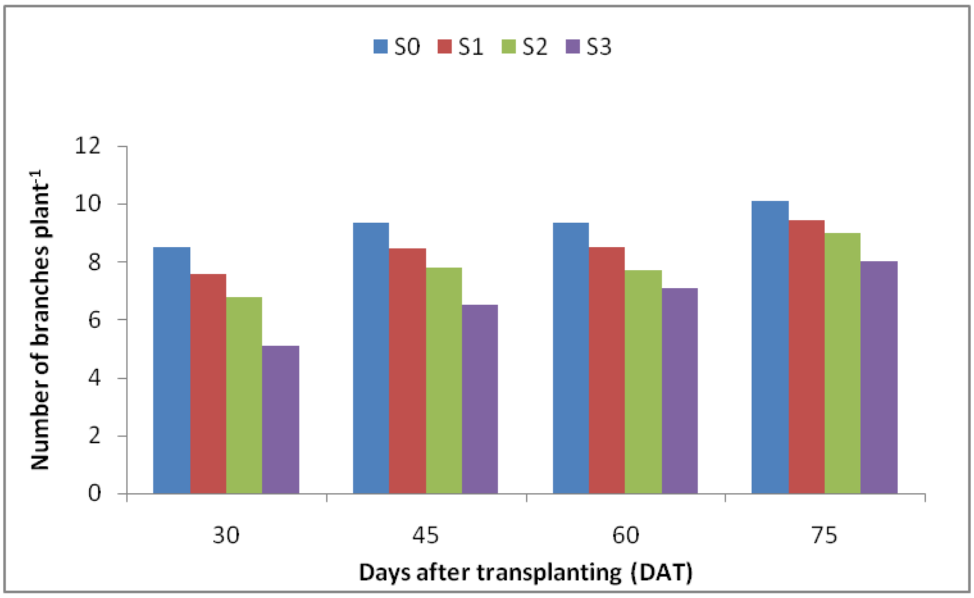

Fig. 3: Number of branches of chilli as influenced by different salinity levels at different days after transplanting.
(S_0 = 0 dSm^{-1}, S_1 = 4 dSm^{-1}, S_2 = 8 dSm^{-1}, S_3 = 12 dSm^{-1})

Number of branches per plant of chilli showed significant differences due to different levels of calcium nitrate at 30, 45, 60 and 75 DAT (Figure 4 and Appendix IV). Data revealed that at 30, 45, 60 and 75, the maximum number of branches per plant (7.42, 8 .42, 8 .42 and 9 .33 respectively) was count from M_2 which was closely followed (7.09, 8.25, 8.33 and 9.25 respectively) by M_1, whereas the minimum number of branches per plant(6.75, 7.84, 7.92 and 9.00 respectively) was found from M_0 for same DAT.

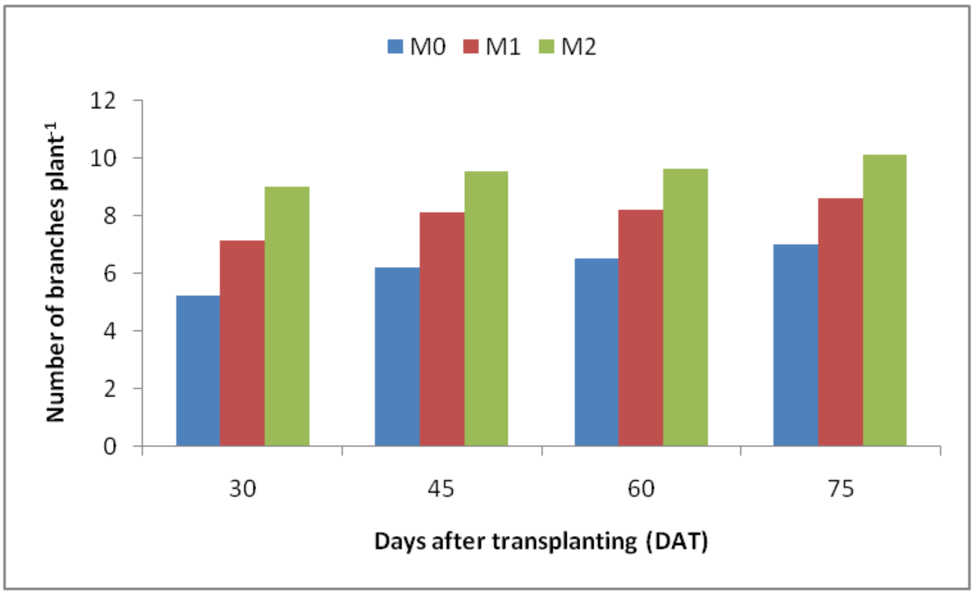

Fig. 4: Number of branches of chilli as influenced by different salinity levels at different days after transplanting.
(M_0 = 0 mM Ca^{2+} (Control), M_1 = 6 mM Ca^{2+}, M_2 = 12 mM Ca^{2+})

Table 3. Combined effect of salinity and calcium on number of branches plant^{-1} of chilli at different days after transplant

Treatments	Number of branches plant^{-1}			
	30 DAT	45 DAT	60 DAT	75 DAT
S_0M_0	8.00 bc	9.00 bc	8.67 b	10.00 a
S_0M_1	8.33 ab	9.33 ab	9.33 a	10.00 a
S_0M_2	8.67 a	9.67 a	9.67 a	10.33 a
S_1M_0	7.33 de	8.00 d	8.00 cd	9.00 bc
S_1M_1	7.67 cd	8.67 c	8.33 bc	9.67 ab
S_1M_2	7.67 cd	8.67 c	8.67 b	9.67 ab
S_2M_0	6.67 fg	7.67 de	7.67 de	9.00 bc
S_2M_1	6.67 g	7.67 de	7.67 de	9.00 bc
S_2M_2	7.00 ef	8.00 d	8.00 cd	9.00 bc
S_3M_0	5.00 i	6.67 f	7.33 e	8.00 c
S_3M_1	5.67 h	7.33 e	7.33 e	8.33 c
S_3M_2	6.33 g	7.33 e	7.33 e	8.33 c
$LSD_{0.05}$	0.3710	0.4007	0.4480	0.7104
CV (%)	7.23	8.56	10.49	9.87

In a column, means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly as per 0.05 level of probability
S_0 = 0 dSm^{-1}, S_1 = 4 dSm^{-1}, S_2 = 8 dSm^{-1}, S_3 = 12 dSm^{-1}
M_0 = 0 mM Ca^{2+} (Control), M_1 = 6 mM Ca^{2+}, M_2 = 12 mM Ca^{2+}

Combined effects of different levels of salt stress and calcium showed significant differences due to their combined effect on number of branches per plant of chilli at 30, 40, 50, 60 and 75 DAT (Table 3 and Appendix IV) at 30, 45, 60 and 75 DAT, the maximum number of branches per plant (8.67, 9.67, 9.67 and 10.33 respectively) was recorded from S_0M_2 treatment combination and the minimum number (5.00, 6.67, 7.33 and 8.00 respectively) was found from S_3M_0 treatment combination.

4.3 Number of leaves per plant

Different levels of salt stress varied significantly in terms of number of leaves per plant of chilli for at 30, 45 and 60 days after transplanting (DAT) under the present trial (Figure 5 and Appendix V). At 30, 45 and 60 DAT the maximum number of number of leaves per plant (33.55, 35.78 and 37.33 respectively) was noted from S_0 which was closely followed (31.00, 33.34 and 34.67 respectively) by $S_{1.}$ On the other hand, the minimum number of leaves (18.33, 21.11 and 23.22 respecively) was counted from S_3 at 30, 45 and 45 DAT respectively .

Number of number of leaves per plant of chilli showed significant differences due to different levels of calcium nitrate at 30, 45 and 60 DAT (Figure 6 and Appendix V). Data revealed that at 30, 45 and 60, the maximum number of number of leaves per plant (28.00, 30.59 and 31.75 respectively) was found from M_1, whereas the minimum number of leaves per plant (25.17, 27.92 and 29.59) was found from M_0 for same DAT.

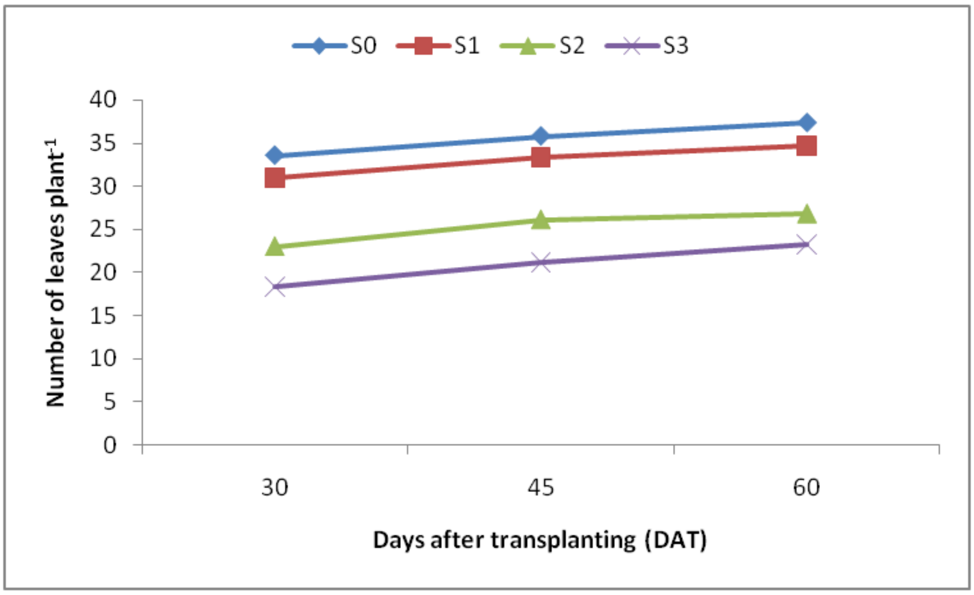

Fig. 5: Number of leaves plant^{-1} of chilli as influenced by different salinity levels at different days after transplanting.

(S_0 = 0 dSm^{-1}, S_1 = 4 dSm^{-1}, S_2 = 8 dSm^{-1}, S_3 = 12 dSm^{-1})

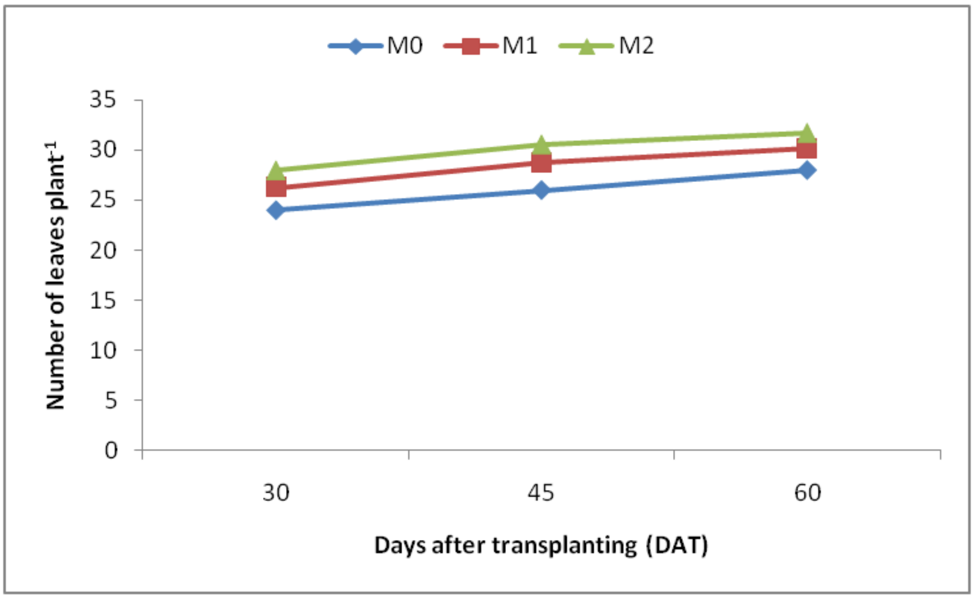

Fig. 6: Number of leaves plant-1 of chilli as influenced by calcium application at different days after transplanting.

(M_0 = 0 mM Ca^{2+} (Control), M_1 = 6 mM Ca^{2+}, M_2 = 12 mM Ca^{2+})

Table 4. Combined effect of salinity and calcium application on number of leaves plant[1] of chilli

Treatments	Number of leaves plant^{-1}		
	30 DAT	45 DAT	60 DAT
S_0M_0	33.33 a	35.33 ab	36.67 ab
S_0M_1	34.00 a	36.67 a	38.33 a
S_0M_2	33.33 a	35.33 ab	37.00 ab
S_1M_0	29.00 c	31.67 c	33.33 c
S_1M_1	32.67 ab	34.67 ab	35.67 bc
S_1M_2	31.33 b	33.67 bc	35.00 bc
S_2M_0	22.33 de	25.33 de	26.67 d
S_2M_1	23.67 d	26.67 d	27.00 d
S_2M_2	23.00 de	26.33 de	26.67 d
S_3M_0	16.00 f	19.33 f	21.67 e
S_3M_1	21.67 e	24.33 e	26.00 d
S_3M_2	17.33 f	19.67 f	22.00 e
$LSD_{0.05}$	1.710	2.030	2.416
CV (%)	9.06	8.63	7.20

In a column, means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly as per 0.05 level of probability

S_0 = 0 dSm^{-1}, S_1 = 4 dSm^{-1}, S_2 = 8 dSm^{-1}, S_3 = 12 dSm^{-1}

M_0 = 0 mM Ca^{2+} (Control), M_1 = 6 mM Ca^{2+}, M_2 = 12 mM Ca^{2+}

Different levels of salt stress and calcium showed significant differences due to their combined effect on number of leaves per plant of chilli at 30, 45 and 60 DAT (Table 4 and Appendix V) at 30, 45 and 60 DAT, the maximum number of leaves per plant (34.00, 36.67 and 38.33 respectively) was recorded from S_0M_1 treatment combination and the minimum number of leaves per plant (16.00, 19.33 and 21.67 respectively) was found from S_3M_0 treatment combination.

4.4 Leaf area

Different levels of salt stress varied significantly in terms of leaf area of chilli for at 30, 45 and 60 days after transplanting (DAT) under the present trial (Figure 7 and Appendix VI) at 30, 45 and 60 DAT the maximum leaf area of chilli per plant (61.17 cm^2, 69.33 cm^2 and 71.35 cm^2) cm^2 was recorded from S_0. On the other hand, the minimum leaf area (27.06 cm^2, 36.11 cm^2 and 37.81 cm^2) cm^2 was recorded from S_3.

Different levels of calcium affected significantly on leaf area (Figure 8 and Appendix VI). The highest leaf area (51.08 cm^2, 57.21 cm^2 and 58.76 cm^2) cm^2 was found from M_2 where the lower leaf area (38.75 cm^2, 46.44 cm^2 and 48.13 cm^2) cm^2 was recorded from M_0.

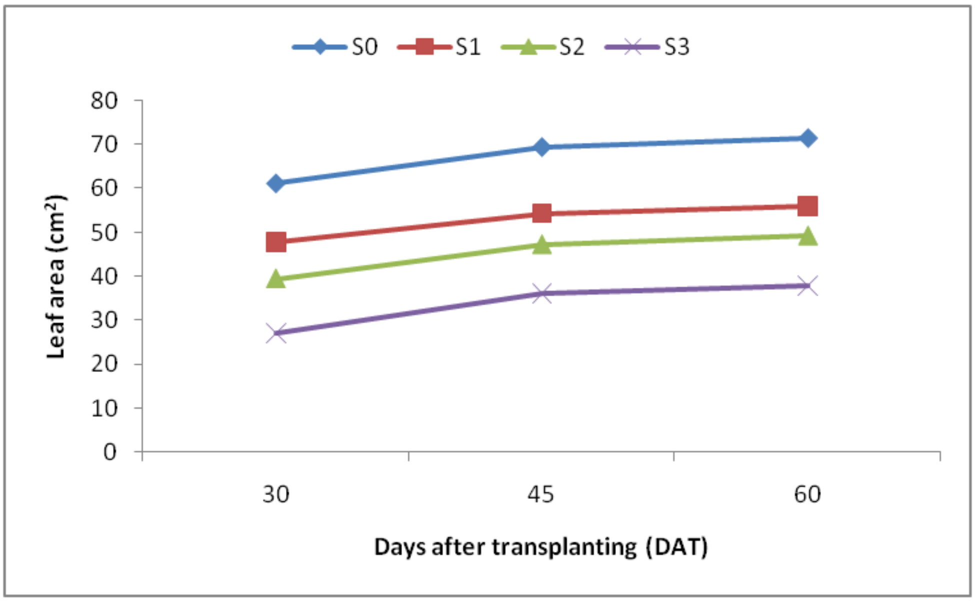

Fig. 7: Leaf area of chilli as influenced by different salinity levels at different days after transplanting.
(S_0 = 0 dSm^{-1}, S_1= 4 dSm^{-1}, S_2= 8 dSm^{-1}, S_3 = 12 dSm^{-1})

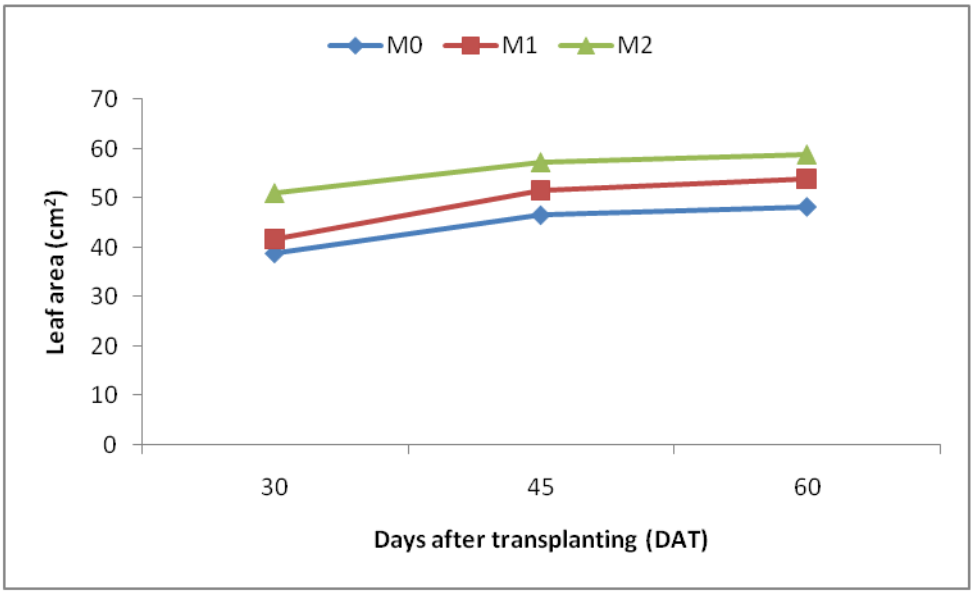

Fig. 8: Leaf area of chilli as influenced by calcium application at different days after transplanting.
(M_0 = 0 mM Ca^{2+} (Control), M_1 = 6 mM Ca^{2+}, M_2 = 12 mM Ca^{2+})

Table 5.Combined effect of salinity and Ca^{2+} on leaf area of chilli

Treatments	Leaf area (cm^2) at		
	30 DAT	45 DAT	60 DAT
S_0M_0	52.00 bc	59.50 c	61.48 c
S_0M_1	55.33 b	68.50 b	70.45 b
S_0M_2	76.17 a	80.00 a	82.12 a
S_1M_0	45.50 d	52.33 de	54.28 def
S_1M_1	47.00 d	53.33 cde	55.38 cde
S_1M_2	50.83 c	57.17 cd	58.20 d
S_2M_0	38.00 ef	44.75 fg	45.70 g
S_2M_1	39.08 ef	47.50 efg	50.52 fg
S_2M_2	41.33 e	49.33 ef	51.30 ef
S_3M_0	19.50 h	29.17 i	31.04 i
S_3M_1	25.67 g	36.83 h	39.00 h
S_3M_2	36.00 f	42.33 gh	43.40 h
$LSD_{0.05}$	3.686	5.901	6.021
CV (%)	11.40	21.63	11.29

In a column, means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly as per 0.05 level of probability
S_0 = 0 dSm^{-1}, S_1 = 4 dSm^{-1}, S_2 = 8 dSm^{-1}, S_3 = 12 dSm^{-1}
M_0 = 0 mM Ca^{+2} (Control), M_1 = 6 mM Ca^{+2}, M_2 = 12 mM Ca^{+2}

The combined effect of salt and calcium played a significant effect on the leaf area (Table 5 and Appendix VI). The maximum leaf area (76.17 cm^2, 80.00 cm^2 and 82.12 cm^2) was recorded from S_0M_2 which was statistically similar (55.33 cm^2, 68.50 cm^2 and 70.45 cm^2) from S_0M_1 and minimum leaf area (19.50 cm^2, 29.17 cm^2 and 31.04 cm^2) was found from S_3M_0.

4.5 Number of flowers plant^{-1}

Number of branches per plant of chilli was significantly affected by the different levels of salinity at harvest DAT (Table 6 and Appendix VII). The highest number of flowers plant^{-1} (43.67) was found from S_0. The lowest value (21.89) was recorded from S_3.

A significant effect of calcium was found on the number of flowers plant^{-1} of chilli at harvest DAT (Table 6 and Appendix VII). The highest number of flowers plant^{-1} (38.67) was found from M_2 and the lowest value (30.25) was recorded from M_0.

The combined effect of salinity and calcium on number of flowers plant^{-1} of chilli showed a significant effect (Table 7 and Appendix VII). The highest number of flowers plant^{-1} (51.00) was found from S_0M_2 and the lowest value (15.00) was found from S_3M_0.

4.6 Number of fruits plant^{-}

Number of fruits plant^{-1} of chilli was significantly affected by the different levels of salinity at harvest DAT (Figure 9, Appendix VII). The highest number of fruits plant^{-1} (37.00) was found from S_0. The lowest value (14.22) was recorded from S_3.

A significant effect of calcium was found on the fruits plant^{-1} of chilli at harvest DAT (Figure 10, Appendix VII). The highest number of fruits plant-1 (29.67) was found from M_2 and the lowest value (22.58) was recorded from M_0.

The combined effect of salinity and calcium on the number fruits plant^{-1} of chilli showed a significant effect (Table 7 and Appendix VII). The highest number of fruits per plant (44.33) was found from S_0M_2 and the lowest value (9.66) was found from S_3M_0.

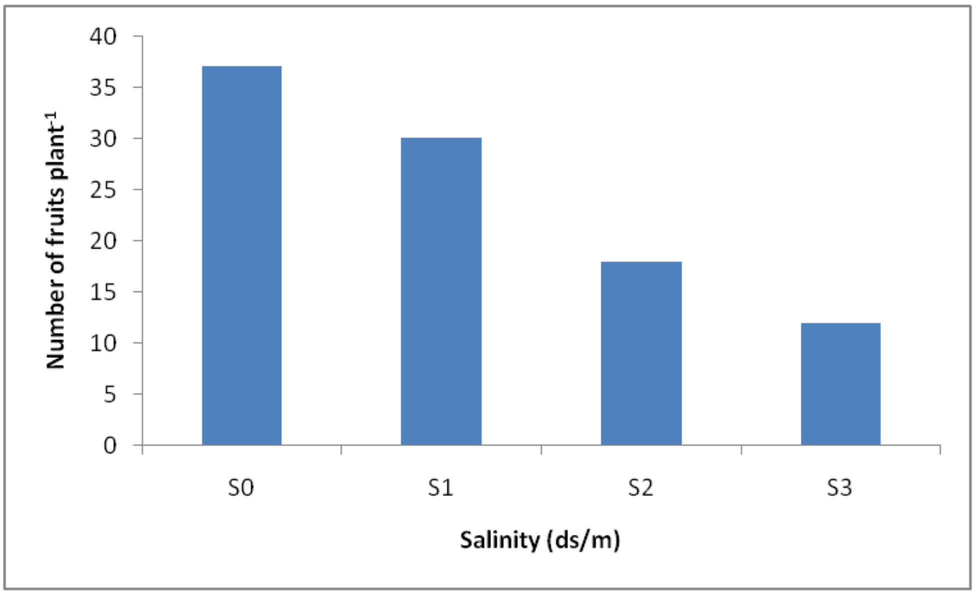

Fig. 9: Number of fruits plant^{-1} of chilli as influenced by different salinity levels at different days after transplanting.
(S_0 = 0 dSm^{-1}, S_1 = 4 dSm^{-1}, S_2 = 8 dSm^{-1}, S_3 = 12 dSm^{-1})

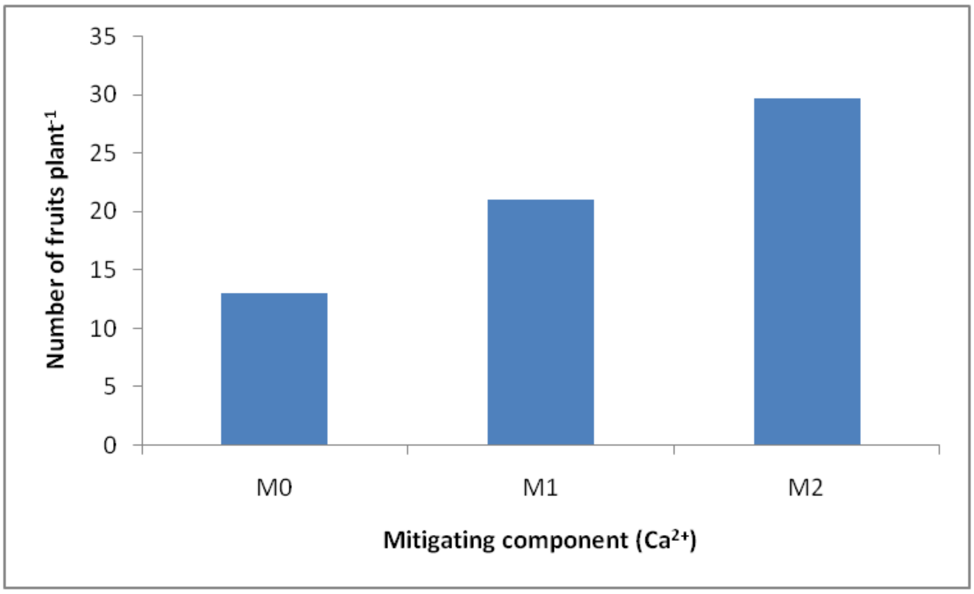

Fig. 10: Number of fruits plant^{-1} of chilli as influenced by calcium application different days after transplanting.

(M_0 = 0 mM Ca^{2+} (Control), M_1 = 6 mM Ca^{2+}, M_2 = 12 mM Ca^{2+})

4.7 Length of fruit (cm)

Length of fruit of chilli was significantly affected by the different levels of salinity at harvest DAT (Table 6 and Appendix VII). The highest length of fruit (8.76 cm) was found from S_0. The lowest value (6.20 cm) was recorded from S_3.

A significant effect of calcium was found on the length of fruit of chilli at harvest DAT (Table 6 and Appendix VII). The highest length of fruit (7.64 cm) was found from M_2 and the lowest value (6.89 cm) was recorded from M_0.

The combined effect of salinity and calcium on the length of fruit of chilli showed a significant effect (Table 7 and Appendix VII). The highest length of fruit (9.30 cm) was found from S_0M_2 and the lowest value (5.90 cm) was found from S_3M_0.

4.8 Diameter of fruit (cm)

Different levels of salinity showed significant effect on diameter of fruit of chilli plant (Table 6 and Appendix VII). The largest diameter of fruit (0.37 cm) was recorded from S_0 application. In comparison, the smallest diameter of fruit (0.25 cm) was observed from S_3 treatment of salinity (Table 6).

Diameter of fruit of chilli plant was significantly influenced by different levels of calcium application (Table 6 and Appendix VII). The largest fruit diameter (0.33 cm) was recorded from M_2 application. In comparison, the smallest fruit diameter (0.30 cm) was observed in M_0 treatment.

Interaction of salinity and salicylic acid significantly affected the plant base diameter of chilli (Table 7 and Appendix VII). The longest fruit diameter (0.40 cm) was recorded from the combination of S_0M_2 treatment. In comparison, the shortest fruit diameter (0.23 cm) was measured in treatment combination of S_3M_0.

4.9 Individual fruit weight (g)

Individual fruit weight of chilli per plant varied significantly due to influence of the different levels of salinity (Figure 11, Appendix VII). The highest individual fruit weight per plant of chilli (2.06 g) was found from S_0 (control). The lowest weight (1.29 g) was obtained from S_3. The results obtained from this experiment showed that salinity stress caused a significant reduction in fruit weight.

Application of different levels of calcium showed statistically insignificant differences for the fruit weight per plant of chilli (Figure 12, Appendix VII). The highest fruit weight (1.67 g) was recorded from M_2 and the lowest yield (1.46 g) was obtained from M_0.

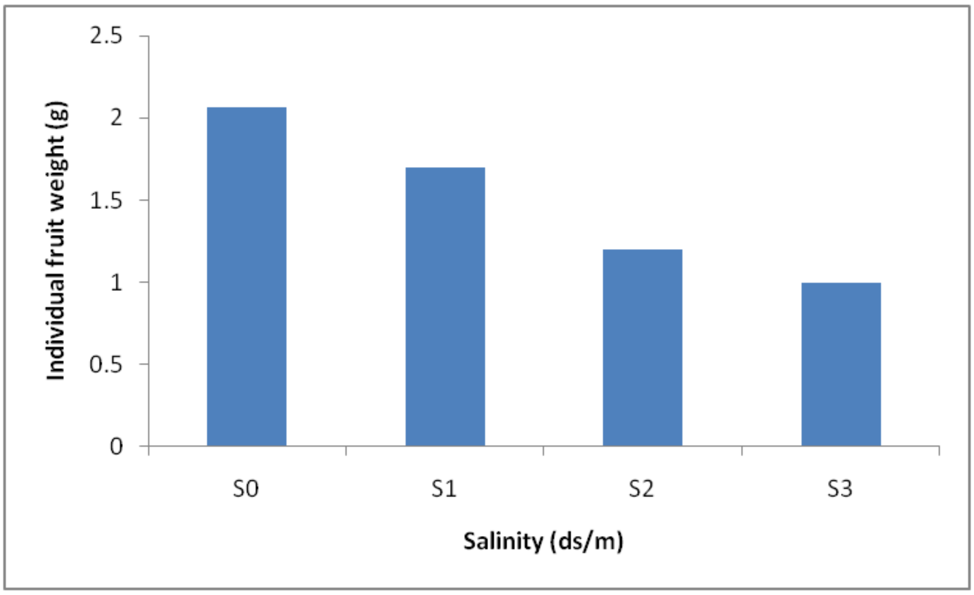

Fig. 11: Individual fruit weight of chilli as influenced by different salinity levels at different days after transplanting.
(S_0 = 0 dSm^{-1}, S_1 = 4 dSm^{-1}, S_2 = 8 dSm^{-1}, S_3 = 12 dSm^{-1})

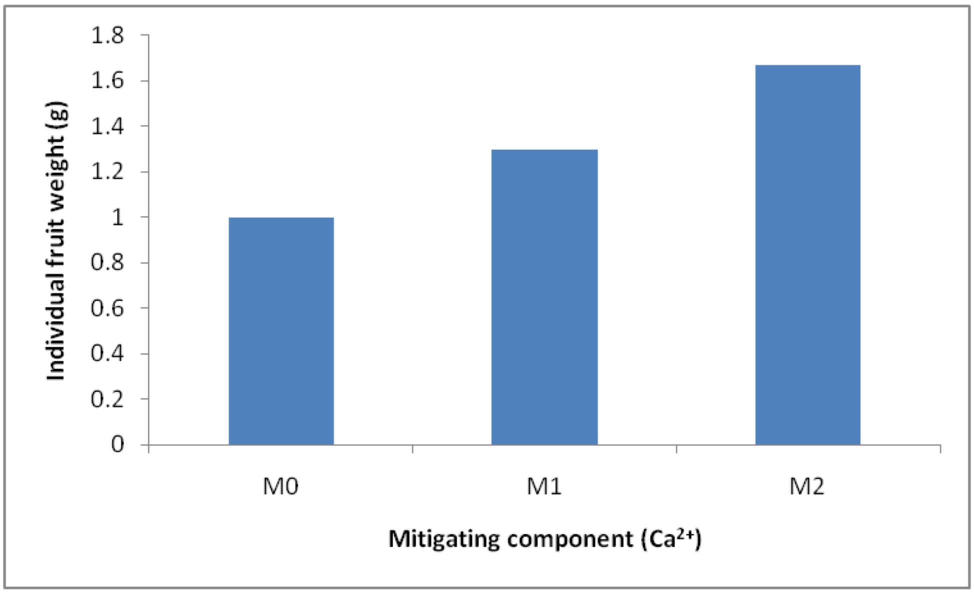

Fig. 12: Individual fruit weight of chilli as influenced by calcium application at different days after transplanting.
(M_0 = 0 mM Ca^{2+} (Control), M_1 = 6 mM Ca^{2+}, M_2 = 12 mM Ca^{2+})

The combined effect of salinity and calcium for the yield of chilli per plant showed significant difference (Table 7 and Appendix VII). The highest fruit weight (2.22 g) was recorded from S_0M_2 whereas the lowest value (1.24 g) was found from the S_3M_0.

4.10 Fruit yield/plant

Fruit yield of chilli per plant varied significantly due to influence of the different levels of salinity (Figure 13 and Appendix VII). The highest fruit yield per plant (68.66 g) was found from S_0 (control). The lowest yield (23.11 g) was obtained from S_3.

Application of different levels of calcium showed statistically insignificant differences for the fruit yield per plant of chilli (Figure 14 and Appendix VII). The highest fruit yield (54.50 g) was recorded from M_2 and the lowest yield (35.83 g) was obtained from M_0. Calcium insignificantly increase the fruit yield mostly at 12mM.

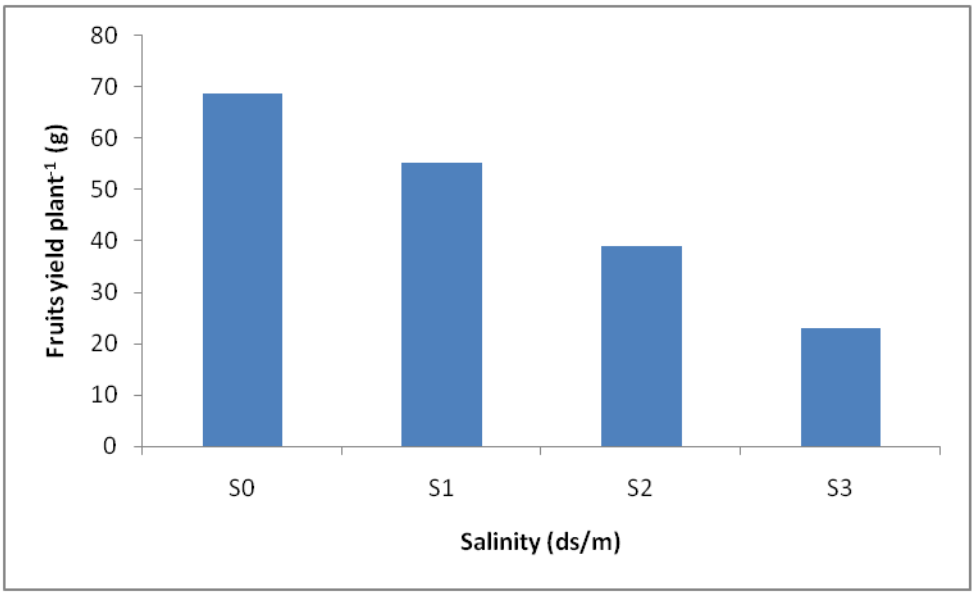

Fig. 13: Fruits yield plant^{-1} of chilli as influenced by different salinity levels at different days after transplanting.
(S_0 = 0 dSm^{-1}, S_1 = 4 dSm^{-1}, S_2 = 8 dSm^{-1}, S_3 = 12 dSm^{-1})

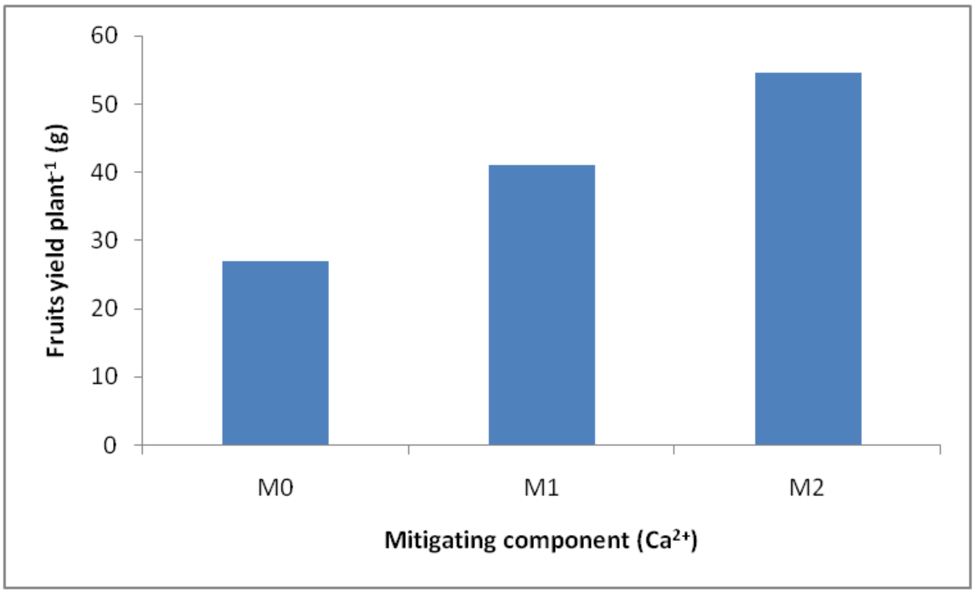

Fig. 14: Fruits yield plant^{-1} of chilli as influenced by calcium application at different days after transplanting.

(M_0 = 0 mM Ca^{2+} (Control), M_1 = 6 mM Ca^{2+}, M_2 = 12 mM Ca^{2+})

Table 6. Effects of salinity and calcium application on yield contributing parameters and yield of chilli

Treatments	Yield contributing parameters					
	Number of flowers plant^{-1}	Number of fruits plant^{-1}	Length of fruit (cm)	Diameter of fruit (cm)	Individual fruit weight (g)	Fruits yield plant^{-1} (g)
Effect of salinity						
S_0	43.67 a	37.00 a	8.76 a	0.37	2.06 a	68.66 a
S_1	36.44 b	28.22 b	7.47 b	0.33	1.55 b	44.22 b
S_2	33.33	23.78 c	6.62 c	0.30	1.44 b	39.11 c
S_3	21.89	14.22 d	6.20 d	0.25	1.29 c	23.11 d
$LSD_{0.05}$	1.355	1.670	0.128	NS	0.1025	2.802
CV (%)	8.59	10.47	11.46	6.87	7.44	10.32
Effect of different levels of mitigating component; Ca						
M_0	30.25 b	22.58 c	6.89 c	0.30	1.46 b	35.83 c
M_1	32.58 b	25.17 b	7.26 b	0.31	1.63 a	41.00 b
M_2	38.67 a	29.67 a	7.64 a	0.33	1.67 a	54.50 a
$LSD_{0.05}$	2.360	2.160	0.231	NS	0.067	2.406
CV (%)	8.59	10.47	11.46	6.87	7.44	10.32

In a column, means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly as per 0.05 level of probability
S_0 = 0 dSm^{-1}, S_1 = 4 dSm^{-1}, S_2 = 8 dSm^{-1}, S_3 = 12 dSm^{-1}
M_0 = 0 mM Ca^{2+} (Control), M_1 = 6 mM Ca^{2+}, M_2 = 12 mM Ca^{2+}

The combined effect of salinity and calcium for the yield of chilli per plant showed significant difference (Table 7 and Appendix VII). The highest yield (99.33 g) was recorded from S_0M_2 whereas the lowest value (17.33 g) was found from the S_3M_0.

Table 7. Combined effect of salinity and calcium (Ca^{2+}) on yield contributing characters and yield of chilli

Treatments	Yield contributing parameters					
	Number of flowers plant^{-1}	Number of fruits plant^{-1}	Length of fruit (cm)	Diameter of fruit (cm)	Individual fruit weight (g)	Fruits yield plant^{-1} (g)
S_0M_0	38.67 c	32.67 b	8.23 bc	0.34 b	1.75 b	47.33 c
S_0M_1	41.33 b	34.00 b	8.73 b	0.35 ab	2.20 a	59.33 b
S_0M_2	51.00 a	44.33 a	9.30 a	0.40 a	2.22 a	99.33 a
S_1M_0	36.00 d	26.33 de	6.88 e	0.31 bc	1.49 cd	42.67 cde
S_1M_1	36.33 d	28.67 cd	7.50 d	0.33 bc	1.55 c	44.00 cd
S_1M_2	37.00 d	29.67 c	8.03 c	0.33 bc	1.60 c	46.00 cd
S_2M_0	31.33 f	21.67 f	6.53 ef	0.29 bcd	1.35 de	36.00 ef
S_2M_1	33.33 ef	24.33 e	6.56 ef	0.30 bcd	1.48 cd	40.00 de
S_2M_2	35.33 de	25.33 e	6.75 ef	0.30 bcd	1.49 cd	41.33 cde
S_3M_0	15.00 h	9.667 h	5.90 g	0.23 e	1.24 e	17.33 g
S_3M_1	19.33 g	13.67 g	6.23 fg	0.24 de	1.28 e	20.67 g
S_3M_2	31.33 f	19.33 f	6.46 ef	0.27 cde	1.35 de	31.33 f
$LSD_{0.05}$	2.14	2.56	0.51	0.05	0.14	6.46
CV (%)	8.59	10.47	11.46	6.87	7.44	10.32

In a column, means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly as per 0.05 level of probability

S_0 = 0 dSm^{-1}, S_1 = 4 dSm^{-1}, S_2 = 8 dSm^{-1}, S_3 = 12 dSm^{-1}

M_0 = 0 mM Ca^{2+} (Control), M_1 = 6 mM Ca^{2+}, M_2 = 12 mM Ca^{2+}

CHAPTER 5
SUMMARY AND CONCLUSION

The experiment was conducted at the Horticulture Farm of Sher-e-Bangla Agricultural University (SAU), Dhaka during the period from October 2018 to April 2019. Seedlings of 30 days of BARI Morich-3 were used as planting material. The two factors experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. The factors are: Factor A: Four levels of sodium (Na^+) salt such as (i) S_0: Control, (ii) S_1: 4 dSm^{-1}, (iii) S_2: 8 dSm^{-1} and (iv) S_3: 12 dSm^{-1}; Factor B: Three levels of calcium (Ca^{2+}) as mitigating agent of salt stress (i) M_0: Control, (ii) M_1: 6 mM and (iii) M_2:12 mM Ca^{2+}.

Data on different growth and yield parameter were recorded and statistically significant variations were found for different level of salt stress and calcium and their combined effect.

Data were collected randomly from each replication. Data on plant height, number of branches plant^{-1}, number of leaves plant^{-1}, leaf area plant^{-1}, number of fruits plant^{-1}, number of flowers plant^{-1}, length of fruit, diameter of fruit, weight of individual fruits, fruit yield plant^{-1} were recorded. All collected data of the present study were analyzed statistically and the mean differences were adjudged by Least Significant Difference (LSD) test.

The result of the experiment revealed that almost all the parameters studied were significantly influenced by different levels of salinity. More or less, all the characters attained highest values from S_0 (0 dSm^{-1}) and S_3 (12 dSm^{-1}) gave the lowest value in all the characters. The maximum yield 68.66 g/plant was obtained by S_0 (Control) and the minimum yield 23.11 g/plant was found in the S_3 (12 dSm^{-1}).

Different concentrations of calcium played vital role on the growth and yield of chilli. Calcium had significant effect on more or less all the characters. The maximum fruit yield plant^{-1} 54.50 g/plant was obtained from M_2 treatment and the minimum fruit yield plant^{-1} 35.83 g was found in M_0 (control).

The combined effect of salinity and calcium for the yield of chilli per plant showed significant difference. The highest yield (99.33 g) was recorded from S_0M_2 whereas the lowest value (17.33 g) was found from the S_3M_0.

CONCLUSION

Considering the previous mentioned results, it may be concluded that, the yield of chilli gradually decreased by the increment of salinity. This detrimental effect was minimized through mitigating agent (Ca^{2+}). Between the calcium levels used for the experiment, M_2 (12 mM Ca^{2+}) showed the highest result in growth, physiology and yield parameters. Therefore, the present experimental results suggested that Ca^{2+} (12 mM) application is relatively more suitable to mitigate salt stress and produce higher yield of chilli.

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APPENDICES

Appendix I. Characteristics of the soil used in pot

A. Morphological characteristics of the experimental site

Morphological features	Characteristics
Location	Horticulture Farm, SAU, Dhaka
AEZ	Madhupur Tract (28)
General Soil Type	Shallow red brown terrace soil
Land type	High land
Soil series	Tejgaon
Topography	Fairly leveled

B. Physical and chemical properties of the initial soil

Characteristics	Value
% Sand	27
% Silt	43
% clay	30
Textural class	Silt loam
Ph	6.1
Organic matter (%)	1.13
Total N (%)	0.03
Available P (ppm)	20.00
Exchangeable K (me/100 g soil)	0.10
Available S (ppm)	23

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

Appendix II. Monthly record of air temperature, relative humidity, rainfall and sunshine hour of the experimental site during the period from November 2018 to March 2019

Month	*Air temperature (ºc)		*Relative humidity (%)	Total Rainfall (mm)	*Sunshine (hr)
	Maximum	Minimum			
November, 2018	25.8	16.0	78	00	6.8
December, 2018	22.4	13.5	74	00	6.3
January, 2019	24.5	12.4	68	00	5.7
February, 2019	27.1	16.7	67	30	6.7
March, 2019	28.1	19.5	68	00	6.8

* Monthly average,

* Source: Bangladesh Meteorological Department (Climate & weather division) Agargoan, Dhaka – 1207

Appendix III. Analysis of variance on plant height of chilli as influenced by salinity and calcium application

Sources of variation	Degrees of freedom	Mean square of plant height (cm)			
		30 DAT	45 DAT	60 DAT	75 DAT
Replication	2	1.052	2.642	3.608	3.201
Factor A	3	NS	112.37*	168.96*	302.444*
Factor B	2	NS	67.19**	62.1820**	179.024*
AB	6	NS	82.54*	87.363*	161.701*
Error	22	1.022	3.293	4.076	4.744

NS = Non-significant * = Significant at 5% level ** = Significant at 1% level

Appendix IV. Analysis of variance on number of branches plant^{-1} of chilli as influenced by salinity and calcium application

Sources of variation	Degrees of freedom	Mean square of number of branches plant^{-1}			
		30 DAT	45 DAT	60 DAT	75 DAT
Replication	2	0.083	0.083	0.333	0.333
Factor A	3	1.657**	2.407*	1.519**	2.546*
Factor B	2	1.083**	1.083**	1.750**	2.083*
AB	6	5.269*	2.935*	2.046*	1.046**
Error	22	1.720	1.356	1.000	2.727

NS = Non-significant * = Significant at 5% level ** = Significant at 1% level

Appendix V: Analysis of variance on number of leaves plant^{-1} of chilli as influenced by salinity and calcium application

Sources of variation	Degrees of freedom	Mean square of number of leaves plant^{-1}			
		30 DAT	45 DAT	60 DAT	75 DAT
Replication	2	0.444	2.194	6.250	3.882
Factor A	3	12.546*	19.00*	92.48*	118.34*
Factor B	2	10.528*	5.861**	6.250**	36.845*
AB	6	26.491*	109.97*	106.06*	288.14*
Error	22	3.020	4.437	5.735	7.738

NS = Non-significant * = Significant at 5% level ** = Significant at 1% level

Appendix VI: Analysis of variance on leaf area of chilli as influenced by salinity and calcium application

Sources of variation	Degrees of freedom	Mean square of leaf area (cm^2)		
		30 DAT	45 DAT	60 DAT
Replication	2	1.044	7.036	6.200
Factor A	3	42.17*	104.696*	605.474*
Factor B	2	44.38*	167.672*	175.023*
AB	6	107.64*	451.741*	461.703*
Error	22	3.055	12.146	12.745

NS = Non-significant * = Significant at 5% level ** = Significant at 1% level

Appendix VII: Analysis of variance on yield contributing parameters and yield of chilli as influenced by salinity and calcium application

Sources of variation	Degrees of freedom	Mean square of yield contributing parameters					
		Number of flowers plant^{-1}	Number of fruits plant^{-1}	Length of fruit (cm)	Diameter of fruit (cm)	Individual fruit weight (g)	Fruits yield plant^{-1} (g)
Replication	2	3.083	5.111	0.105	0.026	0.513	5.444
Factor A	3	176.18*	343.43*	3.624*	NS	0.708**	125.1*
Factor B	2	97.583*	467.52*	8.083*	NS	0.256**	293.7*
AB	6	10.324*	147.49*	1.933**	0.006**	0.146**	17.29**
Error	22	3.598	8.596	0.693	0.005	0.077	9.566

NS = Non-significant * = Significant at 5% level ** = Significant at 1% level

Appendix VIII: Analysis of variance on quality parameters of chilli as influenced by salinity and calcium application

Sources of variation	Degrees of freedom	Mean square of quality parameters	
		TSS (Total soluble solid)	Vitamin C Content
Replication	2	0.194	0.019
Factor A	3	0.991**	0.369**
Factor B	2	0.528**	0.314**
AB	6	3.713**	0.541**
Error	22	0.073	0.006

NS = Non-significant * = Significant at 5% level ** = Significant at 1% level

Appendix IX: Some photographic demonstrations of research work

1. Raising of seedling in the seedbed

2. Weeding in the seedbed

3. Pot preparation for experiment

4. Seedling of chilli after few days of transplanting

5. Application of pesticide on leaves

6. Flower initiation of chilli at about 6 weeks after transplanting

7. Fruit set of chilli at about 8 weeks after transplanting

8. Measuring individual fruit length, diameter and weight