Morpho-physiological study of *Calendula officinalis* L. under saline conditions

BY

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CERTIFICATE-I

This is to certify that this thesis entitled, **"Morpho-physiological study of *Calendula officinalis* L. under saline conditions."** submitted for the degree of **Master of Science** in the subject of **Botany** of the Chaudhary Charan Singh Haryana Agricultural University, Hisar, is a bonafide research work carried out by **Ms Kusum Rani, Admn. No. 2011BS90M** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE-II

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CONTENTS

LIST OF TABLES

LIST OF TABLES IN APPENDIX

LIST OF FIGURES

LIST OF PLATES

CHAPTER-I

INTRODUCTION

Calendula officinalis L. is a herbaceous plant belonging to the family Asteraceae (Compositae). It is commonly known as "pot marigold" in English. The plant is native to the Mediterranean zone, the Middle East and central Europe. The name of *Calendula officinalis* L. comes from Latin word (calend) meaning first day of each month because this plant has a long flowering period. It is not only a spring board for monasteries and gardens but has an array of uses in drugs, foods, feed, beverages, dye, culinary, cosmetics, perfumery industries and at ceremonial religious occasions (Kalvatchev *et al.*, 1997). This plant has been grown in European gardens since 12^{th} century. It was in use as an efficient pesticide (Martin, 2005). It was cultivated as ornamental plant in countries *viz*., Iran, Palestine, Iraq, Saudi Arabia, Egypt, Libya, Tunisia, Algeria, Morocco, Canary Islands, Southern Spain Turkmenistan, Afghanistan, Pakistan and Kashmir until its medicinal properties were known (Mozafariyan, 2003). Pot marigold is being grown as medicinal drug in Germany, Australia, Czech, Austria, Switzerland, Hungary, Egypt, Syria, Eastern Europe, North America, India etc. (Samsamsharit, 2003).

It is an annual herb with simple leaves, bright yellow or orange daisy like flowers that is used as a decorative plant in horticultural industry (Duke *et al*., 2002). It prefers moderately healthy soil with average drainage and a pH of 5-8 but can be grown in a wide range of soils. *Calendula officinalis* is one of the most valued medicinal plant that is used as sudorific, blood refiner, blood sugar reducer and also posses anti-inflammatory skin (Khavarinejad and Lucia, 2004), antiviral, anti HIV, anti-tumor, anti-mutagenic and cytotoxic properties (Boucaud-Maitre *et al*., 1988; Amirghofran *et al.*, 2000). The tincture and sap of its flowers are used locally to hasten the cure of injuries and to reduce swelling. Its sap is also used to reduce the body temperature, cure painful menstruation and cancer. The pot marigold flower has astringent, menstruation, anticonvulsant, energizing, antiseptic nourishing, sopoforic, diuretic, blood thinning and elimination of vomiting effects. It has uses in anemia, kidney problems, grip, mumps, chicken pox, measles, ulcer, jaundice, neurotic problems, acne pimples, skin disease, wounds, snow bites. Its flowers can be used to lower the cholesterol level of the blood or blood pressure because of dilation of surface vessels, relieving stomach ulcer and curing digestive system problems (Mohammad and Kashani, 2012).

Additionally, pot marigold has been found among the most effective drugs to cure extreme bleedings of hemorrhoid. It is suitable to stop gum bleeding. The fresh milk of its leaf in one or two spoons is good to cure regulation and internal laceration. Eating its soup is a common practice so that American calls it 'pot herb'. In France they commonly use it brewed to lower

body temperature and perspiration, as an effective tranquilizer (Mir heydar, 2003). Its resins have antifungal, anti-bacterial, and antivirus effects. Moreover, this plant is used to cure the blisters of todlers' feet and relieve burning in nipples caused by breastfeeding (Zarezadeh, 2003).

A large number of phytochemicals have been found in various parts of the plants that include calendline and oleanolic acid, alpha- and beta- amyrin, taraxasterol, lupeol, brein, faradiol, arnidiol, erythrodiol, calenduladiol, coflodiol, saponins. The main chemical constituents of *Calendula officinalis* include terpenoids, phenolic acids, flavonoides, isorhamnetin, carotinoids, glycosides, vitamin C and sterols (Re *et al*., 2009; Andersen, 2001), which have antioxidant activities and play important role in human health (Meda *et al*., 2005). The leaves and flowers are used in various Homeopathic and Ayurvedic preparations. Flowers are also used in food industry to confer both colour and flavour to foods.

With increasing realization of health hazards and toxicity associated with the indiscriminate use of synthetic drugs and antibiotics, more and more people are interested in the use of plants and plant-based drugs being revived throughout the world. So exploitation of medicinal plants has become more and more popular (Tan *et al.*, 2006).

Salinity stress is one of the most extensive damage stresses in arid and semiarid regions, where limited rainfall, high evapotranspiration and high temperature associated with poor water and soil management contributed to the agricultural production in these regions. Soil salinity is presently one of the major abiotic stresses, reducing the agricultural production globally to a great extent (Desai *et al.*, 2012). Total global area of salt affected soil, including saline and sodic soils is 831 million hectares (Beltran and Manzur, 2005). Currently at least 20 per cent of the worlds irrigated land is salt affected and/or irrigated with water containing elevated levels of salt (Ghassemi *et al*.,1995). In India about 6744968 hectares of land is affected with salinity, whereas, it accounts for 232556 hectares in the state of Haryana (CSSRI, ICAR, Govt. of India report, 2012). Excess accumulation of water soluble salts in the soil adversely affects the plant growth by lowering the osmotic potential of the soil solution, causing nutritional imbalance, specific ionic toxicity or by combination of these factors. All of these factors cause pleiotropic effect on plant growth and development at physiological, biochemical or molecular level (Tester and Davenport, 2003; Kanta and Varshney, 2006). Salinity stress causes adverse effects on germination, growth and vigour, metabolism, flowering and fruiting processes and physiology, ultimately causing diminished economic yield and also quality of produce (Sairam and Tyagi, 2004; Kanta and Varshney, 2008; Deepika and Varshney, 2008).

Salt affected soils are characterized either by the presence of excess levels of water soluble salts (saline soils) and/or high amounts of sodium ions (Na^+) in the soil solution (Qadir *et al*., 2007).

Research efforts are being made toward utilization of such areas which have become unsuitable for raising conventional crops. With the diversification of agriculture, medicinal and aromatic plants are gaining importance in the national scenario. The areas becoming unsuitable for raising conventional crops due to one or the other reasons may be used for growing such plants depending upon their suitability to the prevailing environmental conditions. However, large scale adaption and production of medicinal and aromatic plant species under saline environment remains a challenge due to lack of awareness among farming communities, limited scientific database revealing the potential production capacity of these species at different levels of salinity in soil or irrigation water and lack of appropriate markets. Some studies have been made to assess the physiological responses of medicinal plants like isabgol, chandrashura, german chamomile, senna and and lemon grass to saline conditions (Singh and Anwar, 1985; Varshney and Kohar, 2001; Singh, 2004; Deepika, 2007; Suman, 2010).

Considerable work has already been carried out on this plant with regard to its medicinal importance but the salinity effects have attracted little attention. In order to meet the ever increasing demand of medicinal plants, for the indigenous system of medicine as well as pharmaceutical industry, some medicinal plants need to be cultivated commercially under saline conditions (Jaleel *et al*., 2008a). Research studies made on physiological aspects of *Calendula officinalis* under salinity stress are still meager (Khalid and Teixeira da Silva, 2010; Torbaghan, 2012). Therefore, the present research has been undertaken to enrich scientific database regarding the morhpo-physiology of the medicinal plants *Calendula officinalis*.

The objectives of the present investigation are :

1. To study the phenology, growth and reproductive behavior under field conditions.
2. To study the morpho-physiological attributes under saline conditions.

CHAPTER - II

REVIEW OF LITERATURE

Research studies on medicinal and aromatic plants to assess their suitability of cultivation in salt-affected soils are in progress. Salinity tolerance of medicinal plants has received attention in the recent past (Boucaud-Maitre *et al*., 1988; Kalvatchev *et al.*, 1997; Amirghofran *et al*., 2000; Andersen, 2001; Khavarinejad and Lucia, 2004; Meda *et al*., 2005; Re *et al*., 2009; Mohammad and Kashani, 2012). Such studies are being made considering crop diversification and management for optimal utilization of salt affected soils and saline-sodic water to increase the cultivable area. Several medicinal and aromatic plant species have been reported to have the ability to tolerate levels of salinity in soils and irrigation water (Patra and Singh, 1998, Varshney and Vandana, 2009; Sapna, 2011). The deleterious effects of salinity are both osmotic and ion specific. In general the chloride dominated salinity has been found to be more deleterious than the sulphate dominated salinity by affecting various growth and development and yield parameters in different crop plants (Manchanda and Sharma, 1990; Atam Parkash, 2000; Yadav, 2000 and Sapna, 2011). The deleterious effects of salinity on plant growth are associated with (1) low osmotic potential of soil solution (water stress), (2) nutritional imbalance, (3) specific ion effect (salt stress), or (4) a combination of these factors (Asraf, 1994; Marschner, 1995; Zhu, 2003; Turan *et al*., 2010). Salinity is known to adversely affect the production of most crops worldwide (Hasegawa *et al*., 2000; Bayuelo-Jime nez *et al*., 2002; Asraf, 2009). Response of various crops to salinity is reviewed under the following heads :-

2.1 Effect of salinity on growth attributes

2.2 Effect of salinity on reproductive behavior

2.3 Effect of salinity on physiological attributes

2.4 Effect of salinity on mineral status

2.1 Effect of salinity on growth attributes

Plant growth parameters of *Rosmarinus officinalis* are not affected by low concentration of NaCl, but decreased with higher concentrations. The content of its photosynthetic pigments decreased at all salinity levels (Kiarostami *et al*., 2010). Salinity hampers growth and yield of plants (Demiral and Turkan, 2006). Salinity induced inhibition of plant growth results from osmotic and ionic effect, and different plant species have developed different mechanisms to cope up with these effects (Munns, 2002; Sumithra *et al*., 2006). Salinity significantly reduced the overall growth of chickpea-*kabuli* (Garg and Singla, 2004); chickpea (Singh *et al*., 2001) and tomato (Chookhampaeng *et al*., 2008). Reduction in growth could be attributed to reduction in cell division and/ or cell enlargement (Hopkins, 1999). Eid *et al.* (2011) revealed that 3000 ppm

salinity level reduced plant height, number of branches plant^{-1} and fresh and dry weight plant^{-1} as well as flowering characters (number of flowers plant^{-1} and fresh and dry weights of flowers plant^{-1}) of *Tagetes erecta* L. as compared with control treatment.

Growth parameters such as plant height, leaf area, and fresh weight of aerial parts and percentage of dry weight of aerial parts of tomato hybrids responded negatively as the salinity level increased (Tantawy *et al*., 2009). Salinity also decreased the fresh weight and dry weight of canola plants (Bybordi, 2010). Increase in salinity from 0 and 70 mM NaCl decreased tomato growth, 29 per cent for leaf area, 26 per cent for plant height, 24 per cent for dry matter, but only 11 per cent for number of leaves (Romero-Aranda *et al.*, 2001). Salinity reduced plant height (Achilea, 2002; Agong *et al*., 2004 and Hajer *et al*., 2006), fresh weight (Hassan, 1999; Li, 2000; Sonneveld, 2000, Amico *et al*., 2003 and Hajer *et al*., 2006) as well as dry weight (Li, 2000 and Yustseven *et al*., 2003). The results on plant height, root length, number of leaves plant^{-1}, total number of tillers, dry weight of plant and leaf area reflected that these were reduced relatively more under chloride than sulphate salinity, whereby, affecting growth and development of wheat more adversely. However, percentage of effective tillers and shoot/ root ratio and harvest index showed a reverse trend (Sharma *et al*., 1994). In moong bean, all morpho-physiological characters such as plant height, number of leaf, leaf area were reduced with the increase in salinity levels as compared to control (Hossain *et al*., 2008). Shoot length, shoot fresh and dry weight of *Catharanthus roseus* gradually decreased with increase in salinity as compared to control in salinity treatments (Elfeky *et al*., 2007). Under 100 mM NaCl stress, plant growth of *Catharanthus roseus* was negatively affected, reducing fresh weight and dry weight by about 25 and 26 per cent, respectively. Root length was reduced by up to 30 and 53 per cent under 50 and 100 mM NaCl treatment, respectively, in comparison to untreated plants (Jaleel *et al*., 2008d).

Maize plants grown at 0 mM NaCl, recorded relatively higher dry weight and did not imply toxicity symptoms, however, the plant dry weight was significantly reduced at higher salinity (100 mM) indicating the symptoms of salt toxicity as growth depression. The concentration of 100 mM NaCl significantly reduced dry weight by 45.46 per cent in comparison to the control (Turan *et al*., 2009). Some physiological parameters in marigold (*Calendula officinalis* L.) were studied under 0, 50 and 100 mM NaCl. Higher salinity caused reduction in growth parameters, lipid peroxidation and hydrogen peroxide accumulation (Chaparzadeh *et al*., 2004). Growth parameters such as plant height, number of branches, and leaf area plant^{-1} at different stages of growth of *Chrysanthemum* decreased with increase in salinity level from control to 8 dSm^{-1} (Yadav, 2000) and these parameters were also decreased in marigold from

control to 6 dSm^{-1} (Atam Parkash, 2000). Reduction in all growth parameters was more pronounced in chloride dominated salinity (Yadav, 2000; Atam Parkash, 2000).

Under the two salt treatments (chloride and sulphate dominated salinity) various growth and developmental parameters *viz*., plant height, number of leaves, number of branches, root and shoot dry weight and root/ shoot ratio were adversely affected in isabgol genotypes (Vandana, 2003) and in lemon grass (Sapna, 2011).

i. Plant height : Increasing concentration of NaCl, from 25 mM to 200 mM in tomato cultivar PKM1significantly decreased the plant height (Babu *et al*., 2012). In sunflower 20 days after germination, salinity levels were created by the addition of NaCl of ECe 3.0, 4.5 and 6.0 dSm^{-1}, plant height decreased in each harvest as salinity level increased, however, it increased across the harvest from first harvest to fourth harvest (Khatoon *et al*., 2000). Higher concentrations of 50 and 100 mM NaCl caused 25.1 per cent and 30.9 per cent and 9.88 per cent and 16.3 per cent reduction in plant height and stem diameter of tomato, respectively (Chookhampaeng *et al*., 2008). Plant height of *Catharanthus roseus* decreased by up to 7 per cent and 34 per cent under low and high salinity, respectively, when compared to the control (Jaleel *et al*., 2008d). NaCl stress had an inhibitory effect causing 31.35 and 27.31 per cent reduction in height of plants, exposed to NaCl for 15 days, in *Cleome gynandra* (C_3 plant) and *C. viscosa* (C_4 plant), respectively (Kulya *et al*., 2011).

Significant decline in plant height of senna was found with progressive increase of salinity in the growing medium at the vegetative, flowering and pod maturity stages. Stunting effect of sulphate dominated salinity was more prominent than chloride dominated salinity (Suman, 2010) and similar results were observed in German chamomile (Deepika, 2007).

ii. Number of leaves $plant^{-1}$: Significant decline in number of leaves $plant^{-1}$ was found with the progressive increase of salinity in the growing medium in isabgol (Vandana, 2003), in German chamomile (Deepika, 2007) and in lemon grass (Sapna, 2011).

iii. Leaf biomass, **Stem and branches biomass** : Fresh weight and dry weight of both stems and leaves were significantly inhibited by higher salinity as compared to low and no salinity. Hasni *et al.* (2009) reported that the addition of 200 mM NaCl to the culture medium significantly reduced shoot biomass (33 %) of fenugreek. Maximum reduction (45 %) was observed in stem biomass. The root dry matter was not affected.

Dry weight decreased with increasing salt concentration (0, 2.5 and 5 dSm^{-1}) for all rootstocks of all citrus species, namely alemow, citromelo, rough lemon, volkamer lemon, sour orange and Mexican lime. The highest value of total dry weight observed in the control treatment without salinity (Ghotb Abadi *et al*., 2010). The highest salt concentration (100 mM) used in

cardoon plant induced a deleterious effect on the fresh weight of both roots and shoots (Benlloch-Gonzalez *et al*., 2005). As the salinity levels increased (0, 50, 100, 150 and 200 mM NaCl) in *Catharanthus roseus*, shoot length, shoot fresh and dry weights gradually decreased when compared with the control (Elfeky *et al*., 2007). Low salinity levels (40-80 mM NaCl) stimulated the shoot and root length and the production of dry matter in *Carum carvi* and *Cuminum cuminum*. Thereafter, the values decreased gradually with increase in the NaCl concentrations especially under higher concentrations (Zidan and Elewa, 1995). Garg *et al*. (2002) reported the decline of dry matter of cumin by 19.0, 22.7 and 51.6 per cent of control plants at 8, 12 and 16 dSm^{-1} salinity levels, respectively indicating an abrupt decrease beyond 12 dSm^{-1}.

Application of NaCl (80 and 160 mM) resulted in significant decrease in lengths of root and stem, in dry weight of root, stem and leaves and in the leaf area of *Cichorium intybus*, as compared with control. The reduction was less with the combined application of NaCl and $CaCl_2$ (10 mM) than with the NaCl treatment alone (Arshi *et al*., 2010a). Fresh weight and dry weight of sorghum cultivars were significantly reduced under the influence of increasing salinity induced either by NaCl or Na_2SO_4, however, the sulphate salt of sodium was found to be more detrimental than the chloride (Desai *et al*., 2012). On dry weight basis, 50 and 100 mM NaCl had more deleterious effects on tomato shoot growth (13.9 % and 20.4 % reduction, respectively) than root growth (7.4 % and 14.1 % reduction, respectively) (Chookhampaeng *et al*., 2008). Salt stress was evaluated on gerbera by subjecting plants to 0, 10, 20, 30 and 40 mM NaCl levels for ten weeks and observed a significant decrease in leaf and stem biomass with increased NaCl concentration (Don *et al*., 2010).

Significant decline in leaf dry weight and stem and branches dry weight $plant^{-1}$ of senna was found at vegetative, maximum flowering and pod maturity stages with the build up of salinity in the growing medium (Suman, 2010). Similar results have also been reported in German chamomile (Deepika, 2007)

iv. Flowers and fruits biomass : The application of elevated salt (25, 50, 100, 150 and 200 mM NaCl) stress resulted in decreased leaf area, dry matter content of tomato fruits and number of fruits $plant^{-1}$ (Babu *et al*., 2012). *Calendula* plants treated with different levels of saline irrigation water (0.39, 1.56, 3.13, 4.69, 6.25, 7.81 and 9.38 dSm^{-1}) consisting of NaCl, $CaCl_2$ and $MgCl_2$ salts showed significant reduction in the flower head yield with respect to increase in salinity (Khalid and Teixeira da Silva, 2010).

At the low level of NaCl stress in tomato, the number of mature fruits was reduced from 16.5 to 10.25 per cent $plant^{-1}$ (37.9 % reduction) and the average fruit weight was reduced from 45.56 g to 38.94 g (14.5 % reduction). At 50 to 100 mM NaCl, the number of mature fruits $plant^{-1}$

was drastically reduced by 53.0 and 51.5 per cent, and the average fruit weight was reduced by 54.2 and 58.7 per cent, respectively (Chookhampaeng *et al*., 2008).

In German chamomile (Deepika, 2007) a significant decline in fresh weight as well as dry weight of flower heads/plant was observed with the progressive increase of salinity levels in the growing medium.

v. Root biomass : The root dry matter of fenugreek was not affected by the addition of 200 mM NaCl to the culture medium (Hasni *et al*., 2009). In chickpea cultivars, the root weights showed a greater decline than the shoot mass under saline condition at all stages of growth (Garg and Singla, 2004; Singla and Garg, 2005).

Both root volume and root dry weight plant^{-1} significantly declined with the increase of salinity in the growing medium was observed in senna (Suman, 2010) and in German chamomile (Deepika, 2007). Decline in root and shoot dry weight plant^{-1} at both vegetative and flowering stages was observed in isabgol (Vandana, 2003) and in lemon grass (Sapna, 2011).

vi. Root/ shoot ratio : The reduction in root and shoot dry weights of chickpea directly affected the root to shoot ratio, which also declined with salinity (Singla and Garg, 2005). When maize seedlings were salt shocked by exposure to 50, 100 or 150 mM NaCl, root extension was initially slowed and greater inhibition was observed at higher NaCl concentration (Rodriguez *et al*., 1997).

Decrease in root/ shoot ratio was observed with the increase of salinity in the growing medium in isabgol (Vandana, 2003) and in lemon grass (Sapna, 2011). However increase in root/ shoot ratio was observed with the increase of EC level in senna (Suman, 2010).

2.2 Effect of salinity on reproductive behavior

All yield contributing characters of moong bean such as number of pods plant^{-1}, number of seeds pod^{-1}, 1000 seed weight, and harvest index were reduced with the increase of salinity levels as compared to control (Hossain *et al*., 2008).

i. Days to flower initiation : Researchers have observed a delay in flowering with increased salinity levels (Blits and Gallagher, 1991; Stanton *et al*., 2000). Relatively low levels of salinity can delay flowering by as much as three days (Zandt and Mopper, 2002). Salinity-induced flowering delay have been observed in wild mustard (*Sinapis arvensis*), an annual, non-wetland, salt sensitive species (Stanton *et al*., 2000) as well as in the salt tolerant marsh species *Cakile edentula* (Boyd and Barbour, 1986) and *Sporobolus virginicus* (Blits and Gallagher, 1991). Under 50 and 100 mM NaCl stress, flowering of tomato was significantly delayed for 12 days (Chookhampaeng *et al*., 2008). Increase in salinity caused delay in flower bud initiation, increased the number of days taken for flower initiation and reduced the duration of flowering in

chrysanthemum (Yadav, 2000) and in marigold (Atam Parkash, 2000). Delay in flower initiation was observed with the increase of salinity in German chamomile (Deepika, 2007) and in senna (Suman, 2010).

ii. **Number of flower heads plant^{-1} :** Among the various yield attributes studied, number of flowers plant^{-1} as well as seed weight plant^{-1} decided the maximum quantum of reduction in the harvest index among the *desi* and *kabuli* cultivars of chickpea under salt stress (Singla and Garg, 2005) . Number of pods plant^{-1} of moong bean varied from 9.80 to 8.34 among different salinity levels. The highest number of pods plant^{-1} (9.80) was recorded at control condition and lowest number (8.34) was recorded at 7.82 dSm^{-1} level of soil salinity (Hossain *et al*., 2008).

In chrysanthemum, number of flower buds plant^{-1} and number of flowers plant^{-1} decreased significantly with increasing level of salinity from control to 8 dSm^{-1}. Reduction in per cent flower opening, size of flowers and weight of flowers plant^{-1} were observed with increasing salinity levels from 0 to 10 6 dSm^{-1} particularly in chloride dominated salinity in chrysanthemum (Yadav, 2000) and from 6 dSm^{-1} in marigold (Atam Parkash, 2000).

Significant decrease in number of flower heads/plant of German chamomile occurred with the progressive increase of salinity levels in the growing medium (Deepika, 2007).

iii. Days to maturity: Pod maturity was observed to be delayed in senna by increase of salinity in the growing medium. The delay in flower initiation was relatively more under sulphate dominated salinity (Suman, 2010). However, early maturity was observed in German chamomile under salinity stress (Deepika, 2007).

iv. Number of seeds head^{-1} : The highest number of seeds pod^{-1} (10.00) of moong bean was recorded at control condition and the lowest (8.75) was recorded at 7.82 dSm^{-1} level of soil salinity (Hossain *et al*., 2008). Decline in number of seeds pod^{-1} and 100 seed weight was observed with the build up of salinity in senna (Suman, 2010) and in chickpea (Kiran, 2004).

v. Seeds output plant^{-1} (number basis) : Reduction in seed yield and number of seeds plant^{-1} was recorded in isabgol under sulphate and chloride salt treatments with the increase of EC levels (Vandana, 2003).

vi. Seed yield plant^{-1} : Salt stress affects the plant growth and development thereby affecting the yield quality and quantity (Sattar *et al*., 2010). The various yield attributes and yields were affected by salinity in all chickpea cultivars. The percentage reduction in pod and seed number was more drastic which resulted in a significant decline in weight of seed (Singla and Garg, 2005) as well as yield and some yield quality parameters of tomato hybrids responded negatively as the salinity level increased (Tantawy *et al*., 2009). Garg *et al*. (2002) reported that the increasing salinity progressively decreased both seed yield and shoot dry matter of cumin. The decrease in

seed yield was significant at and above 8 dSm^{-1} salinity levels and was 17.5, 21.8 and 40.7 per cent of control plants at 8, 12 and 16 dSm^{-1}, respectively.

vii. Seed germination (%) : Concentration of 200 mM NaCl delayed germination of fenugreek. Many authors have reported that increase in salinity leads to a reduction and/ or delay in germination of both halophyte and glycophyte seeds (Hasni *et al*., 2009). Zidan and Elewa (1995) observed that during germination cumin could tolerate NaCl salinity up to 200 mM but displayed significant reduction in per cent germination at 240 mM NaCl. Whereas, lower concentration of NaCl (100 mM) reduced the percentage germination of *Linum usitatissimum* to 47 per cent as compared with control. The germination was completely inhibited in response to the higher concentration of NaCl (300 mM). The shoot and radicle lengths decreased to 52.2 and 60.5 per cent, respectively as compared to control. However, application of folic acid, ascorbic acid or coblamine partially overcomes the inhibitory effect of salinity on germination (Emam and Helal, 2008).

West and Francois (1982) reported that the salinities up to 12 dSm^{-1} caused a delay in germination of cowpea but no significant reduction in final germination percentage. However, both 16 and 20 dSm^{-1} significantly reduced final germination percentage. Under the different NaCl dominated salinity levels (0, 1, 3, 5, 7 and 10 dSm^{-1}) *Calendula* seeds (*carenate, alate* and *orbicular*) were sown and the results showed that the maximum germination per cent was observed in control (non salinity treatment) whereas increasing NaCl salinity levels decreased germination per cent by 22.6, 35.5, 72.5, 75.8 83.8, respectively. He also reported that the germination per cent in *orbicular* type was lower than that in *carenate* and *alate* types (Torbaghan, 2012).

Increasing levels of salinity caused significant decline in per cent seed germination, Maguire index and Vigour index of senna (Suman, 2010).

Sorghum cultivars showed a gradual decrease in germination rate with increasing salinity level (Desai *et al*., 2012). Germination of *Crotalaria striata* seeds was carried out at salt concentrations of 0, 0.2, 0.6, 0.6 and 0.8 per cent NaCl. The germination was completely inhibited at 0.8 per cent concentration (Chandrashekar and Sandhyarani, 1995). Two medicinal plants; pot marigold (*Calendula officinalis*) and sweet fennel (*Foeniculum vulgare*) were studied for germination under salinity stress levels *viz*., 0, 2.5, 5, 7.5 and 10 dSm^{-1} and priming levels *viz*., control, GA_3, mannitol, NaCl and distilled water for 24 h at 25°C with three replications. Results indicated that with increasing salinity, germination traits such as germination per cent, rate and plumule length decreased, but seed priming with GA_3 and NaCl showed lower decrease (Sedghi *et al.,* 2010).

viii. Reproductive capacity : The existence of a species in a particular habitat depends to some extents upon its reproductive capacity (Salisbury, 1942). In most annuals the survival depends on viable seed production. Species with wide ecological amplitude have better reproductive equipment which profit them by a large seed output. Such species have in fact the capacity for a high potential progeny. Higher and viable seed production ensures better reproductive capacity of plants. Reproductive capacity of plants has been studied in relation to biotic factors and some edephic factors (Salisbury, 1942; Sant, 1961). But our understanding on this aspect of plant behavior under saline habitat is yet far from complete (Varshney, 1983).

2.3 Effect of salinity on physiological attributes

There was a linear decrease in the level of total cholorophyll, chlorophyll -*a*, chlorophyll -*b*, carotene and xanthophylls in moong bean as well as the intensity of chlorophyll flurorescence under increasing concentration of NaCl treatments. Compared to control, the pigment contents decreased on an average, by 31 per cent for total chlorophyll, 22 per cent for chlorophyll -*a*, 45 per cent for chlorophyll -*b*, 14 per cent for carotene and 19 per cent for xanthophyll (Saha *et al*., 2010). Application of NaCl on maize plant significantly decreased dry mass of plants. Stomatal resistance and proline concentrations were increased by high salinity, while total chlorophyll concentration was decreased. NaCl caused an increase in Na^+ and Cl^- concentrations of plant (Turan *et al*., 2009).

i. Chlorophyll content : Total chlorophyll contents decreased in response to salinity stress. Leaf chlorophyll content (chlorophyll -*a* and chlorophyll -*b* and total chlorophyll) were reduced significantly in all the chickpea cultivars as a result of increasing salinity (Garg and Singla, 2004) and total chlorophyll in tomato hybrids (Tantawy *et al*., 2009) in peanut (Al-Khaliel, 2010). The content of both chlorophyll -*a* and chlorophyll -*b* of *Catharanthus roseus* decreased as compared to the control with increasing salinity level (Elfeky *et al*., 2007). Total chlorophyll content in cumin leaves also progressively and significantly declined with increasing salinity at the flowering stage (Garg *et al*., 2002) and *Albizzia lebbeck* and *Dalbergia latifolia* (Sundaravalli *et al*., 2006).

The low and moderate salinity levels induced insignificant decrease in chlorophyll -*a*, chlorophyll -*b* and carotenoids content in Sorghum *cv*. Dorado and chlorophyll -*a* and carotenoids only in Sorghum *cv*. Hagen Shandawil. Significant decline in all the pigments was observed in Sorghum *cv*. Giza 113, when compared with control/ unsalinized plants (Azooz *et al*., 2004). Moong bean under salt induced stress, also showed a reduced content of chlorophyll -*a* and chlorophyll -*b* and carotenoid under severe stress (-0.5 MPa), but content was higher than that of plant under PEG-induced stress (Zayed and Zeid, 1997). Garg *et al.* (1996) studied the effects of

NaCl, Na_2SO_4 and $NaHCO_3$ each at EC 0, 5 and 10 dSm^{-1} and reported that NaCl at both the levels significantly decreased total chlorophyll in clusterbean but in moth bean and moong bean the decrease was recorded only at higher concentration, however, Na_2SO_4 enhanced chlorophyll content in clusterbean but decreased it in legumes. Bhivare *et al*. (1988) also reported that the chlorophyll content was reduced in French bean subjected to chloride salinity; an opposite trend was observed in sulphate-salinized plants. Desai *et al*. (2012) observed that chlorophyll pigments were drastically reduced in sorghum with progression in the concentration of both Na_2SO_4 and NaCl salts, however, noticeable higher degree of reduction in chlorophyll content was observed under Na_2SO_4 than NaCl. Chlorophyll -*a* and chlorophyll -*b* of canola reduced significantly due to increasing NaCl concentration (Nazarbeygi *et al*., 2011).

With increasing salinity levels in *Catharanthus roseus*, both chlorophyll -*a* and chlorophyll -*b* contents decreased as compared to control (Elfeky *et al*., 2007). The experiment on short term salinity stress on fenugreek was carried out at the laboratory and the results showed that salinity stress caused a significant reduction in chlorophyll and carotenoid contents as compared to control (Pour *et al*., 2013). Halophytes grown under saline soils were significantly lower in chlorophyll -*a* and chlorophyll -*b* contents, compared to other glycophytes; the chlorophyll -*a* being significantly higher than chlorophyll -*b* (Samiullah and Bano, 2011). A decrease in photosynthetic pigment of *Catharanthus* plants under salt stress was observed. There was a decrease of 11 per cent and 38 per cent of chlorophyll -*a* at 90 DAS in response to the 50 and 100 mM NaCl treatment, respectively, when compared to the control. In case of chlorophyll -*b*, the decrease was 16 and 33 per cent in response to 50 and 100 mM NaCl treatment, respectively, as compared to the control. Total chlorophyll content was reduced by 14 and 34 per cent under low and high salinity, respectively (Jaleel *et al*., 2008d).

Chlorophyll -*a*, chlorophyll -*b* and total chlorophyll content suffered a reduction at the two growth stages *i.e*. 60 DAT and 120 DAT with the increment of salinity (Sapna, 2011).

Total chlorophyll, Chlorophyll -*a* and chlorophyll -*b* content of leaf gradually decreased with increasing EC levels in all genotypes of *Plantago ovata*. Chloride salt treatment caused more reduction than sulphate salt treatment (Vandana, 2003). Similar results were also reported by Singh (2004) in chandrashura leaves. Reduction in chlorophyll -*a* and chlorophyll -*b* and total chlorophyll content in leaves of German chamomile with increasing salinity level was found at the vegetative as well as at the flowering stages. Chloride was found more reducing than sulphate salinity (Deepika and Varshney, 2008).

ii. Carotenoids : The effect of salinity stress on chlorophyll and carotenoid of fenugreek was studied with five levels of short term salinity stress (0, 50, 100, 150 and 200 mM) and the

results showed that salinity stress caused a significant reduction in chlorophyll and carotenoid content of fenugreek as compared to control. The reduction decreased with increasing salinity concentration (Pour *et al*., 2013). Salinity caused a decrease in chlorophyll and carotenoid content in rosemary plant (Kiarostami *et al*., 2010).

iii. Proline content : Salinity stress stimulates the accumulation of proline. Biochemical analysis of leaves and mature fruits for proline accumulation indicated that proline accumulation in tomato increased with increase in salt stress (Babu *et al*., 2012) and similar results were reported by Eid *et al*. (2011) in *Tagetes erecta* and Al-Khaliel (2010) in peanut.

The effect of $CaCl_2$ as an ameliorating agent on NaCl stress was studied in *Dioscorea rotundata* plants. Separate NaCl and $CaCl_2$ treatments increased the free amino acid and proline content in *Dioscorea rotundata* plants versus the control (Jaleel *et al*., 2008). Proline and amino acid content increased under NaCl stress in *Dioscorea roseus* plant (Jaleel *et al.,* 2008b). NaCl stressed plant simultaneously treated with $CaCl_2$ had lower free amino acid and proline content than plants treated with NaCl alone (Jaleel *et al*., 2008). The level of 300 mM NaCl promoted proline accumulation in *Linum usitatissimum* up to 170.9 per cent comparing with that of control value (Emam and Helal, 2008). Stress induced by NaCl caused an accumulation of proline, total phenolic and antioxidant in rosemary plants reported by Kiarostami *et al*. (2010).

The proline content in the rice seedling was affected by the presence of NaCl in the growth medium. For the high level of proline, the increment of NaCl concentration from 0 to 513 mM, raised the proline level significantly, by more than 8-fold increase (Wanichananan *et al*., 2003) and the proline content increased as the salinity level increased from 0 mM to 300 mM in sorghum cultivars (Desai *et al*., 2012). Proline content has also been reported to increase in canola (Nazarbeygi *et al*., 2011; Bandeh-hagh *et al*., 2008), in *Brassica juncea* (Saradhi and Mohanty, 1993), in chickpea (Singh *et al*., 2001), in cucumber (El-Baz *et al*., 2003) and in *Carotalaria striata* (Chandrashekar and Sandhyarani, 1995).

A progressively significant increase in the content of both proline and glycine in the salinized plants of lettuce as compared to control was reported by Younis *et al*. (2009). NaCl treatments significantly ($P \leq 0.05$) increased proline contents in roots and leaves of moong bean seedlings (Saha *et al*., 2010). Plants of *Phyllanthus amarus* were raised in pot and salinity stress was imposed by 80 mM NaCl. The NaCl stressed plant showed decreased protein, total sugars, polyphenol oxidase and catalase activity with increased free amino acids, proline and peroxidase activities (Jaleel *et al*., 2008c). Accumulation of proline in the mature leaves of the tomato plants treated with 25, 50 and 100 mM NaCl was 2.55, 2.78 and 11.4 times the leaves found in the controlled plants, respectively (Chookhampaeng *et al*., 2008). High salinity levels caused an

increase in free proline content in leaves of chrysanthemum. Both the cultivars differed in accumulation of proline content in their leaves and the maximum amount of proline accumulated in the plants grown at 8 dSm^{-1} ECe (Yadav, 2000).

An accumulation of proline in leaves of German chamomile under various salinity treatments was found both at vegetative and flowering stages that helped in osmotic adjustment in plants (Deepika, 2007). Proline accumulation has also been reported in leaves of isabgol (Vandana, 2003) and lemon grass (Sapna, 2011).

iv. Relative stress injury : Moong bean grown in pot containing sandy soil were saturated with Cl^- dominated saline irrigation to maintain ECe of 2.5, 5.0, 7.5, dSm^{-1} as compared to control. The H_2O_2, lipid peroxidation (in term of MDA) and RSI increased continuously from 6.73 per cent to 40.24 per cent, 52.72 per cent to 89.47 per cent, 19.16 to 65.49 per cent and from 32.67 per cent to 71.90 per cent, 25.21 per cent to 65.9 per cent, 19.60 per cent to 75.88 per cent in nodules of genotypes Asha and Muskan, respectively (Kumar *et al*., 2008). The increase in relative stress injury (RSI %) of wheat nodule was associated with the increase in lipid peroxidation and can be used as screening of salt tolerance line (Farooq and Azam, 2006). The per cent leakage (RSI) was increased with increasing levels of salinity in chickpea genotypes (Kiran, 2004).

2.4 Effect of salinity on mineral status

Eid *et al*. (2011) reported that the high water salinity level (3000 ppm) led to decreasing of N, P and K per cent to lowest ratios in marigold plant. The results go in line with those of Talaat and Aziz (2005) on chamomile and El-Gabas (2006) on sunflower plant and in peanut (Al-Khaliel, 2010).

Sharma *et al*. (1994) observed higher inhibition in the uptake of essential mineral nutrients like Na^+, Cl^- and SO_4^{2-} in wheat plant parts under chloride than sulphate-salinity. Consequently K^+/ Na^+ ratio was also decreased more under chloride-type than sulphate-type of salinity. It is equally interesting that the accumulation of Cl^- was more than SO_4^{2-} ions at corresponding levels of salinity especially in stem and leaves. Fifteen days old seedlings of soybean were treated with solution of 25 mM, 50 mM and 100 mM NaCl alone and in combination of 10 mM $CaCl_2$ i.e., 25 mM + 10 mM, 50 mM + 10 mM and 100 mM + 10 mM. The Na^+ and Cl^- contents in different plant parts increased with NaCl as well as with NaCl + $CaCl_2$ treatments. The maximum accumulation occurred in roots, followed by the stem and leaves. The K^+ and Ca^{2+} contents decreased under NaCl stress; but NaCl + $CaCl_2$ treatment reduced the extent of decreased caused by NaCl. Thus, calcium ameliorates the deleterious effect of NaCl stress and stimulated plant metabolism and growth (Arshi *et al*., 2010).

Aster tripolium and *Sesuvium portulacastrum* were grown with 0 per cent, 1.5 per cent and 3.0 per cent (*Aster*) or 0 per cent, 2.5 per cent and 5 per cent (*Sesuvium*) NaCl in the watering solution. The growth rate was reduced in both species with increasing NaCl concentrations. The quotient of Na^+/K^+ indicated that *Aster* accumulates more K^+ in comparison to Na^+ while the reverse is true for *Sesuvium*. Osmolality of the leaf sap increased with increasing NaCl concentration in both *Aster* and *Sesuvium* (Ramani *et al*., 2006). With the increase in salinity level from 0 to 8 dSm^{-1}, there was more accumulation of N, P, Cl^-, SO_4^{2-} and proline but K^+ content decreased (Yadav, 2000).

Increase in Na^+ and Cl^- content while a decrease in K^+ content was observed in isabgol shoots with increasing EC levels (Vandana, 2003). Increase in Na^+ and Cl^- content while a decrease in K^+ content was observed in leaves of lemon grass with increasing EC levels (Sapna, 2011).

i. **Sodium (Na^+)** : At high salinity concentration a major amount of sodium content was exported to shoots, whereas, this content decreased in roots. These results suggest that *Mentha pulegium* was able to adopt an excluder. On the other hand, salt treatment induced a significant decrease of K^+ content (Queslati *et al*., 2010). The content of Na^+, K^+, Cl^- and total osmotic activity was measured in roots and shoots of the *Cynara cardnuculus* plants grown at different NaCl or KCl concentrations. When NaCl was used to induce salt stress, the Na^+ content in roots and shoots increased with salinity. At the same time, the K^+ content decreased in the shoots, but no significant change was found in the K^+ content of roots with respect to control plants (Benlloch-Gonzalez *et al.*, 2005).

As the salt content of the soil increased, there was a significant ($P<0.001$) increase of Na^+ concentration in rapeseed, the K^+ concentration did not change, while the Ca^{2+} concentration increased significantly ($P<0.001$). The Na^+ concentration in plant tissue increased several fold as soil sodicity increased from SAR of 12 to 44. As the salinity increased, the K^+/Na^+ ratio in the tissue decreased significantly (Porcelli *et al*., 1995). The increase in Na^+ and decrease in K^+ content with the progressive increase in NaCl concentration was also reported in *Rosmarinus officinalis* by Kiarostami *et al*. (2010) and similar results were reported in canola by Bandeh-hagh *et al*. (2008). Na^+ content responded positively to the increment of salinity levels in tomato leaves (Tantawy *et al*., 2009) and moong bean leaves (Hossain *et al*., 2008). However, Zayed and Zeid (1997) reported that under salinity, the moong bean accumulated Na^+ in roots while the Na^+ in plant shoot was reduced. The Na^+ contents in *Cichorium intybus* increased significantly in the NaCl treated plants with each concentration as compared to control; the maximum accumulation taking place in leaves, followed by the stem and the roots (Arshi *et al*., 2010a). Na^+ per cent in the

dry matter of roots and leaves of cucumber plant were increased by increasing salt stress (El-Baz *et al*., 2003).

ii. Potassium (K^+) : Potassium concentration in the plants increased with increase in salinity levels as compared to low salinity and without salinity. Hasni *et al*. (2009) reported that potassium and calcium content of fenugreek plant decreased significantly in saline conditions whereas Na^+ contents significantly increased. Similar results have been reported for *Ammolei majus* L. (Ashraf *et al*., 2004). Chemical analysis of tomato leaf and mature fruits showed a significant elevation in the level of sodium ion concentration while K^+ and K^+/Na^+ decreased with application of higher concentration of NaCl. The rate of increase in Na^+ content was higher in leaves than in fruits (Babu *et al*., 2012). Similar studies were carried out and same outcomes were found by some other authors (Akram *et al*., 2007; Loukehaich *et al*., 2011). Increasing salinity progressively decreased the concentration of nitrogen, phosphorus and potassium while increased the sodium content both in shoot and seed of cumin (Garg *et al.*, 2002). Decrease in potassium content with increasing salinity level have also been reported in moong bean by Zayed and Zeid (1997); in cucumber by El-Baz *et al*. (2003); in moong bean by Hossain *et al*. (2008) and in Canola by Bybordi (2010). Potassium content in *Triticum aestivum* shoots exhibited an increasing trend whereas the reverse was noted in roots with increasing salt stress levels (Roy *et al*., 2003).

iii. Chloride (Cl^-) : When plants were exposed to salinity, Cl^- concentrations increased in both roots and leaves. West and Francois (1982) reported the concentration of Ca^{2+} and Cl^- increased in cowpea, while K and P concentrations decreased with increasing salinity. Zayed and Zeid (1997) reported that the chloride content increased slightly in moong bean shoots as a result of salt treatment, but its accumulation was high in root cells.

iv. Sulphate (SO_4^{2-}) : Increase in salt concentration in the growing medium from 0 to 10 dSm^{-1} increased the sulphate concentration in chrysanthemum (Yadav, 2000) and in isabgol (Vandana, 2003). Sulphate concentration has also been reported to increase in senna (Suman, 2010) and in lemon grass (Sapna, 2011) with the increase in salinity in growing medium.

CHAPTER - III

MATERIALS AND METHODS

To achieve the objectives of the study entitled, "**Morpho-physiological study of *Calendula officinalis* L. under saline conditions"** experiments were carried out in the screen house of the Department of Botany and Plant Physiology, Chaudhary Charan Singh Haryana Agricultural University, Hisar during the year 2012-13. The details of materials used and techniques followed are described briefly in this chapter,

Climate and weather conditions

Hisar is situated at 29°10′ North latitude and 75°46′ East longitude at an elevation of 215.3 m above the mean sea level. This region has semi-arid climate with severely cold winter and hot dry summer. The average annual rainfall is about 420 mm, bulk of which is mostly received from mid June to mid September. There was no specific pattern of rainfall in winter season during which this investigation was carried out. Mean monthly meteorological data during the growing season of pot marigold recorded at "Meteorological Observatory" located at Research Farm of CCS Haryana Agricultural University, Hisar are given in Table 3.1of this chapter.

Table 3.1 Meteorological data during the experimental period (November, 2012 to June, 2013)

Month	Mean atmospheric Temp (°C)		Mean Relative Humidity (%)		Sunshine hours	Evaporation (mm)	Total rainfall (mm)
	Maximum	Minimum	Morning	Evening			
Nov, 2012	27.4	9.2	92	38	6.0	1.9	0.0
Dec, 2012	20.8	6.0	93	58	5.9	2.1	5.5
Jan, 2013	17.6	4.2	95	58	5.3	1.4	43.0
Feb, 13	21.5	8.9	96	60	5.9	2.1	32.7
March, 13	28.4	12.0	92	47	8.4	3.4	31.1
April, 13	35.0	17.2	68	27	8.7	5.9	2.3
May, 13	41.5	22.7	48	17	9.7	9.2	0.0
June, 13	39.3	27.2	69	44	7.4	7.9	97.3

Plant material : The seeds were collected from the Botanical Garden, Department of Botany and Plant Physiology, CCS Haryana Agricultural University, Hisar in the month of May, 2012.

Experiment 1. Study of the phenology, growth and reproductive behaviour of *Calendula officinalis* under field conditions

Location: Botanical Garden, Department of Botany and Plant Physiology at CCS Haryana Agricultural University, Hisar.

Culture conditions : Seeds were sown directly in field plot of Botanical Garden, CCS Haryana Agricultural University, Hisar.

Physio-chemical properties of the soil

Parameters	**:**	**Soil of Botanical Garden field plot**
pH	:	8.0
EC $_{1:2}$	:	0.20 dSm^{-1}
Organic Carbon	:	0.15 %
Available K_2O	:	375 kg/ha
Available P_2O_5	:	20 kg/ha
Texture	:	Sand

Replicates : Three

Parameters studied

1. **Phenology** (various life cycle events)
2. **Growth attributes** (recorded at vegetative, flowering and maturity stages)**:**
   - i. Plant height (cm)
   - ii. Basal stem diameter (mm)
   - iii. Number of leaves $plant^{-1}$
   - iv. Leaf biomass (g $plant^{-1}$)
   - v. Stem and branch biomass (g $plant^{-1}$)
   - vi. Flower and fruit biomass (g $plant^{-1}$)
   - vii. Root biomass (g $plant^{-1}$)
   - viii. Root/ shoot ratio
3. **Reproductive behavior**
   - ix. Number of flower heads $plant^{-1}$
   - x. Number of seeds $head^{-1}$
   - xi. Seeds output (number $plant^{-1}$)
   - xii. Seed yield (g $plant^{-1}$)
   - xiii. Seed germination (%)
   - xiv. Reproductive capacity

Procedures

1. **Phenology :** Various life cycle events were expressed diagrammatically as shown below

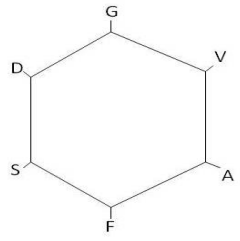
G - Germination;

V - Vegetative growth;

A - Anthesis;

F - Fruiting;

S - Seed maturation;



D - Death /senescence

2. Growth attributes

i. Plant height : Plant height was measured with the help of meter scale from ground level to the tip of the apical shoots or flower of tallest shoot at vegetative (75 days after sowing, DAS), flowering (105 DAS) and maturity stages (155 DAS). Average plant height was calculated and presented in centimeter (cm).

Plants were harvested at the vegetative (75 DAS), flowering (105 DAS) and maturity stages (155 DAS) by taking out soil monoliths. Roots were washed thoroughly under running water to remove the adhering soil particles. Root and shoot portions were separated with the help of scissors and the various parameters were recorded as given below.

ii. Basal stem diameter : Basal stem diameter was measured with the help of digital vernier caliper at two and half centimeter above the ground level and expressed in millimeter (mm).

iii. Number of leaves plant^{-1} : Number of leaves of each plant were counted and the data were expressed on average number of leaves per plant basis.

iv. Leaf biomass (g plant^{-1}) : Leaves were separated plant wise and dried in labeled paper envelops in an oven at 60±2°C for 48 hour and after bringing them to room temperature, their dry weights (g) were determined on digital electronic balance. Average dry weights were expressed on gram plant^{-1} basis.

v. Stem and branches biomass (g plant^{-1}) : Shoot portion (stem and branches except leaves and flower) were dried in labeled paper envelops in an oven at 60±2°C for 48 hour and then dried shoots weight were determined after bringing them to room temperature with the help of digital electronic balance. Average dry weights were expressed on gram plant^{-1} basis.

vi. Flowers and fruits biomass (g plant^{-1}) : Flower and fruit portions of plants were dried in labeled paper envelops in an oven at 60±2°C for 48 hour and weight was determined after bringing them to room temperature with the help of digital electronic balance. Average dry weights were expressed on gram plant^{-1} basis.

vii. Root biomass (g plant^{-1}) : Roots of the plants were dried in labeled paper envelops in an oven at 60±2°C for 48 hour and weight was determined after bringing them to room temperature with the help of digital electronic balance. Average dry weights were expressed on gram plant^{-1} basis.

viii. Root/ shoot ratio : The ratio was determined by using dry weight of roots and shoots.

3. Reproductive behavior

ix. **Number of flower heads plant^{-1} :** Total number of flowers were counted visually on all the experimental plants. Data were expressed on average number of flowers per plant basis.

x. **Number of seeds head^{-1} :** Fruiting heads were harvested after maturity and seeds were separated and then counted for number of seeds per head.

xi. **Seed output (number plant^{-1})** : Seeds were separated from the mature harvested heads and these seeds were counted manually. The average seeds per flower head were multiplied by number of flower heads per plant.

xii. **Seed yield (g plant^{-1})** : Air dried weight of total number of seeds produced per plant were determined and recorded.

xiii. **Seed germination (%)** : Seed collected at the time of maturity were air dried and germinated in Petri dishes on moistened filter paper discs under laboratory conditions in the month of October, 2013. Per cent seed germination was calculated. The criterion for seed germination was the emergence of radicle.

xiv. **Reproductive capacity :** (Salisbury, 1942) It was calculated with the help of following formula.

$$\text{Reproductive capacity} = \frac{\text{Av. seed output plant}^{-1} \text{ x Av. \% seed germination}}{100}$$

Experiment 2: Study of the morpho-physiological attributes of *Calendula officinalis* L. under chloride dominated salinity

Location: Screen house of the Department of Botany and Plant Physiology at CCS Haryana Agricultural University Campus, Hisar.

Culture Conditions

Plants were raised by seeds in polythene bags (12″x15″) filled with eight kilogram dune sand in the screen house of the Department of Botany and Plant Physiology. The sand filled polythene bags were saturated with the solutions of respective salinity treatments along with nutrients (Hoagland and Arnon, 1950) before sowing.

Salinity treatments: Chloride dominated salinity was created by using a mixture of salts *viz*. NaCl, $CaCl_2.2H_2O$, $MgCl_2.6H_2O$, and $MgSO_4.7H_2O$ on meq basis as per the following ratios:

Chloride dominated salinity	:	$Cl^-:SO_4^{-2}$ (7:3); $Na^+:Ca^{2+}+Mg^{2+}$ (1:1); $Ca^{2+}:Mg^{2+}$ (1:3)
EC levels (with added salinity)	:	0 (control), 4, 8, 12 dSm^{-1}
Design	:	(Completely randomized design) CRD
Replicates	:	Three

Physio-chemical properties of the dune sand used in the experiment

Parameters : Dune sand used in polythene bags

pH	:	8.15
EC $_{1:2}$	:	0.21 dSm^{-1}
Organic Carbon	:	0.08 %
Available K_2O	:	185 kg/ha
Available P_2O_5	:	5 kg/ha
Texture	:	Sand

Composition of Hoagland and Arnon nutrient solution

S**tock solution**

Sr. No	**Major salts**	**Quantity**
1	Ca $(NO_3)_2.4H_2O$	364.0 g/l
2	$MgSO_4$. $7H_2O$	217.06 g/l
3	KNO_3	221.3 g/l
4	KH_2PO_4	62.1 g/l
5	**Minor salts**	
	$ZnSO_4$	0.097 g/l
	H_3BO_3	1.269 g/l
	Na_2MoO_4	0.400 g/l
	$CuSO_4.5H_2O$	0.035 g/l
	$MnSO_4$	0.609 g/l
6	Tartaric acid	4.0 g/l
7	$FeSO_4.7H_2O$	5.0 g/l

Nutrients solution was prepared by mixing 62.5 ml each of stock solution from serial number one to five and 15 ml each of stock solution serial number six and seven and finally diluting to 25 litre with distilled water.

Ten seeds per bag were sown in various salinity treated polythene bags. The moisture in the bags was maintained at field capacity by adding water in the required amount. After successful establishment of seedlings, thinning was done to maintain three uniform sized seedlings per polythene bag. All observations were recorded from these plants.

Parameters studied

1. **Growth attributes** (recorded at vegetative, flowering and maturity stages)
    i. Plant height (cm)
    ii. Number of leaves $plant^{-1}$
    iii. Leaf biomass (g $plant^{-1}$)
    iv. Stem and branches biomass (g $plant^{-1}$)

v. Flowers and fruits biomass (g plant^{-1})

vi. Root biomass (g plant^{-1})

vii. Root/ shoot ratio

2. Reproductive behavior

viii. Days to flower initiation

ix. Number of flower heads plant^{-1}

x. Days to maturity

xi. Number of seeds head^{-1}

xii. Seed output (number plant^{-1})

xiii. Seed yield (g plant^{-1})

xiv. Seed germination (%)

xv. Reproductive capacity

3. Physiological attributes

xvi. Chlorophyll content (in leaves at vegetative stage)

xvii. Carotenoids (in flower petals)

xviii. Proline content (in leaves at vegetative stage)

xix. Relative stress injury (in leaves at vegetative stage)

4. Mineral status (in leaves at maturity stage)

xx. Sodium (Na^+)

xxi. Potassium (K^+)

xxii. Chloride (Cl^-)

xxiii. Sulphate (SO_4^{2-})

Procedures

1. Growth attributes

i. Plant height : Plant height was measured with the help of meter scale from ground level to the tip of the apical shoots or flower of tallest shoot at vegetative (75 days after sowing, DAS), flowering (105 DAS) and maturity stages (155 DAS). Average plant height was calculated and presented in centimeter (cm).

ii. Number of leaves plant^{-1} : Number of leaves of each plant were counted and the data were expressed on average number of leaves per plant basis.

Plants were harvested by cutting and removing soil bulk from polythene bags at the vegetative (75 DAS), flowering (105 DAS) and maturity stages (155 DAS). Roots were washed thoroughly under running water to remove the adhering soil particles. Root and

shoot portions were separated with the help of scissors and the various parameters were recorded as given below.

iii. **Leaf biomass (g plant^{-1}) :** Leaves were separated plant wise and dried in labeled paper envelops in an oven at 60±2°C for 48 hour and after bringing them to room temperature, their dry weights (gram) were determined on digital electronic balance. Average dry weights were expressed on gram plant^{-1} basis.

iv. **Stem and branches biomass (g plant^{-1}) :** Shoot portion (stem and branches except leaves and flower) were dried in labeled paper envelops in an oven at 60±2°C for 48 hour and then dried shoots weight were determined after bringing them to room temperature with the help of digital electronic balance. Average dry weights were expressed on gram plant^{-1} basis.

v. **Flowers and fruits biomass (g plant^{-1}) :** Flower and fruit portions of plants were dried in labeled paper envelops in an oven at 60±2°C for 48 hour and weight was determined after bringing them to room temperature with the help of digital electronic balance. Average dry weights were expressed on gram plant^{-1} basis.

vi. **Root biomass (g plant^{-1}) :** Roots of the plants were dried in labeled paper envelops in an oven at 60±2°C for 48 hour and weight was determined after bringing them to room temperature with the help of digital electronic balance. Average dry weights were expressed on gram plant^{-1} basis.

vii. **Root/ shoot ratio :** The ratio was determined by using dry weight of roots and shoots.

2. Reproductive behavior

viii. **Days to flower initiation** : Number of days taken for the formation of first floral bud were recorded.

ix. **Number of flower heads plant^{-1} :** Total number of flowers were counted visually on all the experimental plants. Data were expressed on average number of flowers per plant basis.

x. **Days to maturity :** Number of days taken for the browning of stem was recorded.

xi. **Number of seeds head^{-1} :** Fruiting heads were harvested after maturity and seeds were separated and then counted for number of seeds head^{-1}.

xii. **Seed output (number plant^{-1})** : Seeds were separated from the mature harvested heads and these seeds were counted manually. The average seeds per flower head were multiplied by number of flower heads plant^{-1}.

xiii. **Seed yield (g plant^{-1})** : Air dried weight of total number of seeds produced per plant were determined and recorded.

xiv. **Seed germination (%)** : Seed collected at the time of maturity were air dried and germinated in Petri dishes on moistened filter paper discs under laboratory conditions in the

month of October, 2013. Per cent seed germination was calculated. The criterion for seed germination was the emergence of radicle.

xv. Reproductive capacity : (Salisbury, 1942) It was calculated with the help of following formula.

$$\text{Reproductive capacity} = \frac{\text{Av. seed output plant}^{-1} \times \text{Av. per cent seed germination}}{100}$$

3. Physiological attributes

Plant material : For various biochemical estimation, fourth leaf from the top of each plant was used. Three replicates per treatment were used for each biochemical estimation.

xvi. Chlorophyll content (in leaves at vegetative stage) **:** The chlorophyll a, chlorophyll b and total chlorophyll were estimated according to the method given by Hiscox and Israelstom (1979) using dimethyl sulphoxide (DMSO) as solvent. Calculation was made according to the equation given by Wellburn (1994).

Procedure : One hundred mg of fresh leaf portion was kept into a test tube containing 5 ml of Dimethyl sulphoxide (DMSO). The test tube was then placed in an oven at 60°C for about two hours or more (if required) to facilitate the extraction of the pigment. There after attaining the room temperature absorbance was recorded at 649 and 665 nm wave lengths on computer aided spectrophotometer (Specrtophotometer-119) running a multiple wave lengths programme. DMSO was used as blank. Calculations for different pigments were made according to Wellburn (1994).

Calculation

Chlorophyll 'a' ($\mu g\ ml^{-1}$) = $12.19\ A_{665} - 3.45\ A_{649}$

Chlorophyll 'b' ($\mu g\ ml^{-1}$) = $21.99\ A_{649} - 5.32\ A_{665}$

Total chlorophyll = Chlorophyll 'a' + Chlorophyll 'b'

Chlorophyll -*a*, Chlorophyll -*b* and total Chlorophyll were expressed in ($mg\ g^{-1}$) tissue dry weight.

xvii. Carotenoids (in flower petals) : For carotenoid estimation flower petals were taken as a sample. The carotenoid was estimated according to the method given by Hiscox and Israeldtam (1979) using dimethyl sulphoxide (DMSO) as solvent. Calculation was made according to the equation given by Wellburn (1994).

Procedure : One hundred mg of fresh flower petals were kept into a test tube containing 5 ml of dimethyl sulphoxide (DMSO). The test tube was then placed in an oven at 60°C for about two hours or more (if required) to facilitate the extraction of the pigment. There after attaining the room temperature observance was recorded at 480, 649 and 665 nm

wavelengths on computer aided spectrophotometer (Specrtophotometer-119) running a multiple wavelengths programme. DMSO was used as blank. Calculations for different pigments were made according to Wellburn (1994).

Calculations

Carotenoids ($\mu g\ ml^{-1}$) = $(1000A_{480} - 2.14Ca - 70.16Cb)/ 220$

Ca = Chlorophyll 'a'; Cb = Chlorophyll 'b'

Chlorophyll -*a* and chlorophyll -*b* were calculated as per formulae given under chlorophyll estimation

Carotenoids were expressed in (mg g^{-1}) tissue fresh weight.

xviii. Proline content (in leaves at vegetative stage) : Proline content (mg g^{-1}) was estimated spectrophotometrically according to Bates *et al*. (1973).

Reagents

Acid ninhydrin : acid ninhydrin was prepared by warming 1.25 g of ninhydrin in 30 ml of acetic acid and 20 ml of 6.0 M phosphoric acid. It was stored at low temperature and used within the next 24 hours.

Sulphosalicylic acid 3 per cent (w/v)

Extraction : Each leaf sample (100 mg) was homogenized in 5 ml of 3 per cent sulphosalicylic acid (w/v) and centrifuged at 5000 x g for 20 minutes. The supernatant was used for proline estimation.

Procedure : Two ml of supernatant was taken, and two ml of acid ninhydrin and two ml of concentrated acetic acid were added to it. The mixture was heated in a water bath for one hour at 100°C. Cooling in an ice-bath terminated the reaction and the reaction mixture was shaken vigorously with four ml of toluene. The colored organic phase was separated from the aqueous phase on attaining room temperature. Observance of organic phase was read at 520 nm wave length using toluene as a blank. A calibration curve was prepared by using graded concentration of L-proline. Proline was calculated as given below :

$$\textbf{Proline}\ (\mu g\ g^{-1}) = \frac{\text{Concentration of proline } (\mu g)}{\text{Weight of tissue (g)}} \times \frac{\text{Final volume of aliquot}}{\text{Volume of aliquot used}}$$

Proline was expressed in (mg g^{-1}) tissue dry weight.

xix. Relative stress injury (in leaves at vegetative stage) : Membrane stability in terms of RSI per cent by the method of Sgherri *et al.* (1995).

Procedure : One hundred mg of fresh leaf (third leaf from top) were kept in 20 ml vials containing 10 ml of de-ionised water. After three hours the electrical conductivity (EC) of the solution was measured (ECa) on EC meter (Digital conductivity meter, Naina). Then

the samples were subjected to high temperature regime (50-55°C) for 20 minutes in a water bath. After cooling, the EC of the solution was again measured (ECb). The percent leakage of ions was measured in terms of relative stress injury (RSI %) by the following formula :

$$\textbf{RSI (\%)} = \frac{ECa}{ECa+ECb} \times 100$$

4. Mineral status (in leaves at maturity stage)

Plant material : For mineral studies, the leaves of each treatment were dried in an oven at 60°C for 48 hours. The dried mass was ground to fine powder and used for analysis.

Digestion : One hundred mg oven dried and well ground material was taken in 50 ml conical flask to which 5 ml of HNO_3 : $HClO_4$ (4 : 1) diacid mixture was added. The flasks were heated gently on a hot plate till formation of dense white fumes. At the stage fumes subsided and the samples become transparent. The digest thus obtained was cooled and the final volume was made to 20 ml by adding distilled water. Then it was filtered by using Whatman filter paper and used for analysis of sodium, potassium and sulphate as given below.

xx. Sodium (Na^+) : The diluted and filtered acid digest was used for analyses. Sodium was estimated with a flame photometer (Elico) using standard NaCl. The values measured were then expressed as mg g^{-1} tissue dry weight.

Calculation (Na^+ mg g^{-1}) : (Reading x dilution factor)/1000

Dilution factor = Final volume made (20 ml) / weight of material (0.1 g) = 200 times

xxi. Potassium (K^+) : Estimated in the similar way as sodium above, using standard KCl.

Calculation (K^+ mg g^{-1}) : (Reading x dilution factor)/1000

Dilution factor = Final volume made (20 ml) / weight of material (0.1 g) = 200 times

xxii. Chloride (Cl^-) : Chloride was estimated volumetrically by the method of Chhabra (1973).

Digestion : Five hundred mg of well ground material was taken in 50 ml conical flask and 5 ml of 1N HNO_3-KNO_3 (1:8) solution was added to it. The solution was stirred for 30 min on a magnetic stirrer and then filtered. Filtrate was used for chloride estimation.

Reagents

a. Potassium chromate (5 %) : Prepared by dissolving 5.0 g of potassium chromate in 50 ml of water and adding 1N silver nitrate solution drop wise until a red precipitate was produced. Filter the solution and diluted to 100 ml with distilled water.

b. Silver nitrate solution (N/50) : Dissolved 1.699 g of $AgNO_3$ in distilled water and made volume to one litre. Stored it in amber glass bottle.

c. Preparation of 0.01N NaCl Solution : Dissolved 0.585 g of NaCl in double distilled water and made volume to 1000 ml.

Standardized silver nitrate solution by titrating it against 0.01 N NaCl solution in the presence of K_2CrO_4 indicator.

Procedure : To one ml of aliquot taken in a conical flask, added four drops of K_2CrO_4 indicator and titrated it against N/50 $AgNO_3$ while stirring till brick red colour appeared.

Calculation

Chloride (meq l^{-1}) = $\frac{\text{ml of N/50 } AgNO_3 \text{ used for plant extract– ml of N/50 } AgNO_3 \text{ used for blank x N x 1000}}{\text{Volume of plant extract taken (ml)}}$

Chloride was expressed in (mg g^{-1}) tissue dry weight.

xxiii. Sulphate (SO_4^{2-}) : The sulphate was estimated by Turbidimetric method as digested by Chesin and Yien (1950).

Reagents

a. Gum acacia solution : Dissolved 250 mg of gum acacia in distilled water and diluted to 100 ml.

b. Barium chloride : Ground $BaCl_2.2H_2O$ crystals in a mortar, until they pass through a 20-30 mesh sieve, but retained on 60 mesh sieve.

c. Standard SO_4^{2-} solution : Dissolved 0.1815 g of reagent grade K_2SO_4 in one litre distilled water. This is 100 mg l^{-1} stock solution of SO_4^{2-}. Transferred 1.25, 2.50, 5.0, 7.5, 10.0, 12.5, 15.0 ml of the 100 mg l^{-1} SO_4^{2-} stock solution in a series of 25 ml volumetric flask to obtain 5, 10, 20, 30, 40, 50, 60 mg l^{-1} SO_4^{2-}, respectively. Prepared a standard curve by plotting per cent transmittance (T) on Y -axis and concentration (C) on X -axis on a semi-log graph paper. There should be a straight line relationship between C and T.

Procedure : Transferred five ml aliquot of digest to a 25 ml volumetric flask, added one ml of gum acacia solution, made the volume up to the mark and shake for one minute. Further added one gram of a sieved $BaCl_2$ crystals and shaken for one minute. Measured the turbidity in 25-30 min, after adding $BaCl_2$ crystals, on spectrophotometer (Spectronic- 21) using a blue filter at a wavelength of 420 nm. Simultaneously carried out a blank (without sample). Data expressed as mg g^{-1} tissue dry weight.

Calculations

Weight of plant material	= 100 mg
Volume made after digestion	= 20 ml
Dilution	= 200 times
Volume of water sample taken for turbidity development	= 5 ml
Final volume made	= 25 ml
Dilutions	= 5 times
Total dilution	= 200x5=1000

Transmittance (% T) as read from spectrophotometer = T

SO_4^{2-} content (mg l^{-1}) of sample from the standard curve against T = C

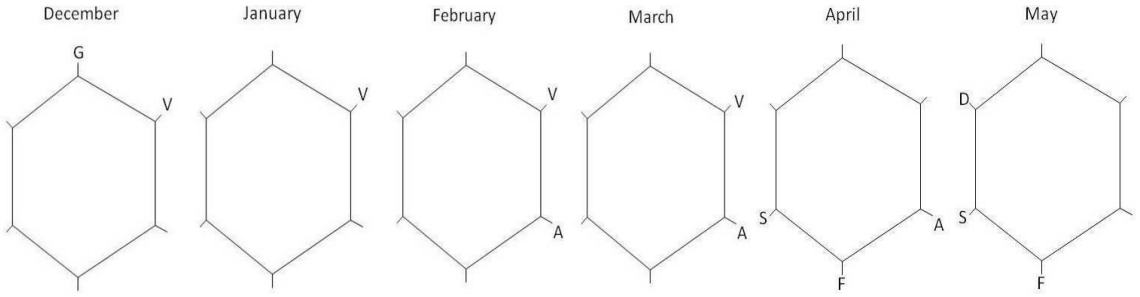
SO_4^{2-} (mg g^{-1}) in sample =(C x dilution factor)/1000

CHAPTER - IV

RESULTS

First Experiment Results

1. Phenology: The germination of *Calendula officinalis* in field conditions occurred in the first week of December. It was followed by the vegetative growth up to March. The flowering (anthesis) initiated in the month of February and continued till mid April (Fig. 1). Fruiting and seed maturation occurred simultaneously from mid April to early May. Death / senescence was observed from early May onwards.



G = germination; V = vegetative growth; A = anthesis (flowering); F = fruiting; S = seed maturation; D = death / senescence

Fig. 1: Phenological study of *Calendula officinalis* under field condition

2. Growth attributes

i. Plant height (cm) : Increase in plant height of *Calendula officinalis* under field conditions was observed with the aging of plants. Significant increase in plant height, however, was recorded up to flowering stage (Table 4.1). Maximum height of the plants (64.00 cm) was achieved at the maturity stage.

ii. Basal stem diameter (mm) : Increase in basal stem diameter was recorded with advancement of growth stage (Table 4.1). The increase in diameter, however, was not significant beyond flowering stage. Maximum basal stem diameter of 13.5 mm was found at maturity stage.

iii. Number of leaves plant^{-1} : The number of leaves plant^{-1} increased significantly with the growth of plants and maximum number of leaves (262.3) was recorded at the maturity stage (Table 4.1).

iv. Leaf biomass (g plant^{-1}) : Increase in leaf biomass (g plant^{-1}) was recorded with the advancement of growth stage (Table 4.1). It reached up to 3.93 g plant^{-1}. The increment in leaf biomass, however, was not significant beyond flowering stage.

v. Stem and branches biomass (g plant^{-1}) : Stem and branches biomass (g plant^{-1}) continued to increase with the advancement of age and became 8.95 g plant^{-1} at the maturity stage (Table 4.1).

Table 4.1 : Growth attributes of *Calendula officinalis* L. under field conditions

Growth parameters	Growth stage (GS)			
	Vegetative Stage	Flowering Stage	Maturity Stage	CD (P≤0.05)
Plant height (cm)	07.83	60.33	64.00	04.93
Basal Stem diameter (mm)	06.33	12.67	13.50	02.16
Number of leaves plant^{-1}	018.0	239.0	262.3	011.5
Leaf biomass (g plant^{-1})	01.21	03.76	03.93	00.81
Stem and branches biomass (g plant^{-1})	00.17	06.29	08.95	00.23
Flowers and fruits biomass (g plant^{-1})	00.00	03.85	07.16	00.42
Root biomass (g plant^{-1})	00.16	01.32	01.75	00.13
Root/ Shoot ratio	00.12	00.10	00.09	0.02

vi. Flowers and fruits biomass (g plant^{-1}) : The flower and fruit biomass (g plant^{-1}) also increased from flowering to maturity stage. It was maximum (7.16 g plant^{-1}) at the maturity stage (Table 4.1).

vii. Root biomass (g plant^{-1}) : Significant increase in root biomass (g plant^{-1}) was obtained from vegetative to flowering stage and then reaching maximum (1.75 g plant^{-1}) at the maturity stage (Table 4.1).

viii. Root/ Shoot ratio : The root/ shoot ratio at the vegetative stage was 0.12. It decreased with the advancement of growth stage (Table 4.1). Significant decrease in root/ shoot ratio, however, was recorded up to flowering stage. Minimum root/ shoot ratio of the plant (0.09) was recorded at the maturity stage.

3. Reproductive behaviour

i. Number of flower heads plant^{-1} : The number of flower heads plant^{-1} in *Calendula officinalis* under field conditions significantly increased from 40.33 at the flowering stage to 51.00 at the maturity stage (Table 4.2).

Table 4.2 : Reproductive behaviour of *Calendula officinalis* L. under field conditions

Parameters	Flowering stage	Maturity stage	CD (P≤0.05)
Number of flower heads plant^{-1}	40.33	51.00	01.54
Number of seeds head^{-1}	-	40.3±0.9*	-
Seed output (number plant^{-1})	-	2055.7±46.8*	-
Seed yield (g plant^{-1})	-	6.1±0.3*	-
Germination of seeds produced (%)	-	86.67±0.35*	-
Reproductive capacity	-	1783.02±15.0*	-

* Mean ±Standard Error

ii. Number of seeds head^{-1} : Number of seeds head^{-1} has been recorded to 40.3±0.9 (Table 4.2).

Plate 1. ***Calendula officinalis*** **L. grown under field conditions**

Plate 2. ***Calendula officinalis*** **L. grown under varying salinity levels**

iii. Seed output (number plant^{-1}) : Seed output was 2055.7±46.8 number plant^{-1} (Table 4.2).

iv. Seed yield (g plant^{-1}) : Seed yield was found to be 6.1±0.3 g plant^{-1} (Table 4.2).

v. Germination (%) : The germinability of seeds of *Calendula officinalis* produced under field conditions was observed to be 86.67± 0.35% (Table 4.2).

vi. Reproductive capacity : The average reproductive capacity of *Calendula officinalis* under field conditions was worked out to be 1783.02± 15.0 % (Table 4.2).

Second Experiment Results

1. Growth attributes

i. Plant height : Progressive increase of EC level from 0 to 12 dSm^{-1} caused a significant decline in plant height of *Calendula officinalis* (Table 4.3). However, with the increase in growth stage (GS) the plant height increased significantly. The interaction effect of GS x EC on the plant height was significant at flowering and maturity stages. The plant height at vegetative stage also decreased significantly up to 4 dSm^{-1} EC level. Thereafter, no significant reduction in plant height was noticed with the progressive increase of EC level.

Table 4.3: Plant height (cm) of *Calendula officinalis* L. at different growth stages under varying salinity

EC level (dSm^{-1}) with added salinity	Growth stage (GS)			Mean
	Vegetative stage	Flowering stage	Maturity stage	
0	06.23	36.80	41.73	**28.26**
4	04.20	33.90	37.87	**25.32**
8	03.10	24.10	28.17	**18.46**
12	02.67	15.27	18.70	**12.21**
Mean	**04.05**	**27.52**	**31.62**	

CD (P≤0.05) GS = 0.89 EC =1.03 GS x EC = 1.78

Table 4.4 : Number of leaves plant^{-1} of *Calendula officinalis* L. at different growth stages under varying salinity

EC level (dSm^{-1}) with added salinity	Growth stage (GS)			Mean
	Vegetative stage	Flowering stage	Maturity stage	
0	13.63	40.47	75.13	**43.08**
4	11.23	35.97	63.90	**37.03**
8	09.63	25.67	49.77	**28.36**
12	06.47	17.00	36.53	**20.00**
Mean	**10.24**	**29.77**	**56.33**	

CD (P≤0.05) GS = 2.15 EC = 2.48 GS x EC = 4.30

ii. Number of leaves plant^{-1} : Number of leaves plant^{-1} was also declined with the build up of salinity level in the growth medium as compared to control. With succeeding growth stage the number of leaves plant^{-1} increased significantly (Table 4.4). The interaction effect of GS x EC on

the number of leaves plant^{-1} although was not significant with succeeding EC levels at the vegetative stage but reduction was quite evident at 12 dSm^{-1} EC level as compared to control and 4 dSm^{-1} EC level. From flowering stage the number of leaves plant^{-1} decreased significantly with succeeding EC levels and increased with growth stage.

iii. Leaf biomass (g plant^{-1}) : Leaf biomass (g plant^{-1}) was decreased significantly with the succeeding EC levels up to 8 dSm^{-1} beyond which the decline in leaf biomass was insignificant. A steady increase in the leaf biomass was evident with the progressing growth stage irrespective of EC level (Table 4.5). Reduction in leaf biomass with the rise of EC level of the growth medium was quite significant at the maturity stage. However, it was not significant beyond 8 dSm^{-1} EC level at the vegetative and flowering stage.

Table 4.5 : Leaf biomass (g plant^{-1}) of *Calendula officinalis* L. at different growth stages under varying salinity

EC level (dSm^{-1}) with added salinity	Growth stage (GS)			Mean
	Vegetative stage	Flowering stage	Maturity stage	
0	0.81	0.99	1.59	**1.13**
4	0.40	0.88	1.25	**0.84**
8	0.14	0.66	1.01	**0.60**
12	0.11	0.63	0.86	**0.53**
Mean	**0.36**	**0.79**	**1.18**	

CD (P≤0.05) GS = 0.08 EC = 0.09 GS x EC = 0.16

Table 4.6 : Stem and branches biomass (g plant^{-1}) of *Calendula officinalis* L. at different growth stages under varying salinity

EC level (dSm^{-1}) with added salinity	Growth stage (GS)			Mean
	Vegetative stage	Flowering stage	Maturity stage	
0	0.20	0.46	0.95	**0.53**
4	0.09	0.38	0.68	**0.38**
8	0.02	0.29	0.57	**0.30**
12	0.02	0.20	0.38	**0.20**
Mean	**0.08**	**0.33**	**0.64**	

CD (P≤0.05) GS = 0.07 EC = 0.08 GS x EC = 0.14

iv. Stem and branches biomass (g plant^{-1}) : The stem and branches biomass (g plant^{-1}) significantly reduced with the rise of EC level in the growth medium (Table 4.6). Significant increase in stem and branches biomass was observed with the advancement of growth stage, in general. The decline in stem and branches biomass with the increase of EC level was although not significant at the vegetative and flowering stage but it showed a significant reduction at the maturity stage. The accumulation of stem and branches biomass was significant with the advancement of stage irrespective of EC level.

v. Flowers and fruits biomass (g plant^{-1}) : With the increase of EC level the flowers and fruits biomass (g plant^{-1}) decreased significantly (Table 4.7). Increase in this attribute was also significant from flowering to maturity stage. The interaction effect of GS x EC on the flowers and fruits biomass was also quite significant.

Table 4.7 : Flowers and fruits biomass (g plant^{-1}) of *Calendula officinalis* L. at different growth stages under varying salinity

EC level (dSm^{-1}) with added salinity	Growth stage (GS)		Mean
	Flowering stage	Maturity stage	
0	0.49	1.32	**0.90**
4	0.35	1.06	**0.70**
8	0.20	0.82	**0.51**
12	0.15	0.57	**0.36**
Mean	**0.30**	**0.94**	

CD (P≤0.05) GS = 0.07 EC = 0.09 GS x EC = 0.13

vi. Root biomass (g plant^{-1}) : Significant increase in root biomass (g plant^{-1}) was found with the growth of plants (Table 4.8). With the increase of EC level of the growth medium the root biomass (g plant^{-1}) declined, in general. The reduction in root biomass with the increase of EC levels was, however, not significant beyond 8 dSm^{-1} EC level at the vegetative and flowering stages. Increment in root biomass with advancement of growth stage was quite significant at every EC level.

Table 4.8 : Root biomass (g plant^{-1}) of *Calendula officinalis* L. at different growth stages under varying salinity

EC level (dSm^{-1}) with added salinity	Growth stage (GS)			Mean
	Vegetative stage	Flowering stage	Maturity stage	
0	0.34	0.98	1.29	**0.87**
4	0.14	0.79	0.98	**0.64**
8	0.04	0.41	0.76	**0.40**
12	0.03	0.33	0.47	**0.28**
Mean	**0.14**	**0.63**	**0.88**	

CD (P≤0.05) GS = 0.05 EC = 0.06 GS x EC = 0.10

vii. Root/ shoot ratio : Root/ shoot ratio of *Calendula officinalis* was found to decline with the rise of EC level beyond 4 dSm^{-1} EC level (Table 4.9). Significant increase in root/ shoot ratio was observed from vegetative to flowering stage. The ratio, however, decreased significantly thereafter, at the maturity stage. The interaction effect of GS x EC on the root/ shoot ratio was not significant.

Table 4.9 : Root/ shoot ratio of *Calendula officinalis* L. at different growth stages under varying salinity

EC level (dSm^{-1}) with added salinity	Growth stage (GS)			Mean
	Vegetative stage	Flowering stage	Maturity stage	
0	0.34	0.51	0.33	**0.39**
4	0.28	0.50	0.34	**0.37**
8	0.27	0.36	0.32	**0.31**
12	0.23	0.33	0.26	**0.28**
Mean	**0.28**	**0.42**	**0.31**	

CD (P≤0.05) GS = 0.05 EC = 0.06 GS x EC = NS

2. Reproductive behavior

viii. Number of flower heads plant^{-1} : Significant increase in number of Flower heads plant^{-1} was recorded from flowering to maturity stages (Table 4.10). Steady reduction in number of flower heads plant^{-1}, however, was observed with the progressive increase of EC level in the growth medium. The decline in number of flower heads plant^{-1} with the increase of from EC level was significant at the maturity stage. While, it was significant beyond 8 dSm^{-1} EC level at the flowering stage. The increase in number of flower heads plant^{-1} with the advancement of growth was significant at every EC level.

Table 4.10: Number of flower heads plant^{-1} of *Calendula officinalis* L. at different growth stages under varying salinity

EC level (dSm^{-1}) with added salinity	Growth stage (GS)		Mean
	Flowering stage	Maturity stage	
0	04.23	18.80	**11.52**
4	03.77	12.77	**08.27**
8	03.43	09.77	**06.10**
12	01.20	07.03	**04.12**
Mean	**02.91**	**12.09**	

CD (P≤0.05) GS = 0.61 EC = 0.86 GS x EC = 1.22

ix. Seed germination (%) : The geminability of seeds produced by plants grown at various EC levels was assessed. The germination of seeds of control plants was 80 per cent which significantly reduced to 60 per cent in plants grown at 12 dSm^{-1} EC levels. Non significant effect of succeeding EC levels was, however, observed (Table 4.11).

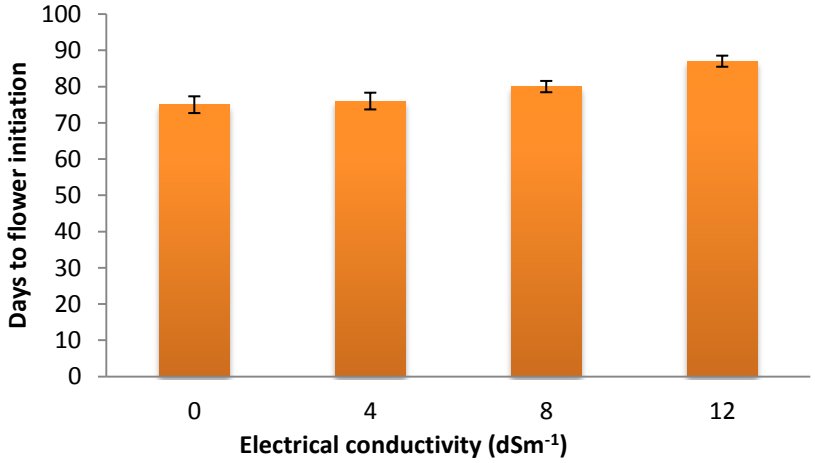
x. Reproductive capacity : The reproductive capacity of *Calendula officinalis* plants grown at various EC levels was worked out (Table 4.11). The reproductive capacity of plants grown under control was 438.77. The results clearly depict a significant decline of reproductive

capacity with the increase of EC levels in the growth medium. The reproductive capacity of plants grown at 12 dSm^{-1} EC level was found to be minimum (101.41).

Table 4.11 : Germination of seeds produced (%) and reproductive capacity of *Calendula officinalis* L. grown under varying salinity

EC level dSm^{-1} with added salinity	Seed germination (%)	Reproductive capacity
0	80.0	438.77
4	73.3	259.18
8	65.0	160.43
12	60.0	101.41
CD (P≤0.05)	8.7	37.38

xi. Days to flower initiation : Flower initiation occurred in 75 days in control. The number of days to flower initiation significantly increased with the increase of EC level beyond 8 dSm^{-1} (Fig. 2; Table A1 – Appendix). It occurred in 87 days under 12 dSm^{-1} EC level.

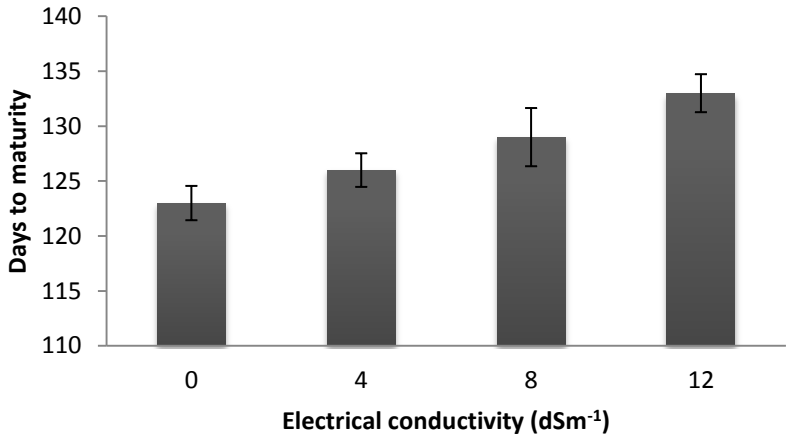


CD (P≤0.05) = 6.3

Fig. 2 : **Days to flower initiation of *Calendula officinalis* L. under varying salinity**

* Bars represent mean ± S. E.

xii. Days to maturity : Maturity of *Calendula officinalis* plants in control occurred in 123 days. An increase in number of days to maturity was also observed with the rise of EC level and this increase was significant at 8 and 12 dSm^{-1} EC levels when compared with control (Fig 3; Table A1 – Appendix). The maturity at 12 dSm^{-1} EC level was achieved in 133 days.

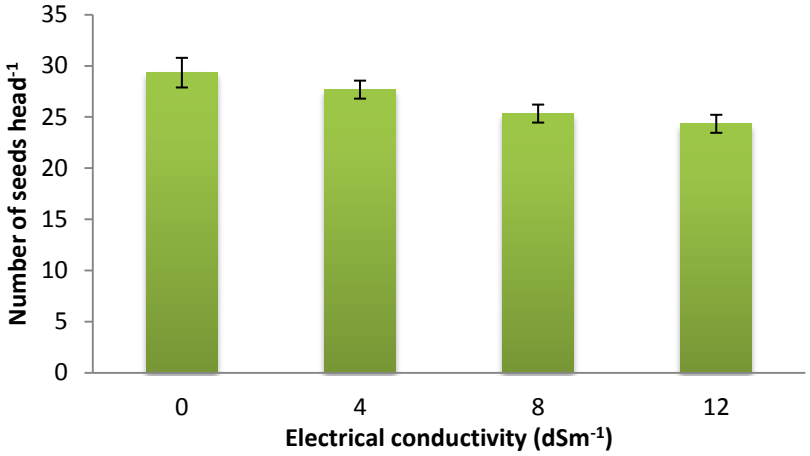


CD (P≤0.05) = 6.1

Fig. 3 : **Days to maturity of *Calendula officinalis* L. under varying salinity**

* Bars represent mean ± S. E.

xiii. Number of seeds head^{-1} : The number of seeds head^{-1} in control was 29.33. It declined with the increase of EC level and the decline was significant at 8 and 12 dSm^{-1} (Fig. 4; Table A1 – Appendix). The number of seeds head^{-1} was reduced to 24.33 at 12 dSm^{-1} EC level.

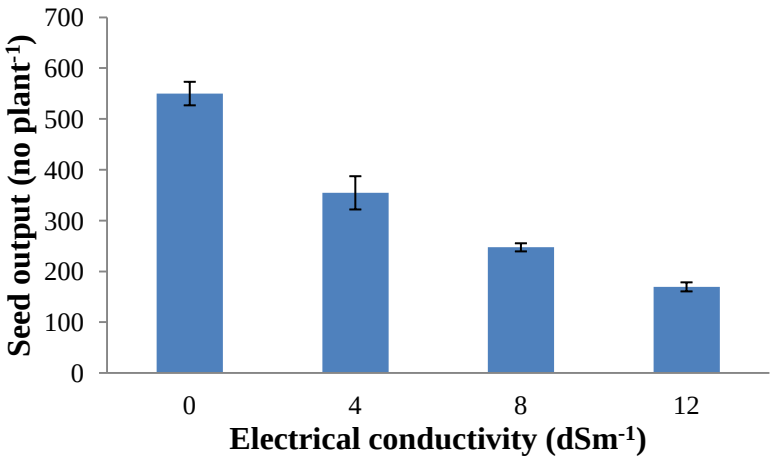


CD (P≤0.05) = 3.49

Fig. 4 : **Number of seeds head^{-1} of *Calendula officinalis* L. under varying salinity**

* Bars represent mean ± S. E.

xiv. Seed output (number plant^{-1}) : Seed output (number plant^{-1}) in control was 549.7 which underwent significant reduction with succeeding EC levels (Fig. 5; Table A1 – Appendix). Seed output was reduced to 169.53 number plant^{-1} at 12 dSm^{-1} EC level.

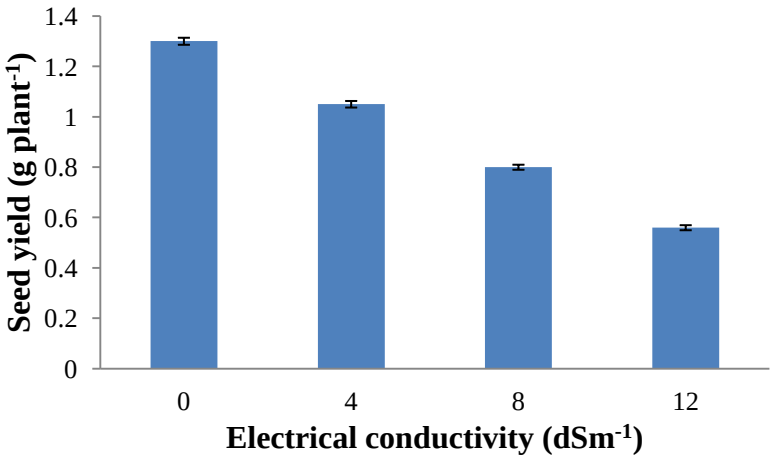


CD (P≤0.05) = 69.26

Fig. 5 : **Seed output (number plant^{-1}) of *Calendula officinalis* L. under varying salinity**

* Bars represent mean ± S. E.

xv. Seed yield (g plant^{-1}) : Maximum seed yield 1.30 g plant^{-1} was recorded in control. The seed yield significantly reduced with the buildup of salinity in the growing medium (Fig. 6; Table A1 – Appendix). At 12 dSm^{-1} EC level the seed yield was 0.56 g plant^{-1}.



CD (P≤0.05) = 0.04

Fig. 6 : **Seed yield (g plant^{-1}) of *Calendula officinalis* L. under varying salinity**

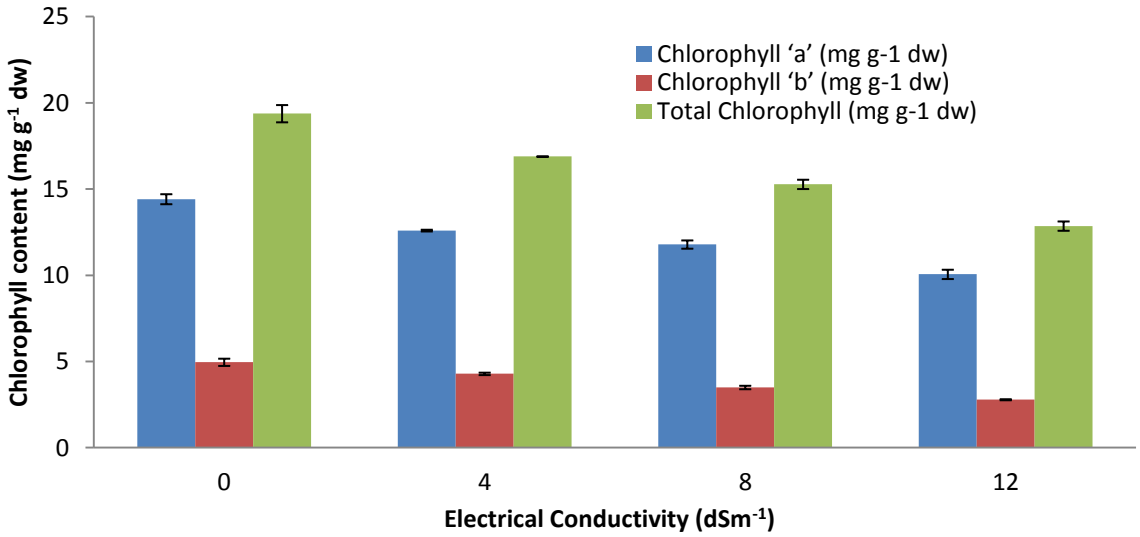
* Bars represent mean ± S. E.

3. Physiological attributes

i. Chlorophyll : The chlorophyll *-a*, chlorophyll *-b* and total chlorophyll contents were determined in leaves of *Calendula officinalis* at the vegetative stage.

a) Chlorophyll –*a* : Significant decline in chlorophyll *-a* content of leaves was found with the rise of EC level in the growing medium right from control (14.42 mg g^{-1} dw). The chlorophyll *-a* content was declined to 10.06 mg g^{-1} dw at 12 dSm^{-1} EC level (Fig. 7; Table A2 – Appendix).

b) Chlorophyll –*b* : Chlorophyll -*b* remained less than chlorophyll -*a* in leaves of plants grown under different EC levels. Chlorophyll -*b* showed significant reduction with the increase of EC levels of the growth medium. The decline in chlorophyll -*b* content was observed to be from 4.96 mg g^{-1} dw in control to 2.79 mg g^{-1} dw at 12 dSm^{-1} EC level (Fig. 7; Table A2 – Appendix).



CD (P≤0.05)

Chl 'a' = 0.77 Chl'b' = 0.40 Total Chl = 1.04

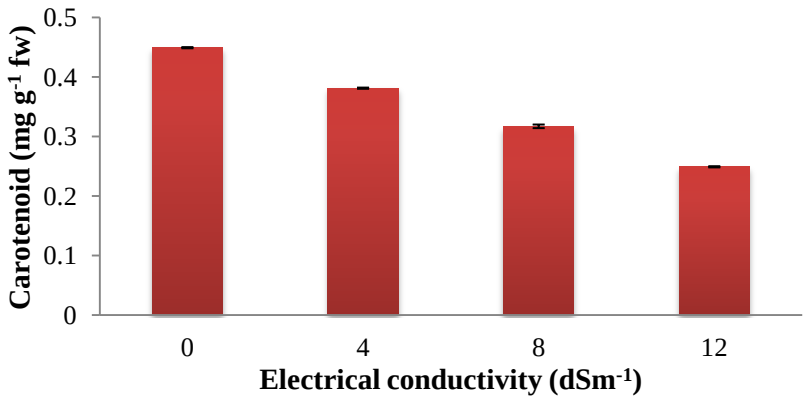
Fig. 7 : **Chlorophyll content (mg g^{-1} dw) in leaves of *Calendula officinalis* L. at vegetative stage under varying salinity**

* Bars represent mean ± S. E.

c) Total Chlorophyll : A steady decline in total chlorophyll content of leaves was observed from 19.38 mg g^{-1} dw in control with the increase of EC level. It gone down to 12.86 mg g^{-1} dw at 12 d Sm^{-1} EC level (Fig. 7; Table A2 – Appendix).

ii. Caroteniods : A significant decline in carotenoid content of flower petals was observed with the increase of EC levels (Fig. 8; Table A2 – Appendix) in the growth medium. It declined from 0.449 mg g^{-1} fw (control) to 0.249 mg g^{-1} fw (12 dSm^{-1}).

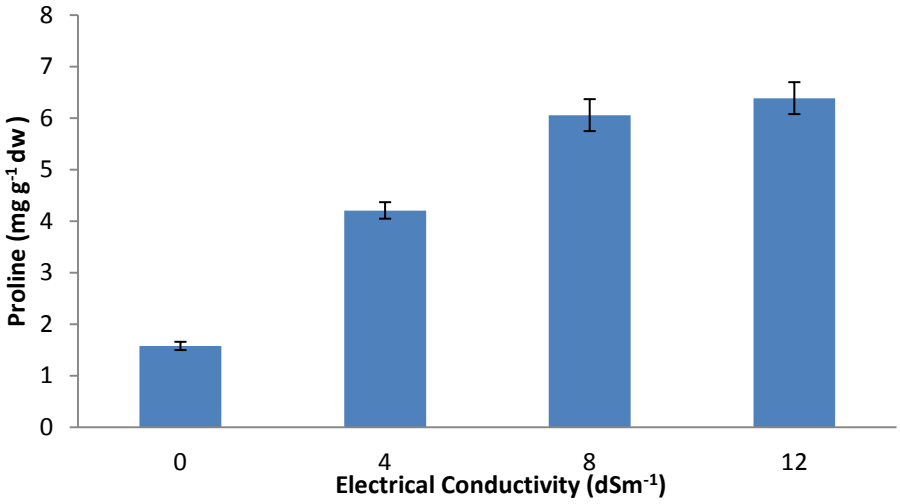
iii. Proline content : Increase in leaf proline content with the progressive increase of salinity (EC level) was observed. Proline level increased from 1.58 mg g^{-1} dw in control to 6.39 mg g^{-1} dw at 12 dSm^{-1} EC level. The increment in proline content was, however, not significant beyond 8 dSm^{-1} EC level (Fig. 9; Table A2 – Appendix).



CD (P≤0.05) = 0.005

Fig. 8 : **Carotenoid content (mg g^{-1} fw) in flower petals of *Calendula officinalis* L. at flowering stage under varying salinity**

* Bars represent mean ± S. E.

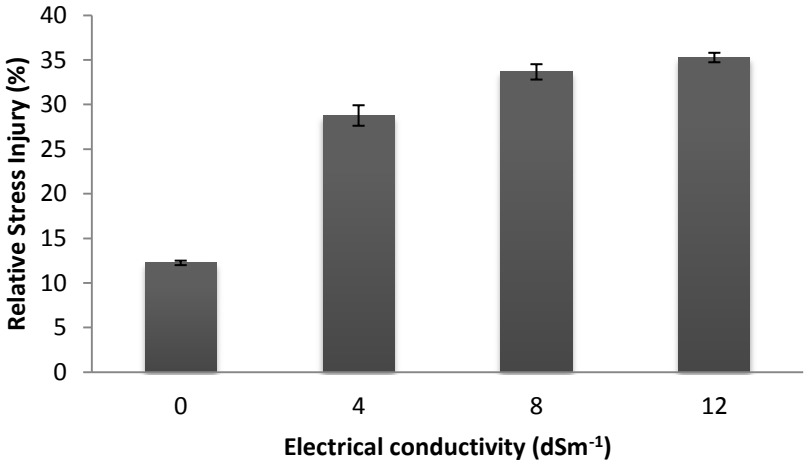


CD (P≤0.05) = 0.78

Fig. 9 : **Proline content (mg g^{-1} dw) in leaves of *Calendula officinalis* L. at vegetative stage under varying salinity**

* Bars represent mean ± S. E.

iv. Relative stress injury : An increase in relative stress injury (%) in leaves with the progressive increase in EC levels (Fig. 10; Table A2 – Appendix) was observed. Relative stress injury (%), however, was not significant beyond 8 dSm^{-1}. It increased from 12.25 per cent (control) to 35.27 per cent (12 dSm^{-1}).



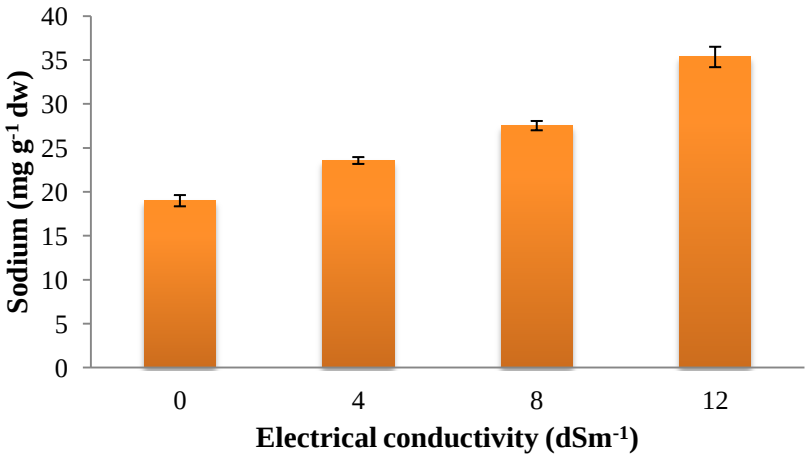
CD (P≤0.05) = 2.57

Fig. 10 : **Relative stress injury (%) in leaves of *Calendula officinalis* L. at vegetative stage under varying salinity**

* Bars represent mean ± S. E.

4. Mineral status

i. Sodium : An increase in sodium content (mg g^{-1} dw) in leaves at maturity stage was observed with the increase of EC levels in the growing medium (Fig. 11; Table A3 – Appendix). This increase was 18.99 mg g^{-1} (control) to 35.34 mg g^{-1} (12 dSm^{-1}).

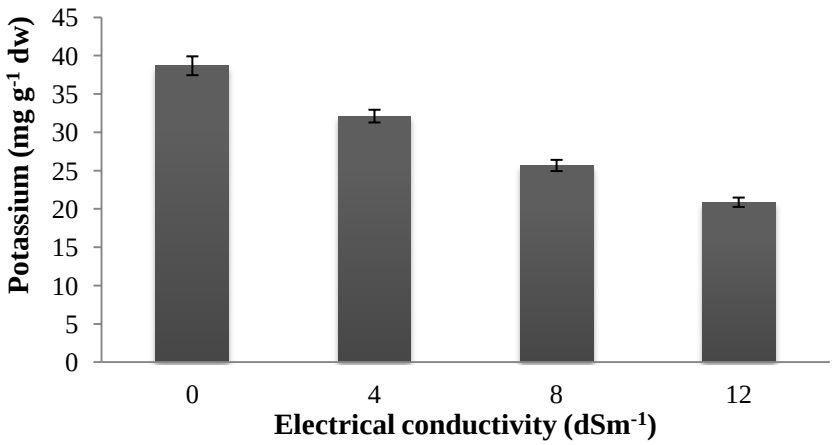


CD (P≤0.05) = 2.44

Fig. 11 : **Sodium (Na^+) content (mg g^{-1} dw) in leaves of *Calendula officinalis* L. at maturity stage under varying salinity**

* Bars represent mean ± S. E.

ii. Potassium : A steady and significant decline in the potassium content (mg g^{-1} dw) in leaves was observed with the rise of EC levels (Fig. 12; Table A3 – Appendix). The potassium content decreased from 38.68 mg g^{-1} (control) to 20.87 mg g^{-1} (12 dSm^{-1}).

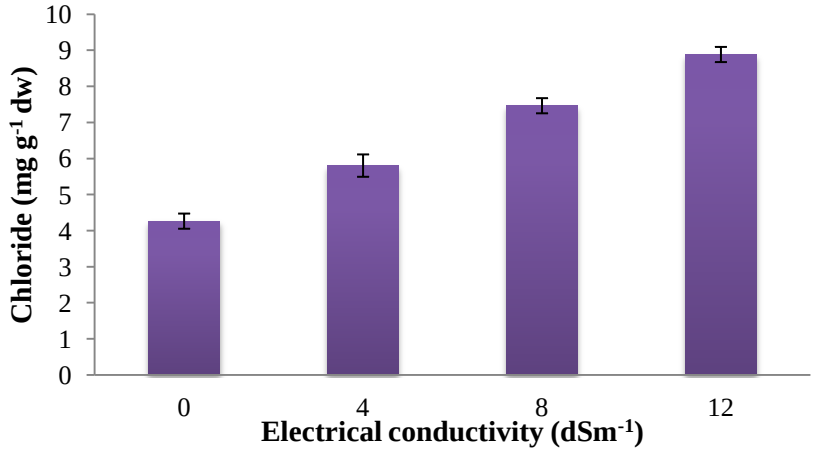


CD (P≤0.05) = 2.92

Fig. 12 : **Potassium (K^+) content (mg g^{-1} dw) in leaves of *Calendula officinalis* L. at maturity stage under varying salinity**

* Bars represent mean ± S. E.

iii. Chloride : A significant increase in chloride content (mg g^{-1} dw) in leaves at maturity stage was observed with the increase of EC levels in the growing medium (Fig. 13; Table A3 – Appendix). This increase was 4.26 mg g^{-1} (control) to 8.88 mg g^{-1} (12 dSm^{-1}).

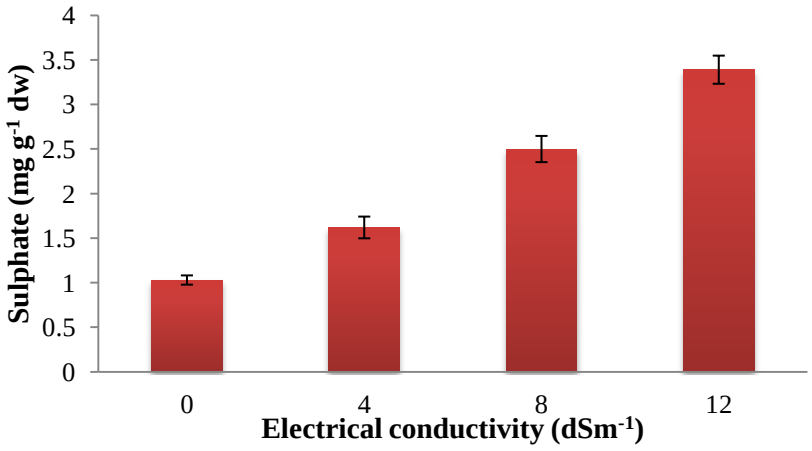


CD (P≤0.05) = 0.78

Fig. 13 : **Chloride (Cl^-) content (mg g^{-1} dw) in leaves of *Calendula officinalis* L. at maturity stage under varying salinity**

* Bars represent mean ± S. E.

iv. Sulphate : A steady and significant increase in sulphate content (mg g^{-1} dw) in leaves at maturity stage was observed with the increase of EC levels in the growing medium (Fig. 14; Table A3 – Appendix). This increase was 1.03 mg g^{-1} (control) to 3.39 mg g^{-1} (12 dSm^{-1}).



CD (P≤0.05) = 0.42

Fig. 14 : **Sulphate (SO_4^{2+}) content (mg g^{-1} dw) in leaves of *Calendula officinalis* L. at maturity stage under varying salinity**

* Bars represent mean ± S. E.

CHAPTER-V

DISCUSSION

Salient experimental findings are discussed in this chapter in the light of recent literature.

Study of pot marigold (*Calendula officinalis* L.) under non-saline field conditions revealed an increase in plant height, basal stem diameter, number of leaves plant^{-1}, leaf biomass (g plant^{-1}), stem and branches biomass (g plant^{-1}), flowers and fruits biomass (g plant^{-1}) and root biomass (g plant^{-1}) with the advancement of growth stage from vegetative to maturity stage. Decline in root/ shoot ratio, however, occurred from vegetative to maturity stage. The seed output plant^{-1} was 2055.7±46.8 and the germinability of seeds produced was 86.67±0.35. The reproductive capacity of pot marigold under non-saline field conditions has been worked out to be 1783.02±15.00.

Results of pot culture experiment made to assess the morphological attributes of *Calendula officinalis* under chloride dominated salinity are discussed below.

1. Growth attributes

The pot culture studies revealed a steady decrease in growth parameters *viz*., plant height (56.79%), number of leaves plant^{-1} (53.57%), leaf biomass (53.10%), stem and branches biomass (62.26%), flowers and fruits biomass (60.0%), root biomass (67.82%) and root/ shoot ratio (28.21%) with the rise of EC level up to 12 dSm^{-1} in the growth medium. All the above parameters significantly increased with the advancement of growth stage.

The deleterious effects of salinity on plant growth has been reported by Asraf, 1994; Marschner, 1995; Zhu, 2003; Turan *et al*., 2010. Salinity reduced plant height (Achilea, 2002; Agong *et al*., 2004 and Hajer *et al*., 2006), fresh weight (Hassan, 1999; Li, 2000; Sonneveld, 2000, Amico *et al*., 2003 and Hajer *et al*., 2006) as well as dry weight (Li, 2000 and Yustseven *et al*., 2003) in plants. The decline in various growth parameters with the rise of salt stress was also recorded by many workers in palmarosa and lemon grass (Prasad *et al*., 1998), barley (Naseer *et al*., 2001), isabgol genotypes (Vandana, 2003), chandrashura (Singh, 2004), *Khaya senegalensis* (Abd El-Aziz *et al*., 2006), ajwain (Ashraf and Orooj, 2006), *Matricaria chamomilla* (Razmajoo *et al*., 2008; Deepika and Varshney, 2008), *Atriplex hortensis* Var. (Kachout *et al*., 2009) and morocco (Belaqziz *et al*., 2009).

Salt stress can lead to stomatal closure, which reduces CO_2 assimilation in the leaves and inhibit carbon fixation resulting in reduction in photosynthesis rate and plant growth (Kiarostami *et al*., 2010). The reduction in growth might be due to toxicity of the ions or low osmotic potential as well as decrease in wall extensibility (Grieve *et al*., 2001; Haplerin and Lynch, 2003).

NaCl inhibited the growth of plant, and led to a decrease in biomass. This may be related to the effect of salt-stress which resulted in the limitation of water absorption and biochemical processes (Cusido *et al*., 1987; Parida and Das, 2005). In addition, a decline in the rates of net photosynthesis occurs, due to adverse affect on CO_2 assimilation, which leads to decrease in nutrient uptake and ultimately growth of plants (Seeman and Sharkey, 1986; Cha- Um and Kirdmanee, 2009).

The reduction in plant growth due to salinity was attributed not only to inhibition of water absorption and ion toxicity, but also to the nutrient disturbance under such conditions (Helal *et al*., 1975; Zaho *et al*., 2007; Sairam *et al*., 2002; Jenifer and Franklin-Janus, 2002). Dunlap and Binzel (1996) also mentioned that increasing NaCl concentration can reduce the endogenous level of IAA, which may be critical to water movement through the root system of plants.

Progressive increase in EC level from 0 to 12 dSm^{-1} caused a significant decline in plant height of *Calendula officinalis.* Reduction in plant height and lateral stem number might be due to the fact that water stressed stomata become closed or half closed leading to decrease in CO_2 absorption. On the other hand, the plants consume a lot of energy to absorb water, which result into reduction in production of photosynthetic matters (Rahmani *et al*., 2009).

Furthermore, salinity can also reduce hydraulic conductivity, decrease the extensibility and increase the yield threshold of the cell wall (Cramer, 1992). These changes in the cell growth parameters may be responsible for the decreased expansion growth of plants under salt stress (Bandeh-hagh *et al*., 2008).

In plants grown under saline conditions, as soon as the new cell starts its elongation process, the excess of salts modify the metabolic activities of the cell wall causing the deposition of various materials which limit the cell wall elasticity. Secondary cell wall sooner becomes rigid and consequently the turgor pressure efficiency in cell enlargement is decreased (Ali *et al*., 2004).

The other expected causes of the reduction in yield $plant^{-1}$, leaf area and yield components in rice could be the shrinkage of the cell contents, reduced development and differentiation of tissues, imbalanced nutrition, damage of membrane and disturbed avoidance mechanism (Ali *et al*., 2004) . This may be due to slower development of stress in salinized plants, which in turn, led to osmotic adjustment (Sundaravalli *et al*., 2006) and water stress in salinized plants compared to non -salinized plants, and also that salinized plants utilized less water because of reduced leaf area and overall reduced plant growth. This reduction in growth might be due to toxicity of the ions or low osmotic potential as well as a decrease in wall extensibility (Azooz *et al*., 2009). It seems that reduction in height due to decreasing turgor

pressure in cells (Heidari *et al*., 2011). El and Saffan (2008) reported that osmotic effects of salinity might cause a stir in the water relations of plants, reduce turgor potential and decline growth due to stomatal closure and reduced photosynthesis. The major reason for the detrimental effects may be the negative osmotic pressure caused by the salt in the root zone (Jacoby, 1994) or the growth inhibition due to injury of cells in transpiring leaves (Tuteja, 2007).

Generally plant biomass is inhibited by an excess of the solutes taken up by plants from the saline growth media (Arshi *et al*., 2002 & 2004). The reduction in leaf area, yield and yield components under saline conditions were also due to reduced growth as a result of decreased water uptake, toxicity of sodium and chloride in the shoot cell as well as reduced photosynthesis (Ali *et al*., 2004). The salinity- induced reduction in the leaf area might be due to inhibition of cell division and cell expansion under salt stress (Serraj and Sinclair, 2002).

Plant biomass significantly decreased with NaCl treatment because salinity can inhibit plant growth by altering the water potential, increasing ion toxicity or causing an ion imbalance (Arshi *et al*., 2010). Salinity can also reduce biophysical restraints to cell wall expansion which, in turn, inhibits root growth and plant biomass (Singh *et al*., 1995; Arshi *et al*., 2002).

The reduction in dry weight under salt stress may also be attributed to inhibition of hydrolysis of reserved food and their translocation to the growing shoots (Razmjoo *et al.*, 2008).

The root growth inhibition was poorly influenced by salt induced water deficit, and it was mainly due to ionic toxicity (Apse and Blumwald, 2007). Reduction in root extension rates might also come from the marked lowering of root radical hydraulic conductivity that occur with salinization (Joly, 1989; Azaizeh and Steudle, 1991; Azaizeh *et al*., 1992; Evlagon *et al*.,1992). But Neumann *et al*. (1994) concluded that despite the drastic decline in conductivity, water movement into cells was still sufficiently rapid so as not to restrict their rate of expansion. With increasing NaCl concentration, the damage of roots enhanced with decrease in decrease in number of lateral roots, increase in girth and brittleness accompanied with browning of tissues (Saha *et al.*, 2010). Decline in root/ shoot ratio (dry weight basis) with increase of salinity was due to relatively better shoot growth as compared to root growth (Sapna, 2011).

Reduction in flower dry weight due to salinity may be a cumulative effect of decline in the number of flowers (Razmjoo *et al*., 2008).

2. Reproductive performance

Results of the pot culture experiment indicate a significant decline in the reproductive parameters of *Calendula officinalis viz*., number of flower heads plant^{-1} (64.24%), number of seeds head^{-1} (17.05%), seed output (69.17%), seed yield (56.92%), germinability of produced seeds (25.0%) and reproductive capacity (76.89%) with the increase of salinity from 0 to 12 dSm^{-}

^{1} in the growth medium. However, days to flower initiation (16.0%) and days to maturity (8.13%) increased with the increase of salinity from 0 to 12 dSm^{-1} in the growth medium.

Similar results were observed by Atam Parkash (2000) in marigold, Yadav (2000) in chrysanthemum, Kiran (2004) in chickpea and Suman (2010) in senna.

Salinity caused reduction in the number of flowers, the number of branches and head diameter. The main reason for this reduction may be attributed to suppression of growth under salt stress during the early developmental stages (shooting stage) of the plants (Razmjoo *et al.*, 2008). According to Munns (2003), suppression of plant growth under saline conditions may either be due to decreased availability of water or to the toxicity of sodium chloride.

Salinity inhibits seed germination through accumulation of toxic ions and/or reduced water uptake which arrested radical emergence (Hampson and Simpson, 1990; Begum *et al*., 1992). Furthermore excess of Na^{+} might cause problems with membranes, enzyme inhibition, disturbance in metabolism which disorganize cell division, elongation and structure as recorded by Ghoulam and Fares (2001); Nuran and Husnu (2002); Abo-Kassem (2006).

Decrease in germination percentage of seeds due to the effect of increasing salt concentration may be resulted from decreasing osmosis potential of solution, increasing toxic ions and changing in the remobilization balance of seed reservoirs. Decreasing germination percent by the effect of increasing salt concentrations is in consistence with the results of Flowers and Lauchli (1983) who interpreted this fact on the basis of replacement of K^{+} by Na^{+} at the cell membrane level. These types of exchanges certainly had differential inhibiting effects under chloride and sulphate salinity as has also been observed by other workers (Rathert and Doering, 1983; Weimberg *et al*., 1982). In other words, at higher osmotic potential, the moisture will be accessible to seed and its germination will decrease (Sedghi *et al*., 2010).

Plants of *Dactyloctenium aegyptium* growing in saline habitat possessed higher reproductive capacity as compared to those in non saline habitat. Which was remarkably due to higher germinability of seeds under increasing level of sodium as well as chloride ions. The reproductive of both the non saline as well as saline population was although decreased but the plant of saline population exhibited relatively better reproductive capacity (Varshney, 1983).

The seeds produced by plants grown under salinity were found viable although 25 per cent reduction in germinability was recorded up to 12 dSm^{-1}. Heavy reduction (76.89%) in the reproductive capacity of *Calendula officinalis* found up to 12 dSm^{-1} was due to more reduction (69.17%) in the seed output.

3. Physiological attributes

Increase of salinity from 0 to 12 dSm^{-1} in the growth medium decreased physiological parameters such as chlorophyll -a, chlorophyll -b, total chlorophyll and carotenoids.

Similar response on physiological parameters of medicinal plants under salinity conditions were observed in isabgol (Vandana, 2003), chandrashura (Singh, 2004), German chamomile (Deepika, 2007), senna (Suman, 2010) and lemon grass (Sapna, 2011).

Reduction in chlorophyll concentrations is probably due to the inhibitory effect of the accumulated ions of various salts on the biosynthesis of the different chlorophyll fractions. Salinity affects the strength of the forces bringing the complex pigment protein-liquid in the chloroplast structure. As the chloroplast is membrane bound its stability depends on the membrane stability which under high salinity condition seldom remains intact due to which reduction in chlorophyll was recorded (Ali *et al*., 2004).

Even increase in leaf membrane permeability under chloride salinity might contribute towards disturbances in the synthetic processes. In fact, these deleterious effects on chlorophylls substantiated the adverse effects of salinity on photosynthetic and respiration rates of wheat also (Sharma *et al*., 1994). A decline in the chlorophyll content may be correlated to the indirect effects of Na^+ and Cl^- ions on the contents of essential nutrients (Arshi *et al*., 2010a). NaCl treatment caused a progressive increasing displacement of calcium from the membrane of isolated protoplasts (Arshi *et al*., 2010).

Decrease in chlorophyll level under salt stress may be due to reduction in pigment biosynthesis or enzymatic chlorophyll degradation (Yang *et al*., 2009). The chlorophyll level is an index of the photosynthesis (Xu *et al*., 2008) and decrese in chlorophyll level leads to reduction in growth parameters (Kiarostami *et al*., 2010). Decrease in growth parameters may be due to the formation of proteolytic enzymes such as chlorophyllase, which is responsible for the chlorophyll degradation (Sabater and Rodriguez, 1978) as well as damaging to the photosynthetic apparatus (Yasseen, 1983).

The reduction in leaf chlorophyll content under NaCl stress has been attributed to the destruction of chlorophyll pigments and the instability of the pigment protein complex (Levit, 1980). It is also attributed to the interference of salt ions with the *de novo* synthesis of proteins, the structural component to chlorophyll, rather than the breakdown of chlorophyll (Jaleel *et al*., 2007). It is, therefore, proven that soil salinity had negative effects on the growth and photosynthetic metabolism of *Catharanthus roseus* (Jaleel *et al*., 2008a).

Reduction in chlorophyll concentrations is probably due to the inhibitory effect of the accumulated ions (Ali *et al*., 2004). The inhibitory effects of salts on chlorophylls could be due to

suppression of specific enzymes responsible for the synthesis of green pigments (Netondo *et al*., 2004). The decrease in chlorophyll may be attributed to increased activity of chlorophyllase (Reddy and Vora, 1986; Sudhaker *et al*., 1997; Nazarbeygi *et al.*, 2011).

The degradation of chlorophyll -b at a higher rate than chlorophyll -a can be explained by the fact that chlorophyll -b degradation begins with its conversion into chlorophyll -a. (Saha *et al.*, 2010).

Increase of chloride dominated salinity in the growing medium caused a decline in carotenoid content of flower petals *Calendula officinalis.*

Slight reduction in carotenoid contents may be due to their protective role against reactive oxygen species. Salinity can lead to oxidative stress and causing significant decrease to photosynthetic systems (Kiarostami *et al*., 2010). Marta *et al*. (2008) believed that the mineral fertilizers applied in pot marigold plants had positive effect on pigments and stimulated both the photosynthesis and the accumulation of carotenoid pigments, and increased mobilization of carotenoid and other pigments presented in leaf and flower.

An accumulation of proline in the leaves of *Calendula officinalis* was found with the rise of salinity. The results corroborate the findings of Deepika and Varshney (2008) in German chamomile. Similar results were obtained in isabgol genotypes by Vandana (2003) and in chandrashura by Singh (2004).

One of the most important mechanisms by higher plants under salt stress is the accumulation of compatible solutes such as proline. Proline accumulation in salt stressed plants is a primary defense response to maintain the osmotic pressure in a cell. Several reports show a significant role of proline in osmotic adjustment, protecting cell structure and its function in plants in salt-tolerant and salt-sensitive cultivars of many crops (Desingh and Kanagaraj, 2007; Koca *et al*., 2007; Veeranagamallaiah *et al*., 2007; Turan *et al*., 2007).

The accumulation of nitrogen-containing compatible solutes including proline is known to function in osmotic adjustment, protection of cellular macromolecules from damage by salts, storage of nitrogen and scavenging of free radicals. Many plants, both halophytes and glycophytes accumulate proline as a non toxic and protective osmolyte under saline conditions, including mangrove (Parida *et al*., 2002), maize (Cicek and Cakirlar, 2002), sorghum (de Lacerda *et al*., 2005) and mulberry (Harinasut *et al*., 2003). Proline accumulation in response to lower salt concentrations may contribute positively to salt tolerance, whereas the extremely high concentration in leaf tissues under high salinity treatment may be partly due to leaf damage. Moreover, the high growth reduction in high- salt treatment may be related to the higher

partitioning of metabolic energy needed for synthesis of proline, which costs a much large amount of ATP than accumulation of inorganic solutes (Raven, 1985).

Proline is the most stable amino acid, resisting acid hydrolysis to toxins and is least inhibitory to cell growth among all the amino acids, presumably because of these qualities it accumulates in plants, under several above conditions and may serve as reserve material for later use on emergence from the stress (Savitskaya, 1976).

The accumulation of proline during NaCl stress has an adaptive significance as it suppresses the stress-induced enhancement in free radical production by the thylakoid membranes and thereby reduces the lipid peroxidation linked membrane deterioration (Saradhi and Mohanty, 1993). The accumulation of nitrogen- containing compatible solutes including proline, is known to function in osmotic adjustment, protection of cellular macromolecules from damage by salts, storage of nitrogen and scavenging of free radicals (Chookhampaeng, 2011). Many plants accumulate proline as a non-toxic and protective osmolyte under salinity, including mangrove (Parida *et al*., 2002), maize (Cicek and Cakirlar, 2002), sorghum (de- Lacerda *et al*., 2005) and canola (Bandeh- hagh *et al*., 2008).

Its accumulation is caused both by the activation of its biosynthesis and inactivation of its degradation (Mattioni *et al*., 1997). Proline protects membranes and protein against the adverse effects of high concentration of inorganic ions (Paleg *et al*., 1984 and Santoro *et al*., 1992). Proline also functions as hydroxyl radical scavenger (Smirnoff and Cumbes, 1989; Hoque *et al*., 2007).

Proline, an amino acid, besides acting as a cytoplasmic osmoticum, may function as a carbon and nitrogen source of post stress recovery and growth, as a stabilizer for membranes and protein synthesis machinery (Kardpol and Rao, 1985), as a scavenger of free radicals (Smirnoff and Cumbes, 1989), as a sink for energy to regulate redox potential (Alia and Saradhi, 1993) and also serves to protect the protein against denaturation (Ravi Kishor *et al*., 2005).

An increase in relative stress injury (%) in leaves of *Calendula officinalis* was observed with the progressive increase of EC levels from 0 to 12 dSm^{-1} in the growing medium that is ascribed to increased damage of cell membranes resulting in electrolyte leakage.

An increase in electrolyte leakage is usually an expression of modification of physical properties of cell membrane. Acevado *et al*. (1997) reported that NaCl salts stress affected the mechanical properties of cell wall.

High electrolyte leakage is generally accompanied by enhanced lipid peroxidation as an evidence of oxidative damage (Aroca *et al*., 2003; Shanker *et al*., 2004; Mandhania *et al*., 2006).

NaCl treatment cause a progressive increasing displacement of calcium from the membrane of isolated protoplasts (Arshi *et al*., 2010).

4. Mineral status

Salinity affects plant growth by causing toxic accumulation of sodium, chloride or sulphate ions, reducing nutrient availability, decreasing water uptake and by creating poor soil physical conditions.

Mineral contents such as sodium (86.10%), chloride (108.45%) and sulphate (229.13%) in leaves of *Calendula oficinalis* were increased with the increase of salinity from 0 to 12 dSm^{-1}. Potassium content (46.04%) on the other hand was decreased with the increase of salinity in the growth medium.

Increased salinity level, in general, have been reported to enhance the accumulation of sodium in different crops (Yadav, 2000, Atam Parkash, 2000, Kara and Kaser, 2001, Surajkala, 2010). Many worker have reported an increase of sodium, chloride and concomitant decrease of potassium under salt stress in isabgol (Kanta Rani, 2000 and Vandana, 2003), chrysanthemum (Yadav, 2000), marigold (Atam Prakash, 2000), ajwain (Ashraf and Orooj, 2006), senna (Suman, 2010) and lemon grass (Sapna, 2011). However, Asha and Dhingra (2007) in chickpea, Hussain *et al.* (2009) in chasku (*Cassia absus*) reported the accumulation of both sodium as well as potassium under salt stress. Increase in sulphate content under salinity stress have been reported in chrysanthemum (Yadav, 2000), senna (Suman, 2010) lemon grass (Sapna, 2011) and chickpea (Kiran, 2004).

Accumulation of Cl^- and Na^+ in salt stressed leaves was related to reductions in net CO_2 assimilation rate in leaves of grafted and ungrafted plants. It has been proposed that the reduction of leaf gas exchange in response to salinity is due to increase in leaf Na^+ concentration (Garcia-Legaz *et al*., 1993; Walker *et al*., 1993). However, other authors associated reductions in photo-synthetic capacity and stomatal conductance with high concentrations of Cl^- (Banuls *et al*., 1997; Garcia-Sanchez *et al*., 2002).

Under salt stress, membranes become leakier because of Na^+, therefore, the uptake of K^+ decreases, leading to the decrease of K^+/ Na^+ ratios. A lower K^+/ Na^+ ratio is an index of toxicity because Na^+ impairs the activity of K^+ requiring enzymes, thus determining a low growth rate (Chaparzadeh *et al*., 2003). Decline in K^+ accumulation because of salt stress has been reported in wheat, sorghum, Swiss chard (*Beta vulgaris* L.), barley and rice (Zhao *et al*., 2007).

Na^+ and Cl^- may readily cross the cell membrane into the cytoplasm, and they are able to accumulate or decrease availability of some essential nutrients (Levitt, 1972). Specific ion toxicity of the Na^+ and Cl^- ions to cell membrane, cytoplasm or nucleus of the cells may partly be

related to the fact that NaCl was greatly inhibitory to the growth than growth (Razmjoo *et al.*, 2008). The accumulation of sodium, calcium and chlorine in plant tissues might mean that salinity is linked to its limited efficiency in keeping Na^+ and Cl^- in leaf tissue below toxic levels and compensating for the lower water potentials associated with salinity by increasing tissue levels of organic solutes (Rush and Epstein, 1976).

The increasing trends of sodium content is due to the fact that plant roots might have been injured considerably at higher salinity level or the osmotic pressure of the solution was too high to affect the semi-permeability characteristics of the membrane allowing higher uptake of salts (Roy *et al.*, 2003). The absorption of sodium was higher in chloride salinity resulting into poor crop growth. This may be due to NaCl induced shrinkage of chloroplasts, that increased photosynthetic phosphorylation, and the rate of hill reaction decreased with increasing osmotic potential (Roy *et al*., 2003). Excessive Na^+ concentration in the plant tissue hinders nutrient balance, osmotic regulation and causes toxicity (Bernstein, 1963). Flowers and Lauchli (1983) interpreted this fact on the basis of replacement of K^+ by Na^+ at the cell membrane level. These types of exchanges certainly had differential inhibiting effects under chloride and sulphate salinity, as observed by other workers (Rathert and Doering, 1983; Weimberg *et al*., 1982).

K^+ plays an important role in osmotic adjustment during the early stages of growth under NaCl stress (Bernstein, 1977). The reduced growth was probably due to excess of Na^+ and Cl^- which might have resulted in specific ion deficiencies *viz*., K^+, the dominant cation in the present system. It will be interesting to study the relative distribution of ions in the cells to find out whether inadequate compartmentation of excessive ions led to resultant growth inhibition in this system (Pandey and Ganapathy, 1984). The decrease in internal K^+ concentration has been associated with a competition between external K^+ and high external Na^+ in saline solution (Grattan and Grieve, 1999).

When NaCl was applied to the soil, the levels of K^+ in plants were reduced in accordance with the antagonism between Na^+ and K^+ (Alberico and Cramer, 1993; Azevedo and Tabosa, 2000). Cramer *et al*. (1985) showed that excess NaCl leads to the loss of potassium due to membrane depolarization by sodium ions. Under salinity, high NaCl uptake competes with the uptake of K^+ and leads to reduction of K^+/ Na^+ ratio and Na^+ toxicity (Kiarostami *et al*., 2010). Accumulation of proline in the cytoplasm is accompanied by a reduction in the concentrations of less compatible solutes, *e.g.* K^+ and glucamate, and an increase in cytosolic water volume (Samaras *et al*., 1995).

According to Weimberg (1987) high levels of sodium inhibit the potassium uptake resulting in decreased K^+/ Na^+ ratio. The decrease in the K^+/ Na^+ ratio may be attributed to the

fact that sodium causes a disturbance in the ion balance in plant by an increase in the sodium uptake. Accumulation of Cl^- in the root tissue is disruptive to membrane uptake mechanisms, leading to increased translocation of Cl^- to the shoots (Yousif *et al*., 1972).

CHAPTER- VI

SUMMARY AND CONCLUSION

The information given in the preceding pages may now be summarized into following points :

1. The germination of *Calendula officinalis* in non-saline field conditions occurred in the first week of December. It was followed by the vegetative growth up to March. The flowering (anthesis) initiated in the month of February and continued till mid April. Fruiting and seed maturation occurred simultaneously from mid April to early May. Death/ senescence was observed from early May onwards.
2. The overall growth of *Calendula officinalis* L. under non-saline field conditions was better than under pot conditions. All plant growth attributes *viz*., plant height (cm), basal stem diameter (mm), number of leaves plant^{-1}, leaf biomass (g plant^{-1}), stem and branches biomass (g plant^{-1}), flowers and fruits biomass (g plant^{-1}) and root biomass (g plant^{-1}) studied in the present experiment were found to increase with the advancement of growth stage under field conditions. The root/ shoot ratio, however, decreased with the advancement of growth stage.
3. The reproductive performance of *Calendula officinalis* L. in general, was better under field conditions as compared to pot culture. Reproductive behaviour such as number of seeds head^{-1} (40.3±0.9), seeds output (2055.7±46.8 number plant^{-1}), seed yield (6.1±0.3 g plant^{-1}), germination of seeds produced (86.67±0.35%) and reproductive capacity (1783.02±15.0) were also recorded at field conditions. Number of flower heads plant^{-1} was increased from flowering (40.33) to maturity stage (51.00).
4. Progressive increase of EC level from 0 to 12 dSm^{-1} in the growing medium caused a significant decline in plant height from 28.26 to 12.21 cm (56.79%) in *Calendula officinalis*. However, with the increase of growth stage (GS) the plant height increased significantly.
5. Number of leaves plant^{-1} was declined from 43.08 to 20.00 (53.57%) with the build up of salinity level in the growth medium as compared to control. With succeeding growth stage the number of leaves plant^{-1} increased significantly.
6. Leaf biomass (g plant^{-1}) was decreased from 1.13 to 0.60 g plant^{-1} (53.10%) with the succeeding EC levels up to 8 dSm^{-1} beyond which the decline in leaf biomass was insignificant. A steady increase in the leaf biomass was evident with the progressing growth stage irrespective of EC level.

7. The stem and branches biomass (g plant^{-1}) reduced from 0.53 to 0.20 g plant^{-1} (62.26%) with the rise of EC level in the growth medium. Significant increase in stem and branches biomass was observed with the advancement of growth stage, in general.
8. With the increase of EC level the flowers and fruits biomass (g plant^{-1}) decreased from 0.90 to 0.36 g plant^{-1} (60.00%). Increase in this attribute was also found from flowering to maturity stage.
9. Significant increase in root biomass (g plant^{-1}) was found with the growth of plants. With the increase of EC level of the growth medium up to 12 dSm^{-1} the root biomass (g plant^{-1}) declined from 0.87 to 0.28 g plant^{-1} (67.82%), in general.
10. Root / shoot ratio of *Calendula officinalis* was found to decline from 0.51 to 0.34 (33.33%) with the rise of EC level. Significant increase in root/ shoot ratio was observed from vegetative to flowering stage. Thereafter, an insignificant reduction in the ratio was noticed.
11. Significant increase in number of flower heads plant^{-1} was recorded from flowering to maturity stage. Steady reduction in number of flower heads plant^{-1} from 11.52 to 4.12 heads plant^{-1} (64.24%), however, number of flower heads plant^{-1} was observed with the progressive increase of EC level in the growth medium up to 12 dSm^{-1}.
12. Flower initiation occurred in 75 days in control. The number of days to flower initiation significantly increased from 75 to 87 days (16%) with the increase of EC level.
13. Maturity of *Calendula officinalis* plants in control occurred in 123 days. An increase in number of days to maturity was observed from 123.0 to 133.0 days (8.13%) with the rise of EC level up to 12 dSm^{-1}.
14. The number of seeds head^{-1} in control was 29.33. It declined to 24.33 seeds head^{-1} (17.05%) up to 12 dSm^{-1} of EC level.
15. Seed output (number plant^{-1}) in control was 549.97 which underwent significant reduction from 549.97 to 169.53 plant^{-1} (69.17%) with increase of salinity level up to 12 dSm^{-1}.
16. Maximum seed yield 1.30 g plant^{-1} was recorded in control. The seed yield significantly reduced from 1.30 to 0.56 g plant^{-1} (56.92%) with the buildup of salinity up to 12 dSm^{-1} in the growing medium.
17. The geminability of seeds produced by plants grown at various EC levels was assessed. The germination of seeds of control plants was 80 per cent which significantly reduced to 60 per cent in plants grown at 12 dSm^{-1} EC levels.
18. The reproductive capacity of *Calendula officinalis* decreased from 438.77 to 101.41 per cent (76.89%) with increase in EC level up to 12 dSm^{-1}. The reproductive capacity of plants grown under control was 438.77.

19. Chlorophyll -a, chlorophyll -b and total chlorophyll content decreased with the increase of salinity in the growing medium. The decline in Chlorophyll -a, chlorophyll -b and total chlorophyll content was 30.2 per cent, 43.8 per cent and 33.6 per cent respectively, up to 12 dSm^{-1} EC level.
20. A significant decline in carotenoid content of flower petals was observed from 0.449 to 0.249 mg g^{-1} fw (44.54%) with the increase of EC levels in the growth medium.
21. Increase in leaf proline content from 1.58 to 6.39 mg g^{-1} dw (304.43%) with the progressive increase of salinity up to 12 dSm^{-1} was observed.
22. An increase in relative stress injury (%) in leaves with the progressive increase in EC levels was observed. It increased from 12.25 per cent (control) to 35.27 per cent (12 dSm^{-1}).
23. Accumulation of in sodium in leaves at maturity stage was observed from 18.99 to 35.34 mg g^{-1} dw (86.10%) with the increase of EC levels in the growing medium up to 12 dSm^{-1}.
24. Decline in the potassium content in leaves was observed from 38.68 to 20.87 mg g^{-1} dw (46.04%) with the rise of EC levels.
25. Increase in chloride content in leaves at maturity stage was observed from 4.26 to 8.88 mg g^{-1} dw (108.45%) with the increase of EC levels in the growing medium up to 12 dSm^{-1}.
26. Sulphate content in leaves at maturity stage was observed to increase from 1.03 to 3.39 mg g^{-1} dw (229.13%) with the increase of EC levels in the growing medium up to 12 dSm^{-1}.

Conclusion

The present study revealed that *Calendula officinalis*, a high valued medicinal plant despite reduction in growth, seed yield and reproductive capacity under saline conditions performed well up to the salinity of 12 dSm^{-1} EC level. Its survival and successful reproductive capacity at 12 dSm^{-1} EC level shows its salt tolerance. It may be put on field trial in salt-affected areas.

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APPENDIX

Table A1 : Reproductive behavior of *Calendula officinalis* L. at different growth stages under varying salinity

EC level dSm^{-1} with added salinity	Days to flower initiation	Days to maturity	Number of seeds $head^{-1}$	Seed output (no $plant^{-1}$)	Seed yield (g $plant^{-1}$)
0	75.0	123.0	29.33	549.97	1.30
4	76.0	126.0	27.67	354.60	1.05
8	80.0	129.0	25.33	247.33	0.80
12	87.0	133.0	24.33	169.53	0.56
CD (P≤0.05)	06.3	006.1	03.49	069.26	0.01

Table A2 : Physiological attributes of *Calendula officinalis* L. at different growth stages under varying salinity

EC level dSm^{-1} with added salinity	Chlorophyll 'a' (mg g^{-1}dw)	Chlorophyll 'b' (mg g^{-1} dw)	Total Chlorophyll (mg g^{-1} dw)	Carotenoid (mg g^{-1} fw)	Proline (mg g^{-1} dw)	Relative Stress Injury (%)
0	14.42	4.96	19.38	0.449	1.58	12.25
4	12.60	4.29	16.89	0.381	4.21	28.77
8	11.79	3.50	15.28	0.317	6.06	33.66
12	10.06	2.79	12.86	0.249	6.39	35.27
CD (P ≤0.05)	0.77	0.40	1.04	0.005	0.78	02.57

Table A3 : Minerals status of *Calendula officinalis* L. at maturity stage under varying salinity

EC level dSm^{-1} with added salinity	Sodium (mg g^{-1} dw)	Potassium (mg g^{-1} dw)	Chloride (mg g^{-1} dw)	Sulphate (mg g^{-1} dw)
0	18.99	38.68	4.26	01.03
4	23.56	32.11	5.80	01.62
8	27.53	25.67	7.46	02.50
12	35.34	20.87	8.88	03.39
CD (P ≤0.05)	02.44	02.92	0.78	00.42

ABSTRACT

Title of the thesis	:	**Morpho-physiological study of *Calendula officinalis* L. under saline conditions**
Full name of the degree holder	:	**Kusum Rani**
Title of the degree	:	Master of Science
Name and address of Major Advisor	:	**Dr. U. K. Varshney,** Department of Botany & Plant Physiology, CCS HAU, Hisar – 125 004, India
Degree awarding University/ Institute	:	CCS HAU, Hisar – 125 004, India
Year of award of degree	:	2013
Major Subject	:	Botany
Total number of pages in the thesis	:	55+xiv
Total number of words in the abstract	:	570 (Approx.)

Key words : *Calendula officinalis*, pot marigold, salinity, growth, yield parameters, reproductive capacity, chlorophyll, carotenoids, proline, RSI, sodium, potassium, chloride, sulphate

Calendula officinalis L. commonly known as pot marigold in English belongs to the family Asteraceae (Compositae). It is an important medicinal herb indigenous to central, eastern and southern Europe. It is cultivated commonly in North America, Eastern Europe, Germany and India for medicinal as well as ornamental purpose. The present investigation was undertaken to enrich the scientific database regarding the morpho-physiology of *Calendula officinalis* under non-saline field and saline conditions.

Two experiments were planned. First experiment was performed to study the phenology, growth and reproductive behavior of *Calendula officinalis* under natural non-saline field conditions. Seeds were sown in the first week of December. Phenological study depict the vegetative growth up to March. Flowering (anthesis) initiated simultaneously in the month of February and continued till mid April. Fruiting and seed maturation also occurred simultaneously from mid April to early May. Death / senescence was observed from early May onwards.

The overall growth of *Calendula officinalis* L. under field conditions was better than under pot conditions. All plant growth attributes *viz*., plant height (cm), basal stem diameter (mm), number of leaves plant^{-1}, leaf biomass (g plant^{-1}), stem and branches biomass (g plant^{-1}), flowers and fruits biomass (g plant^{-1}) and root biomass (g plant^{-1}) studied in the present experiment were found to increase with the advancement of growth stage under field conditions. The root/ shoot ratio, however, decreased with the advancement of growth stage.

The reproductive performance of *Calendula officinalis* L. in general, was better under field conditions as compared to pot culture. Reproductive behaviour was studied in terms of the number of flower heads plant^{-1}, number of seeds head^{-1}, seeds output (number plant^{-1}), seed yield (g plant^{-1}), germination of seeds produced and reproductive capacity in field conditions.

The second experiment was performed to study the morpho-physiological attributed of *Calendula officinalis* under chloride dominated salinity. The plants were raised in sand filled polythene bags under varying EC levels *viz*., 0 (control), 4, 8 and 12 dSm^{-1} of nutrients supplemented chloride dominated salinity. Progressive increase of EC level from 0 to 12 dSm^{-1} caused a significant decline in growth parameters *viz*., plant height (cm), number of leaves plant^{-1}, leaf biomass (g plant^{-1}), stem and branches biomass (g plant^{-1}), flowers and fruits biomass (g plant^{-1}), root biomass (g plant^{-1}) and root / shoot ratio. However, these parameters increased significantly with the advancement of growth stage (GS).

Reproductive attributes such as number of flower heads plant^{-1}, number of seeds head^{-1}, seed output (number plant^{-1}), seed yield (g plant^{-1}), geminability of seeds produced and reproductive capacity suffered a reduction with the rise of salinity. Both days to flower initiation and days to maturity were increased with the progressive increase of EC level in the growth medium.

Physiological parameters *viz*., chlorophyll -a (mg g^{-1} dw), chlorophyll -b (mg g^{-1} dw) and total chlorophyll content (mg g^{-1} dw) of leaves and carotenoid content (mg g^{-1} fw) of flower petals significantly decreased with the increase of salinity in the growing medium. Accumulation of proline (mg g^{-1} dw) and increase in relative stress injury (%) in the leaves was observed with the progressive increase of salinity.

Enhancement of sodium and concomitant decline of the potassium content (mg g^{-1} dw) in leaves was found with the rise of EC level in growing medium. Accumulation of both chloride and sulphate was observed with the increase of salinity. The accumulation of chloride was relatively less in leaves at the maturity stage than sulphate.

Major Advisor Signature of Student

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I hereby, declare that all the information provided in the resume is true to best of my knowledge.

(Kusum Rani)

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Place : Hisar

UNDERTAKING OF COPY RIGHT

I, **Kusum Rani**, Admn. No. **2011BS90M** undertake that I give copy right to the CCS Haryana Agricultural University, Hisar of my thesis entitled, **"Morpho-physiological study of *Calendula officinalis* L. under saline conditions"**.

I also undertake the patent if any, arising out of the research work conducted during the programme shall be filed by only with due permission of the competent authority of CCS Haryana Agricultural University, Hisar.

Signature of the student

Date : 27-12-2013

Place : Hisar

***Calendula officinalis* L. (Pot marigold)**