The impact of salinity on the growth, productivity and physiology in *Silybum marianum* L. is enumerated in this chapter. The results obtained are given under following heads:

4.1 Growth

4.2 Biomass and productivity

4.3 Physiological aspects

4.4 Nutrients

4.5 Plant antioxidant system and biochemical analysis

### 4.1 GROWTH

#### 4.1.1 Seed Germination

The seeds of *Silybum marianum* L. were subjected to salinity (25mM, 50mM, 100mM, 150mM, and 200mM) to determine the seed germination. Seeds of *S.marianum* were non-dormant and germination initiated within 4 days of incubation. Seeds responded to salinity treatments (Fig. 4.1; 4.2). The percent germination and days to germination varied with stress conditions. The first emergence of seedling was observed on 4th day in control, 25mM, 50mM 100mM, 150mM, and 200mM NaCl. During the initial period (until 12 day in case of 25mM and 50mM the germination were recorded 85% and 80% respectively as compared to untreated plants. After that the germination percentage were observed at 18th day of incubation of seed the highest in the case of 50mM and lowest in 150mM salinity treatments 88% and 73% as compared to untreated plants where 78% germination.
Fig. 4.1: The time-course of germination of *Silybum marianum* seeds as affected by NaCl. Values are mean ± S.E n=3. Values are mean ± SE; n=3.
Fig. 4.2: Germination of *Silybum marianum* seeds as affected by NaCl after 24 days of incubation
Fig. 4.3: The seedling of *Silybum marianum* L. is influenced after 24 days different concentration of NaCl (a) Control (b) 25mM NaCl (c) 50mM NaCl (d) 100mM NaCl (e) 150mM NaCl (f) 200mM NaCl.

Seed germination is enhanced by all concentration of salinity stress as compared to untreated plants. In 25mM NaCl it enhanced by 7%; in 50mM NaCl by 10%; in 100mM NaCl by 4%; in 200mM NaCl by 2%; except 150mM NaCl where seed germination decreased to 5% as compared to control. The degree of increase was maximum in case of 50mM NaCl i.e. after 24 days of incubation 88% seed germinated as against 78% in control. Data was subjected to two-way analysis of variance (ANOVA). The individual performance of treatments calculated with the help of Bonferroni multiple comparison post tests.
4.1.2 Seedlings Shoot and Root Lengths and Plant Height

Stem length was measured after 24 days of seed germination. Salt stress adversely affected the seedling growth (Fig. 4.3 (A)). Elevated NaCl concentrations decreased the length of shoot in concentration dependent manner i.e., in 25mM NaCl by 5.73% in 50mM NaCl by 5.6% in 100mM NaCl by 4.13% in 150mM NaCl by 2.13% in 200mM NaCl by 0.67% NaCl all concentration were inhibit for stem length. The maximum effect intensified with decrease in concentration. After 24 days of incubation the degree of decrease in stem length was minimum in 25mM NaCl (1.46cm) and maximum in 200mM NaCl (1.8 cm) as against 1.84 cm stem length in control. The one-way ANOVA analysis was performed and means comparison analysis was achieved using Tukey’s Multiple Comparison Test (P<0.05).

Root length in seedlings of *S. marianum* was measured after 24 days of seed incubation (Fig. 4.3 (B)). Seeds responded differently to salinity (NaCl) treatments and enhanced in root length were reported in all applied concentration. The root growth was does not affected more than the shoot growth. The root length after 24 days of incubation increased as compared to untreated plants, i.e., by 13.6% in 25mM NaCl in 50mM NaCl by 22% in 100mM NaCl 19.06% in 150mM NaCl by 10.53% in 200mM NaCl 5.33%. The degree of enhancement in root length was minimum in 200mM NaCl (0.4 cm) and maximum in 50mM NaCl (1.5 cm). The root length was (7.4 cm) after 24 days of incubation in control. Data was analysed by one-way analysis of variance (ANOVA) and multiple comparisons between treated and untreated plants by Tukey’s analysis of variance.

Plant height of *Silybum marianum* L. under salt stress was measured after 24 days of seed incubation (Fig. 4.3 (C)). The height of plant is enhanced in applied concentration of salt stress i.e., by 5.76% in 25mM NaCl in 50mM NaCl by 5.93% in 100mM NaCl by 8.89% in 150mM NaCl by 5.99% in 200mM NaCl by 4.33% as compared to untreated plant. The degree of enhancement in plants height was minimum in 200mM NaCl (0.29 cm) and maximum in 100mM NaCl (0.6 cm). The plant height was (3.98 cm) after 24 days of incubation in control. Data was analysed by one-way analysis of variance (ANOVA) and multiple comparisons between treated and untreated plants by Tukey’s analysis of variance.
Fig. 4.4: Effect of NaCl on stem length (A) root length (B) and plant height (C) of *Silybum marianum* L. Values are mean ± SE; n=3. Analyzed by One-way ANOVA followed by Tukey’s multiple comparison test.
4.2 BIOMASS AND PRODUCTIVITY

4.2.1 Total Fresh Weight

Total fresh weight of *Silybum marianum* L. under salinity stress after 24 days was measured and enhanced as compared to untreated plants, i.e., 25mM NaCl by 14% in 50mM NaCl by 44% in 100mM NaCl by 32% in 150mM NaCl by 29% in 200mM NaCl by 73%. Applied salinity stress concentration may enhance the fresh weight of plants as compared to control. The degree of enhancement was maximum in i.e., by 200mM NaCl 1.26% in 150mM NaCl by 1.06 in 100mM NaCl by 1.06 in 50mM NaCl by 1.13 in 25mM NaCl by 0.93% compared to untreated plants (Fig. 4.5 (A, B). Data were analysed by one-way analysis of variance (ANOVA) and multiple comparisons between treated and untreated plants by Tukey’s analysis of variance.

4.2.2 Total Dry Weight

Total dry weight of plant showed a continuous decrease in applied salt stress concentration except 25mM NaCl where the dry weight is enhanced i.e., by 2.8% in 50mM NaCl. It decreased by 19.4% in 50mM NaCl by 28.4% in 100mM NaCl by 32.2% in 150mM NaCl by 26.6% in 200mM NaCl compared to untreated plants. The degree of decrease was maximum in 150mM NaCl was 32.6% and the effect intensified with an increase in concentration as compared to control (2.2gm). Data were analysed by one-way analysis of variance (ANOVA) and multiple comparisons between treated and untreated plants by Tukey’s analysis of variance.
Fig. 4.5: Total fresh (A) and dry weight (B) of *Silybum marianum*, respectively after 24 days of incubation growth under salinity stress conditions. Values are mean ± SE; n=3. Analyzed by One-way ANOVA followed by Tukey’s multiple comparison test.
Fig. 4.6: 10 days old seedlings of *Silybum marianum* under different concentration NaCl (a), 50ppm Trehalose (TH) (b), 100ppm Trehalose (TH) (c), 50ppm Mannitol (MT) (d), 100ppm Mannitol (MT) (e), 50ppm Sorbitol (SOR) (f), 100ppm Sorbitol (SOR) (g).
4.3 PHYSIOLOGICAL ASPECTS

4.3.1 Relative Water Content
The results of relative water content (RWC) are presented in (Fig. 4.7.) It is evident from the results that with an increase in the concentration of salinity stress relative water content is decreased. When the osmolyte is applied they enhanced the relative water content of leaf in some concentration. There was a progressive decrease in the relative water content of leaves and enhanced with osmolyte. RWC of leaves was measured after 45 days of seed incubation. It decreased by 31% in 25mM in 50mM by 22.15% in 100mM by 27.24% in 150mM by 8.47% in 200mM by 33.24% as compared to control 87.37%. When osmolyte trehalose is applied with salt concentration then enhanced the (RWC) in 200mM +50ppm by i.e., 5.51% then progressively decreases in 25mM+50ppm by 11.28% in 50mM +50ppm by 1.1% in 100mM +50ppm by 4.1% in 150mM +50ppm by 4.5% in 25M+100ppm by 22.28% in 50mM+100ppm by 0.39% in 100mM+100ppm by7.05% in150mM+100ppm by 33.17% in 200mM+100ppm trehalose by 10.11% as compared to control 72.98% then in mannitol with salinity it increases by 25mM +50ppm by 1.56% in 100mM+50ppm by 6.07% in 200mM +50ppm by 3.99% in 0.11% in 100mM+100ppm by 2.24% then decreases in 25mM+50ppm by 3.95% in 150mM+50ppm by 22.49% in 25mM+100ppm by 15.16% in 150mM +100ppm by 21.19% in 200mM+100ppm by 3.38% as compared to control 76.25% then in case of sorbitol enhanced in 25mM +50ppm by 25.61% in 100mM +50ppm by 9.05% in150mM +50ppm by 9.51% it decreases in 50mM+50ppm by 10.39% in 200mM +50ppm sorbitol by 2.7% in 25mM+100ppm by 27.62% in 50mM+100ppm by 16.67% in 100mM +100ppm by 25.97% in 150mM +100ppm by 8.86% in 200mM NaCl+100ppm sorbitol by 3.28% as compared to control 65%. The degree of decrease in RWC was minimum in 50mM NaCl+100ppm mannitol and maximum in150mM NaCl+100ppm trehalose. The data for relative water content was analysed by two-way analysis of variance (ANOVA) Individual performances for significance between treated and untreated plants was calculated by Bonnferoni multiple comparison test (P<0.05).
Fig. 4.7: Relative water content under salinity stress in combination with Trehalose (A) Mannitol (B) and Sorbitol (C). Values are mean ± SE; n=3. Analyzed by Two-way ANOVA followed by Bonferroni multiple comparison tests.
4.4 NUTRIENTS

Sodium and potassium nutrients were analysed in the leaves of *Silybum marianum* L. after 45 days and results are presented in Fig. 4.8 (A) Plants responded differently to salinity stress and osmolyte treatment with respect to Na\(^+\) content. It increased only salinity concentration by 0.014\% in 25mM by 0.014\% in 50mM by 0.014\% in 100mM by 0.014\% in 150mM by 0.014\% in 200mM NaCl by 0.030\% as compared to control. Then with respect to osmolyte it enhanced 0.004\% in 200mM+50ppm trehalose except other concentration of trehalose like 50 ppm and 100ppm with respect to salinity is equal to control then with respect to mannitol. It enhanced 0.014\% in 25M+50ppm in50mM by 0.014\% and decreases in 100mM+50ppm mannitol except these all concentration equal to control then in sorbitol enhanced in 50mM+50ppm sorbitol and decreases in 100mM+100ppm by 0.001\% in 200mM by 0.001\% except these all concentration is equal to control as compared to untreated plants. The degree of decrease was minimum in 100M+100ppm (0.001mmolg\(^{-1}\) DW) and maximum in200mM+100ppm sorbitol (0.001mmolg\(^{-1}\) DW) as compared to control. Data was analysed by two-way analysis of variance (ANOVA) Individual performances for significance between treated and untreated plants was calculated by Bonnferoni multiple comparison test (P<0.05).

K\(^+\) content in *Silybum marianum* L. is varied in salinity concentration decreased as well as enhanced by the applied osmolyte are presented in Fig. 4.8 (B) it decreased in 25mM by 8\% in 50mM by 8\% enhanced in 7.2\% in 50mM in 150mm by 7\% in 200mM by 11\% as compared to control in case of osmolyte it decreased by 4.9\% in 25mm+50ppm in 50M+100ppm by 5\% in100mm +50ppm by 9\% in150mm +50ppm by 4.8\% in 25mm +100ppm by14.8\% in 150mm +100ppm by 14.1\% in100mm +100ppm and 200mM +100ppm is equal to control enhanced in 50mM +50ppm by 3.3\% in 200mM +50ppm by 15.2\% as compared to control in case of mannitol it decreases by 4.9\% in 25mM +50ppm in 50mM +50ppm by 4.7\% in 100M +50ppm mannitol by 5.2\% in 150mM +50ppm by15.2\% in 200mM +50ppm by 9.8\% in 25mM +100ppm by 9.8\% in 50mM +100ppm by 10.2\% in 100mM +100ppm by 1.2\% in 150mM +100ppm by 10.2\% in 200mM +100ppm by 15.1\% in compared to control. In case of sorbitol it decreases by
5.4% in 50mM +50ppm in 100mM +50ppm by 10.4% in 150mM +50ppm by 0.5% in 25mM +100ppm by 5.2% in 100mM +100ppm by 0.1% in 200mM +100ppm by 5.1% then enhanced in 25mM +50ppm by 4.9% in 200mM +50ppm by 3.5% in 50mM +100ppm by 4.5% in 150mM +100ppm by 5.1% as compared to control. The degree of decrease was minimum in 100mM +100ppm (0.1mmolg⁻¹ DW) and maximum in 150mM NaCl+50ppm mannitol (15.2mmolg⁻¹ DW) as compared to control. Data was analysed by two-way analysis of variance (ANOVA) Individual performances for significance between treated and untreated plants was calculated by Bonferoni multiple comparison test (P<0.05).
Fig. 4.8 (A): Na\(^+\) content under salinity stress in combination with (A) Trehalose, (B) Mannitol and (C) Sorbitol. Values are mean ± SE; n=3. Analyzed by Two-way ANOVA followed by Bonferroni multiple comparison tests.
Fig. 4.8 (B): \( K^+ \) content under salinity stress in combination with (A) Trehalose, (B) Mannitol and (C) Sorbitol. Values are mean ± SE; n=3. Analyzed by Two-way ANOVA followed by Bonferroni multiple comparison tests.
4.5 PLANT ANTIOXIDENT DEFENCE SYSTEM

4.5.1 Enzymatic Antioxidants

The enzymatic antioxidants analyzed in the leaves of *Silybum marianum* L. Catalase (CAT) and peroxidase(POD).

4.5.1.1 Catalase (CAT)

Catalase activity of *Silybum marianum* L. leaves was measured after 45 days of incubation and the results obtained are given in Fig. 4.9. There was variable in result is obtained from different concentration of NaCl. Salt stress along with three osmolyte trehalose, mannitol and sorbitol. There is significant role of osmolyte is observed on plants under salinity stress. It increased in 25mM by 0.1% in100mM by 7.46% in 150mM by 4.53% in 200 by 3.94% decreased in 50mM by1.46% as compared to control. Then increased in case of osmolyte (trehalose) in 25mM +50ppm by 64.54% in 50mM +50ppm by 64.56% decreased in 100mM +50ppm by 28.38% in 150mM +50ppm by 28.46% in 200mM +50ppm by 25. 69% in 25mM +100ppm by 22.24% in 50mM+100ppm by 23.94% in 100mM+100ppm by 23.92% in 150mM +100ppm by 5.62% in 200mM +100ppm by15.64% in case of mannitol increased in 50M+50ppm by 9.73% then decreased in 25mM +50ppm by 10.36% in100mM +50ppm by7.22% in 150mM +50ppm by 7.17% in 200mM +50ppm by 7.62% in 25mM+100ppm by7.83% in 50mM +100ppm by 4.83% in 100mM +100ppm by 10.27% in150mM+100ppm by10.95% in 200mM +100ppm by 7.97% then in case of sorbitol it decreased in 25mM +50ppm by 18.25% in 50mM +50ppm by 17.82% in 100mM +50ppm by 20.15% in150mM +50ppm by 20.44% in 200mM +50ppm by 22.33% 25mM +100ppm by 9.65% in50mM +100ppm by 13.89% in100mM +100ppm by 15.85% in150mM +100ppm by 24.41% in150mM +100ppm sorbitol by 33.15% as compared to control. After 45 days the degree of decrease in catalase activity per minute was minimum in 50mM NaCl+100ppm mannitol (4.83 U/mg protein) and maximum in 200mM NaCl+100ppm sorbitol (33.15 U/mg protein) against 61.47U/mg protein in control. The two-way ANOVA showed significant difference between treatments and Bonferroni Multiple Comparison Test (P<0.05) showed significance differences between treated and untreated plants.
4.5.1.2 Peroxidase (POD)

Total peroxidase content of *Silybum marianum* L. leaves was measured after 45 days of incubation. Quantitatively the POD activity increased when salinity is applied along with different concentration of osmolyte (Trehalose, Mannitol, and Sorbitol) compared to control plants (Fig. 4.10. The increase in POD content was more in plants kept at high salt stress conditions and in case of trehalose. The POD content increased by 0.015% in 25mM in 50mM by 0.02% in 100mM is decreased by 0.018% in 150mM by 0.07% in 200mM by 0.064% as compared to control then in case of trehalose it increased in 50mM +50ppm by 0.057% in 100mM +50ppm by 0.0386% in 150mM+50ppm by 0.104% in 200mM +50ppm by 0.018% in 25mM +100ppm by 0.039% in 50mM +100ppm by 0.004% in 100mM +100ppm by 0.016% in 150mM +100ppm by 0.035% in 200mM+100ppm by 0.02% and decreased in 25mM NaCl+50ppm trehalose by 0.035% as compared to control then in case of mannitol decreased in 25mM +50ppm by 0.0186% in 50mM +50ppm by 0.0096% in 100mM +50ppm by 0.1356% in 150mM +50ppm by 0.0626% in 200mM +100ppm by 0.050% in 150mM+100ppm by 0.0916% in 200mM +100ppm by 0.1576% then increased in 200mM +50ppm by 0.0295% in 25mM +100ppm by 0.1464% in 50mM +100ppm by 0.094% as compared to control then in case of sorbitol increased in 25mM +50ppm by 0.085% in 50mM +50ppm by 0.075% in 100mM +50ppm by 0.261% in 200mM +50ppm by 0.083% then decreased in 150mM +50ppm by 0.0082% in 25mM+100ppm by 0.010% in 50mM +100ppm by 0.032% in 100mM +100ppm by 0.014% in 150mM +100ppm by 0.018% in 200mM +100ppm by 0.024% as compared to control. The two-way ANOVA showed significant difference between treatments and Bonferroni Multiple Comparison Test (P<0.05) showed significance differences between treated and untreated plants.
Fig. 4.9: Catalase activity of *Silybum marianum* leaves under salinity stress in combination with (A) Trehalose, (B) Mannitol and (C) Sorbitol. Values are mean ± SE; n=3. Analyzed by Two-way ANOVA followed by Bonferroni multiple comparison tests.
Fig. 4.10: Peroxidase activity of *Silybum marianum* leaves under salinity stress in combination with (A) Trehalose, (B) Mannitol and (C) Sorbitol. Values are mean ± SE; n=3. Analyzed by Two-way ANOVA followed by Bonferroni multiple comparison tests.
4.5.2 Non Enzymatic Antioxidants

4.5.2.1 Phenol

Total phenol content of *Silybum marianum* L. leaves were measured after 45 days of incubation. The impact of different concentrations of NaCl and osmolyte on the phenol content is given in (Fig.4.11). It is evident from results that the phenol content increased by 1.003% in 25mM in 50mM by 1.323% in 100mM by 0.007% in 150mM by 1.437% and decreased in 200mM by 0.143% as compared to control then in case of trehalose 25mM +50ppm by 0.64% in 50mM +50ppm by 0.696% in 100mM +50ppm by 0.19% in 150mM +50ppm by 0.326% in 200mM +50ppm by 0.186% in 25mM +100ppm by 0.48% in 50mM +100ppm by 0.353% in 100mM +100ppm by 0.49% in 150mM +100ppm by 0.22% in 200mM +100ppm by 0.318% then in case of trehalose 25mM +50ppm by 0.226% in 100mM +50ppm by 0.036% in 150mM +50ppm by 0.026% in 25mM +100ppm by 0.105% then increased in 50M+50ppm in 200mM +50ppm by 0.034% in 50mM +100ppm by 0.067% in 100mM +100ppm by 0.72% in 150mM +100ppm by 0.924% in 200mM +100ppm by 0.954% then in case of sorbitol increased by 0.286% in 25mM +50ppm in 50mM +50ppm by 0.39% in 100mM +50ppm by 0.063% in 150mM +50ppm by 0.333% in 200mM +50ppm by 0.346% in 25mM +100ppm by 0.403% in 50mM +100ppm by 0.27% in 100mM +100ppm by 0.383% in 150mM +100ppm by 0.153% in 200mM NaCl +100ppm by 0.8% as compared to control. A maximum of 5.89 mgg⁻¹ FW phenol content was observed in 150mM NaCl and a minimum of 4.31 mgg⁻¹ FW in 200mM NaCl. The two-way ANOVA showed significant difference between treatments and Bonnferoni Multiple Comparison Test (P<0.05) showed significance differences between treated and untreated plants.

4.6 BIOCHEMICAL ANALYSIS

4.6.1 Lipid peroxidation

Estimation of lipid peroxidation was performed in terms of total malonoaldehyde (MDA) content in the leaves of *Silybum marianum* L. after 45 days of incubation. Total MDA content due to different concentrations of NaCl and osmolyte are shown in Fig.4.12. It is evident that MDA increased by 0.384% in 25mM in 50mM by 0.194% in 100mM by 0.321% then decreased in 0.209% in 150mM in 200mM by 0.199% as
compared to control then in case of osmolyte (trehalose) decreased in 25mM+50ppm by 0.037% in 50mM+50ppm by 0.19% in 25mM+100ppm by 0.01% in 150mM+100ppm by 0.027% then increased in 100mM +50ppm by 0.473% in150mM+50ppm by 0.056% in 200mM+50ppm by 0.013% in 50mM+100ppm by 0.016% in 150mM+100ppm by 0.01% in 200mM+100ppm by 0.016% as compared to control then in case of mannitol increased in 25mM+50ppm by 0.19% in 150mM +50ppm by0.41% in 25mM+100ppm by 0.004% in 50mM+100ppm by 0.004% in 100mM+100ppm by 0.06% in 150mM+100ppm by 0.307% in 200mM+100ppm by 0.027% then decreased in 50mM +50ppm by 0.046% in 100mM+50ppm by 0.033% as compared to control then in case of sorbitol decreased in 25mM+50ppm by 0.02% in 100mM+50ppm by 0.174% in 150mM+50ppm by 0.14% in 200mM +50ppm by 0.16% in 25mM+100ppm by 0.004% in 150mM+100ppm by 0.09% then increased in 50mM+50ppm by 0.01% in 25mM +100ppm by 0.563% in 100mM+100ppm by 0.05% in 200mM NaCl+100ppm by 0.01% as compared to control. After 45days the degree of increase in total MDA content was minimum in 50mM NaCl (0.19mg g⁻¹FW) and maximum in 25mM NaCl (0.38mg g⁻¹FW). For lipid peroxidation the data was analyzed by two-way ANOVA and Bonnferoni multiple comparison tests.
Fig. 4.11: Phenol contents of *Silybum marianum* leaves under salinity stress in combination with (A) Trehalose, (B) Mannitol and (C) Sorbitol. Values are mean ± SE; n=3. Analyzed by Two-way ANOVA followed by Bonferroni multiple comparison tests.
Fig. 4.12: Malonoaldehyde content of *Silybum marianum* leaves under salinity stress in combination with (A) Trehalose, (B) Mannitol and (C) Sorbitol. Values are mean ± SE; n=3. Analyzed by Two-way ANOVA followed by Bonnferoni multiple comparison tests.