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# EFFECT OF MANNITOL ON *LEPIDIUM SATIVUM* L. UNDER ABIOTIC STRESS (WATER STRESS) CONDITION

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## Abstract

The effects of mannitol to water stress on physiological , nutritional, secondary metabolites and antioxidant capacity were investigated in *Lepidium sativum* L. Plants were grown under controlled temperature (25°C) and light conditions (16 hours light and 8 hours dark). Physiological parameters (carbohydrate, protein and proline) nutritional analysis (sodium and potassium) secondary metabolites (alkaloid and saponin) and antioxidant activity (ABTS and DPPH) were determined after 35, 75 and 110 days. Exogenous application of mannitol (50µg/l, 100µg/l, and 250µg/l) were standardized and applied to different water potential -0.01$\Psi_w$ MPa, -0.02$\Psi_w$ MPa and -0.03$\Psi_w$ MPa. *Mannitol enhanced the physiological parameters, nutritional factors, secondary metabolites and antioxidant activity in stressed plant; it is play a major role in cellular osmotic adjustment. Present study indicating that the mannitol play pivotal role to water stress in Lepidium sativum* L. Therefore, it found that the plants are able to cope with abiotic stress when exogenous mannitol is applied.

***Key words:*** Mannitol, water stress, abiotic stress, protein, carbohydrate. ABTS and DPPH

## Introduction

Environmental factors influence the characters, composition, growth and development of plant and plants communities. When any environmental influence exceeds the optimum tolerance of a plant, the result is stress to that plant (Lawlor, 2002; Jaleel, 2007). Plants, as of their sessile nature, are the leading creatures, which continuously face several environmental stresses such as extreme temperature, drought, water logging, salinity, heavy metal etc., which affect productivity (Heidari, 2009). Plants exposed to various abiotic stresses because of unescapable environmental conditions, which harmfully affect their growth and development and generate a series of morphological, physiological and biochemical changes (Ahmad *et al.,* 2008). In the consequence of global climatic change, different biotic and abiotic stresses are severe threats to the agricultural production worldwide. In nature, plants unceasingly stressed by exposure to multiple adverse conditions. The collective effect of multiple biotic and abiotic stresses is a major yield-limiting factor in agriculture. The stress concept in plants described according to physiological and ecological requirements of a plant throughout its life cycle (Godbold, 1998). The required resources can be any environmental factor, and hence include chemical, physical and biotic factors. Such stress factors distinct as extreme environmental conditions that induce functional changes in plants to such an extent that stress on the plants develops, subsequent in inhibited growth, reduced bio production, physiological acclimatization and adaptation of species. ROS generated in plant cells by ordinary cellular metabolism or due to unfavorable environmental conditions such as drought, salinity, heavy metals, drought, herbicides, nutrient deficiency, or radiation. Their fabrications controlled by various enzymatic and non-enzymatic antioxidant defense systems. Enzymatic antioxidant defense systems, including catalase, ascorbate peroxidase, peroxidase, super oxide dismutase, monodehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase and non-enzymatic antioxidant defense systems, including phenolic, carotenoids, ascorbate, glutathione, compounds, proline, glycine betaine, sugar, and polyamines (Ahmad *et al.,* 2008; Gill and Tuteja, 2010; Karuppanapandian *et al.,* 2011). Antioxidant defense mechanisms allow plants to acclimatize and survive stressful events. However, long-

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term exposure of plants to biotic and abiotic stress induces an interruption in plant metabolism implying physiological costs and thus, leading to a reduction in suitability and eventually in productivity (Shao *et al*., 2008). Osmolytes are small molecules that exploited by cells as a defensive system against stress conditions. Most osmolyte compounds divided into three chemical classes: polyhydric alcohols and sugars (polyols), amino acids and their derivatives, and methyl ammonium compounds (Macchi *et al*., 2012). Many plants accumulate organic osmolytes in comeback to the imposition of abiotic stresses that cause cellular dehydration. Mannitol, trehalose and sorbitol exhibit marginal improvements in salt and/ or drought tolerance (Garg *et al.,* 2002). Metabolic assistances of osmolyte accumulation may augment the classically accepted roles of these compounds (Hare *et al.,* 1998). Glycine betaine and proline are two foremost organic osmolyte that accumulate in a variety of plant species in comeback to environmental stresses such as drought, salinity, extreme temperature, UV radiation and heavy metal. To improve plant forbearance to abiotic stresses such as excess light, drought, extreme environmental temperatures or salinity, the osmotic potential of plant cells must increase, usually by increasing the concentration of cell solutes. However, increasing the deliberations of common solutes, such as organic acids, carbohydrates and inorganic ions, can inhibit enzymatic activity. These solutes consequently found within plant cell vacuoles where their increasing concentration does not harm cell metabolism. In contrast to common solutes, compatible osmolytes (osmoregulators) are membrane-impermeable solutes, which accumulate in the cytoplasm at high concentrations (C > 0.2 M), do not adversely affect functional activities in the cell and as such, they considered valuable osmoprotectors (Yancey *et al*., 1982).

## Materials and Methods

### Plant Growth

The seeds propagated in seed trays comprising soil placed in a polyhouse with regulated temperatures ranging among 20 to 25°C, under a long-day photoperiod (16h light/8h dark). 10days old seedling shifted to different pots. After sowing of 20 days water stress and osmolyte, treatments started. Water stress imposed by watering while weighing method. Different water potential (-0.01 $\Psi_w MPa$, -0.02 $\Psi_w MPa$, *and* -0.03 $\Psi_w MPa$) were achieved at 20 days of seed sowing. Seedling fertilized by adding Hoagland nutrient solution to each pot after every seven days. Plants parts (Leaves) sampled to determine carbohydrate, protein, proline, sodium, potassium, alkaloid, saponin, ABTS and DPPH of plant after 35, 75 and 110 days. Osmolyte concentration used for treatments 50µg/l, 100µg/l and 250µg/l apply through foliar spray.

### Estimation of Carbohydrates

Total carbohydrates were determined in plant tissue method described by (Hedge and Hofreiter, 1962). Weighed 100 mg of the sample. Hydrolyzed via keeping it in hot water bath aimed at 180 minutes through 5 mL of 2.5N HCl then cooled. Deactivated it through dense sodium carbonate until the bubbliness finishes. Centrifuged at 10,000 rpm for 5 minutes. Collected the supernatant and took 0.5 then 1ml aliquots for examination. Made up the volume toward 1ml in all the tubes comprising the sample tubes by addition of refined water. Then, added 4 ml of anthrone reagent. Heated aimed at eight minutes in a hot water bath. Cooled rapidly and read the green to dark green color at 630nm. Drawn a normal graph by scheming absorption of the standard on the X-axis versus absorbance proceeding the Y-axis from the graph calculated the quantity of carbohydrate existing in the sample tube.

### Estimation of Protein Content

Protein estimated by method as described by (Lowry *et al.,* 1951). Considered 0.5gm of the sample then grind well through a pestle, mortar in 5-10 ml of the phosphate buffer. Centrifuged and cast-off the supernatant aimed at protein approximation. Pipette obtainable 0.2, 0.4, 0.6, and 0.8 besides 1 ml of the operational standard hooked on a series of test tube. Pipette obtainable 0.1 ml and 0.2 ml of the sample extract in dualistic test tubes. A tube through 1mL of water aided as the blank. Added 5 ml of mixture C (alkaline copper solution) to each tube including the blank. Mixed well and permissible to stance for 10min. Then added 0.5 ml of reagent D (Folin-Ciocalteau Reagent) mixed well and kept at room temperature in the dark aimed at 30min. Blue color developed. Took the reading at 660nm. Drawn a standard graph using BSA and calculated the amount of protein in the sample.

### Estimation of Proline Content

Proline measured by the method given by (Bates *et al.,* 1973). Extracted 0.5g of plant material by homogenizing in 10 ml of 3% aqueous sulphosalicylic acid. Filtered the homogenate through Whattman No. 2 filter paper. Took 2 ml of remainder in a test tube and added 2 ml of glacial acetic acid and 2 ml acid ninhydrin. Heated it in the boiling water bath for 1h. Terminated the reaction by retaining the tube in ice bath. Added 4 ml toluene to the reaction mixture and stirred well for 20-30sec

separated the toluene layer and warmed to room temperature. Measured the red color intensity at 520nm. Ran a series of standards with pure proline in a similar way and prepared a standard curve.

**Determination of Sodium and Potassium**

Potassium and sodium in the acid-digest of plant sample (leaf) was determined using Flame photometer. Weighed 500 mg dried plant sample in 100 ml conical flask. Additional 10 ml of conc. $HNO_3$ placed funnel on the flask and kept for about 6-8 hrs or overnight at a covered place for pre-digestion. After pre-digestion when the solid sample was no more visible, additional 10 ml of conc. $HNO_3$ and 2-3 ml $HClO_4$. Kept on a hot plate in acid proof chamber having fume exhaust system, heated at about 100°C for first 1 hr and then raised the temperature to 200°C. Continued digestion until the contents became colorless and only white dense fumes appeared. Reduced the acid contents to about 2-3 ml by continuing heating at the same temperature. Filtered through Whattman No. 42 filter paper. Gave 3-4 washings of 10-15 ml portions of refined water then made the total volume 100 ml. Measured $Na^+$ and $K^+$ concentrations in the remainder by using Flame photometer. Recorded the flame photometer readings aimed at each of the operational standards of Na and K subsequently adjusting blank to zero. Drawn a standard curve by scheming the readings against Na and K readings.

**Determination of Alkaloid**

Adopted the method given by (Omoruyi *et al.,* 2012). 5 g of plant extract mixed with 200 mL of 10% acetic acid in ethanol. The mixture covered then permissible toward stand for 4 h. This mixture filtered than the remainder stood concentrated on a hot water bath to a quarter of its original volume. Rigorous ammonium hydroxide added in droplets to the extract until precipitation (cloudy fume) accomplished. The solution remained permissible to settle, washed through diluted ammonium hydroxide then filtered. The residue collected was dried and weighed then the alkaloid content calculated by means of the equation:

% Alkaloid = Weight of precipitate/Weight of original sample × 100

**Determination of Saponins**

Saponin content estimated as method described by (Obadoni and Ochuko, 2001). 5 g of the crushed plant sample added to 50 mL of 20% ethanol, retained on a shaker aimed at 30 min and then heated in a water bath on 55$^0$C for 4 h. The subsequent mixture filtered and then remainder re-extracted through additional 200 mL of 20% aqueous ethanol. The remainders were collective and condensed to 40 mL in a boiling water bath at 90$^0$C. The concentrate shifted into a splitting funnel, 20 mL of diethyl ether added and then shaken enthusiastically. The ether film, which was the upper film, discarded and then the aqueous (bottom) layer retained in a beaker. The retained layer re-introduced into a splitting funnel and 60 mL of n-butanol added then shaken enthusiastically. The butanol extract, which is the upper layer, reserved although the bottom layer thrown away. The butanol layer was wash away twice with 10 mL of 5% aqueous sodium chloride. The residual solution collected and heated to evaporation in a boiling water bath, formerly dehydrated to constant weight at 40°C in an oven. The saponin content remained calculated by means of the equation:

% Saponin content = Weight of residue/Weight of original sample× 100

**Determination of 2, 2-azinobis (3-ethylbenzthiazoline) -6-sulfonic acid (Assay)**

The 2, 2-azinobis (3-ethylbenzthiazoline)-6-sulfonic acid (ABTS) free radicals scavenging assay was used to define the antioxidant potential of extracts (Re *et al.,* 1999). Solutions in 100 ml of methanol were prepared for ABTS (7 mM) besides potassium per sulphate (2.45 mM) these two solutions thoroughly mixed aimed at the creation of free radicals and kept in the dark overnight. Around 3 ml of this stock solution was taken and its absorbance at 745 nm was set to 0.76 (control solution). Approximately 200 µl of the test sample mixed through three ml of ABTS solution then incubated at 25°C for 15 min. Absorbance measured using a spectrophotometer with a double beam of 745 nm. The similar way followed for the preparation of various ascorbic acid dilution (positive control). The data collected in triplicates, and the formula used to measure the percentage of ABTS free radicals scavenging activity:

% Inhibition = (Ac-As/Ac) x 100

**Determination of 2, 2-diphenyl-1-picrylhydrazyl (Assay)**

The method given by (Barros *et al.,* 2007) calculated the radical scavenging behavior of the extract DPPH. Elucidation was prepared by dissolving DPPH in methanol. 3ml was taken from this solution, and its absorbance at 515 nm (control solution) was set to 0.75.To prevent free radicals, the DPPH stock solution was coated with aluminum foil and kept in the shady for 24 hours. 5 mg of separately extract was liquefied in 5 ml methanol aimed at the preparation of stock solutions. Approximately 200µl of test sample mixed by 2 ml DPPH

then incubated aimed at 15 min in dark. Ascorbic acid used as a typical antioxidant compound in all the assays for comparative analysis. The percentage inhibition of DPPH free radical by extracts remained calculated by means of the following formula:

% Inhibition = (Ac-As/Ac) x 100

Where (Ac) remains the absorbance of control and (As) absorbance of extract/standard.

At the end of experiment, data subjected to analysis of variance (ANOVA) and mean separation. The statistical analysis done using Graph Pad Prism®5.2. The least significance difference (LSD) at 5% level used to compare the means of different test parameters. Data are mean ± SD of three replicates (n=3) remained examined using graph pad prism 5.2 by Two way Anova followed through Bonferroni multiple comparison post-test $P<0.05$*, $P<0.01$**, $P<0.001$*** significance level.

## Results

### Carbohydrate

**Table 1:** Effect of mannitol and water stress on carbohydrate content (mg/g) of *Lepidium sativum* L. Data are mean ± SD, of three replicates (n=3) were analyzed using graph pad prism 5.2 by Two way Anova followed by Bonferroni multiple comparison post – test $P<0.05$*, $P<0.01$**, $P<0.001$*** significance level. Different lower case letters in a table indicate significant difference between control and treatments.

| Treatments | 35 Days | 75 Days | 110 Days |
|---|---|---|---|
| Control | 4.328±0.570a | 6.110±0.321b,a | 8.258±0.440c,a |
| Control Mannitol (50) µg/l | 5.420±0.275a | 7.121±0.401b,a | 9.157±0.248c,a |
| Mannitol 50 µg/l and -0.01 $\Psi_w MPa$ | 5.086±0.358a | 7.080±0.342b,a | 9.114±0.205c,a |
| Mannitol 50 µg/l and -0.02 $\Psi_w MPa$ | 4.853±0.358a | 7.033±0.489b,a | 9.078±0.470c,a |
| Mannitol 50 µg/l and -0.03 $\Psi_w MPa$ | 4.486±0.326a | 6.970±0.252b,a | 9.010±0.404c,a |
| Control Mannitol (100) µg/l | 5.625±0.327a | 7.258±0.561b,a | 9.390±0.598c,a |
| Mannitol 100 µg/l and -0.01 $\Psi_w MPa$ | 5.291±0.352a | 7.211±0.518b,a | 9.347±0.673c,a |
| Mannitol 100 µg/l and -0.02 $\Psi_w MPa$ | 4.958±0.781a | 7.171±0.684b,a | 9.288±0.591c,a |
| Mannitol 100 µg/l and -0.03 $\Psi_w MPa$ | 4.625±0.530a | 7.071±0.565b,a | 9.217±0.417c,a |
| Control Mannitol (250) µg/l | 6.291±0.352a | 7.265±0.543b | 9.431±0.358c,a |
| Mannitol 250 µg/l and -0.01 $\Psi_w MPa$ | 5.958±0.228a | 7.175±0.575b,a | 9.321±0.714c,a |
| Mannitol 250 µg/l and -0.02 $\Psi_w MPa$ | 5.625±0.450a | 7.132±0.465b,a | 9.284±0.520c,a |
| Mannitol 250 µg/l and -0.03 $\Psi_w MPa$ | 5.291±0.352a | 6.986±0.671b,a | 9.251±0.602c,a |

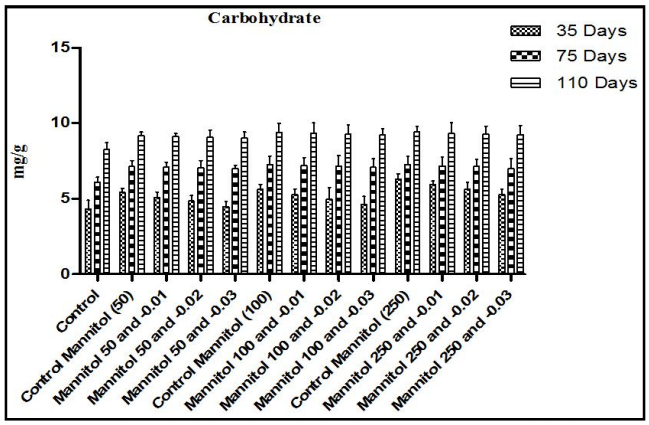


**Fig. 1:** Effect of mannitol and water stress on carbohydrate content.

The effects of mannitol and water stress treatments on carbohydrate content of *Lepidium sativum* L. illustrated in Fig. 1 and table 1. The carbohydrate content enhanced in concentration dependent manner. While mannitol 50, 100 and 250µg/l applied with water potential -0.01, -0.02 and -0.03 $\Psi_w MPa$ the carbohydrate content is significantly increased as compare to their respective control at 35, 75 and 110 days.

### Protein

The effects of mannitol and water stress treatments on protein content of *Lepidium sativum* L. illustrated in Fig. 2 and table 2. The protein content enhanced in concentration dependent manner. While mannitol 50, 100, and 250µg/l applied with water potential -0.01, -0.02 and -0.03 $\Psi_w MPa$ the protein content is significantly increased as compare to their respective control at 35, 75 and 110 days.

### Proline

The effects of mannitol and water stress treatments on proline content of *Lepidium sativum* L. illustrated in Fig. 3. and table 3. The proline content enhanced in concentration dependent manner. While mannitol 50, 100, and 250µg/l applied with water potential -0.01, -0.02 and -0.03 $\Psi_w MPa$ the proline content is significantly increased as compare to their respective control at 35, 75 and 110

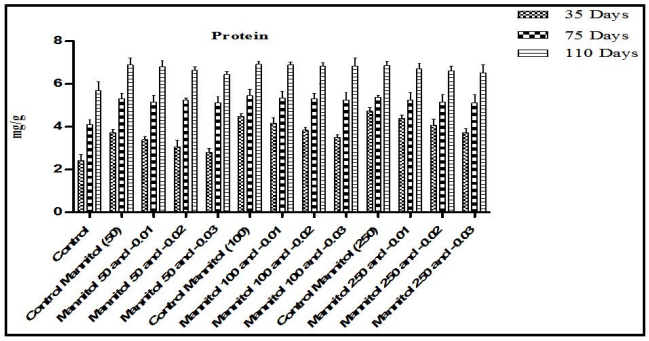


**Fig. 2:** Effect of mannitol and water stress on protein content.

days.

**Sodium**

The effects of mannitol and water stress treatments on sodium content of *Lepidium sativum* L. illustrated in Fig. 4. and table 4. The sodium content enhanced in concentration dependent manner. While mannitol 50, 100, and 250µg/l applied with water potential -0.01, -0.02 and -0.03 $\Psi_w MPa$ the sodium content is significantly increased as compare to their respective control at 35, 75 and 110 days.

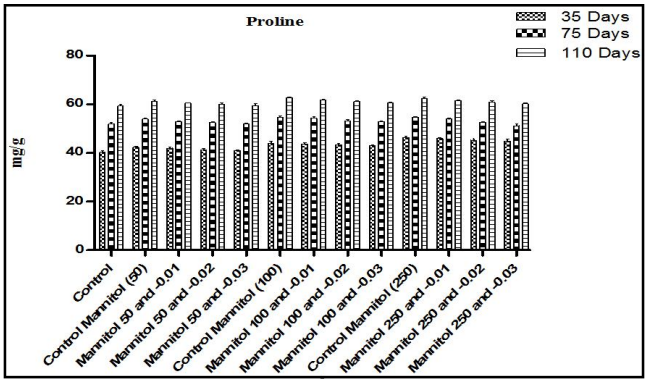


**Fig. 3:** Effect of mannitol and water stress on proline content.

**Table 2:** Effect of mannitol and water stress on protein content (mg/g) of *Lepidium sativum* L. Data are mean ± SD, of three replicates (n=3) were analyzed using graph pad prism 5.2 by Two way Anova followed by Bonferroni multiple comparison post – test P<0.05*, P<0.01**, P<0.001*** significance level. Different lower case letters in a table indicate significant difference between control and treatment.

| Treatments | 35 Days | 75 Days | 110 Days |
|---|---|---|---|
| Control | 2.431±0.470a | 4.115±0.386b,a | 5.705±0.715c,a |
| Control Mannitol (50) µg/l | 3.733±0.278a | 5.319±0.445b,a | 6.895±0.545c,a |
| Mannitol 50 µg/l and -0.01 $\Psi_w MPa$ | 3.400±0.251a | 5.161±0.549b,a | 6.811±0.512c,a |
| Mannitol 50 µg/l and -0.02 $\Psi_w MPa$ | 3.066±0.520a | 5.236±0.181b,a | 6.645±0.275c,a |
| Mannitol 50 µg/l and -0.03 $\Psi_w MPa$ | 2.800±0.345a | 5.120±0.475b,a | 6.440±0.262c,a |
| Control Mannitol (100) µg/l | 4.501±0.211a | 5.460±0.515b | 6.923±0.215c,a |
| Mannitol 100 µg/l and -0.01 $\Psi_w MPa$ | 4.168±0.425a | 5.352±0.521b,a | 6.889±0.248c,a |
| Mannitol 100 µg/l and -0.02 $\Psi_w MPa$ | 3.835±0.219a | 5.310±0.447b,a | 6.852±0.282c,a |
| Mannitol 100 µg/l and -0.03 $\Psi_w MPa$ | 3.501±0.211a | 5.266±0.557b,a | 6.836±0.656c,a |
| Control Mannitol (250) µg/l | 4.735±0.286a | 5.382±0.135b | 6.861±0.354c,a |
| Mannitol 250 µg/l and -0.01 $\Psi_w MPa$ | 4.401±0.249a | 5.241±0.610b | 6.701±0.439c,a |
| Mannitol 250 µg/l and -0.02 $\Psi_w MPa$ | 4.068±0.514a | 5.164±0.582b,a | 6.621±0.364c,a |
| Mannitol 250 µg/l and -0.03 $\Psi_w MPa$ | 3.735±0.286a | 5.115±0.663b,a | 6.518±0.661c,a |

**Potassium**

The effects of mannitol and water stress treatments on Potassium content of *Lepidium sativum* L. illustrated in Fig. 5. and table 5. The potassium content enhanced in concentration dependent manner. While mannitol 50, 100, and 250µg/l applied with water potential -0.01, -0.02 and -0.03 $\Psi_w MPa$ the potassium content is significantly

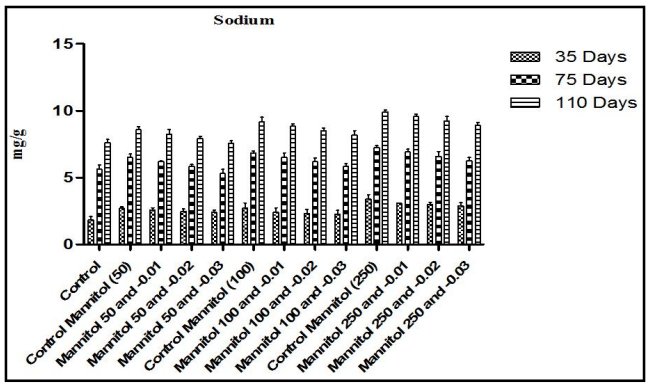


**Fig. 4:** Effect of mannitol and water stress on sodium content.

**Table 3:** Effect of mannitol and water stress on proline content (mg/g) of *Lepidium sativum* L. Data are mean ± SD, of three replicates (n=3) were analyzed using graph pad prism 5.2 by Two way Anova followed by Bonferroni multiple comparison post – test P<0.05*, P<0.01**, P<0.001*** significance level. Different lower case letters in a table indicate significant difference between control and treatments.

| Treatments | 35 Days | 75 Days | 110 Days |
|---|---|---|---|
| Control | 40.226±0.651a | 51.836±0.547b,a | 59.256±0.850c,a |
| Control Mannitol (50) µg/l | 42.256±0.458a | 53.916±0.345b,a | 61.106±0.553c,a |
| Mannitol 50 µg/l and -0.01 $\Psi_w MPa$ | 41.922±0.384a | 52.913±0.301b,a | 60.473±0.243c,a |
| Mannitol 50 µg/l and -0.02 $\Psi_w MPa$ | 41.256±0.552a | 52.484±0.362b,a | 60.110±0.528c,a |
| Mannitol 50 µg/l and -0.03 $\Psi_w MPa$ | 40.922±0.340a | 51.916±0.345b,a | 59.473±0.793c,a |
| Control Mannitol (100) µg/l | 43.922±0.823a | 54.593±0.667b,a | 62.696±0.283c,a |
| Mannitol 100 µg/l and -0.01 $\Psi_w MPa$ | 43.589±0.615a | 54.220±0.629b,a | 61.796±0.376c,a |
| Mannitol 100 µg/l and -0.02 $\Psi_w MPa$ | 43.256±0.548a | 53.230±0.560b,a | 61.096±0.364c,a |
| Mannitol 100 µg/l and -0.03 $\Psi_w MPa$ | 42.922±0.282a | 52.906±0.353b,a | 60.556±0.336c,a |
| Control Mannitol (250) µg/l | 46.256±0.548a | 54.586±0.323b,a | 62.363±0.708c,a |
| Mannitol 250 µg/l and -0.01 $\Psi_w MPa$ | 45.922±0.282a | 53.913±0.353b,a | 61.466±0.273c,a |
| Mannitol 250 µg/l and -0.02 $\Psi_w MPa$ | 45.256±0.606a | 52.580±0.316b,a | 60.823±0.664c,a |
| Mannitol 250 µg/l and -0.03 $\Psi_w MPa$ | 44.922±0.866a | 51.216±0.577b,a | 60.156±0.410c,a |

increased as compare to their respective control at 35, 75 and 110 days.

## Alkaloid

The effects of mannitol and water stress treatments on alkaloid content of *Lepidium sativum* L. illustrated in Fig. 6. and table 6. The alkaloid content enhanced in concentration dependent manner. While mannitol 50, 100, and 250µg/l applied with water potential -0.01, -0.02 and -0.03 $\Psi_w MPa$ the alkaloid content is significantly increased as compare to their respective control at 35, 75 and 110 days.

## Saponin

The effects of mannitol and water stress treatments on saponin content of *Lepidium sativum* L. illustrated in Fig. 7. and table 7. The saponin content enhanced in concentration dependent manner. While mannitol 50, 100,

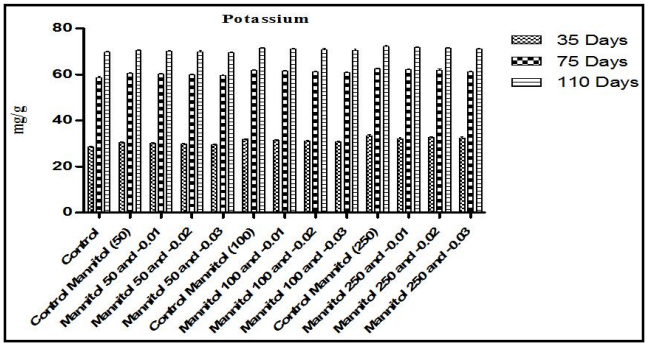


**Fig. 5:** Effect of mannitol and water stress on potassium content.

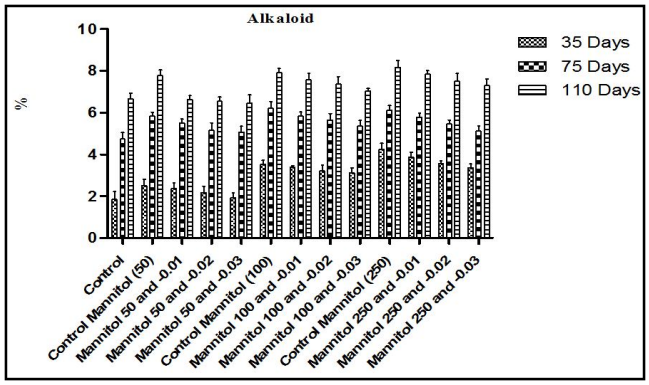


**Fig. 6:** Effect of mannitol and water stress on alkaloids content.

**Table 4:** Effect of mannitol and water stress on sodium content (mg/g) of *Lepidium sativum* L. Data are mean ± SD, of three replicates (n=3) were analyzed using graph pad prism 5.2 by Two way Anova followed by Bonferroni multiple comparison post – test P<0.05*, P<0.01**, P<0.001*** significance level. Different lower case letters in a table indicate significant difference between control and treatments.

| Treatments | 35 Days | 75 Days | 110 Days |
|---|---|---|---|
| Control | 1.842±0.410a | 5.625±0.546b,a | 7.620±0.413c,a |
| Control Mannitol (50) µg/l | 2.702±0.209a | 6.513±0.436b,a | 8.580±0.387c,a |
| Mannitol 50 µg/l and -0.01 $\Psi_w MPa$ | 2.569±0.290a | 6.179±0.117b,a | 8.246±0.623c,a |
| Mannitol 50 µg/l and -0.02 $\Psi_w MPa$ | 2.469±0.325a | 5.846±0.242b,a | 7.913±0.279c,a |
| Mannitol 50 µg/l and -0.03 $\Psi_w MPa$ | 2.402±0.258a | 5.313±0.525b,a | 7.580±0.311c,a |
| Control Mannitol (100) µg/l | 2.729±0.622a | 6.846±0.242b,a | 9.180±0.575c,a |
| Mannitol 100 µg/l and -0.01 $\Psi_w MPa$ | 2.396±0.571a | 6.513±0.571b,a | 8.846±0.325c,a |
| Mannitol 100 µg/l and -0.02 $\Psi_w MPa$ | 2.329±0.510a | 6.179±0.523b,a | 8.513±0.320c,a |
| Mannitol 100 µg/l and -0.03 $\Psi_w MPa$ | 2.263±0.508a | 5.846±0.348b,a | 8.180±0.565c,a |
| Control Mannitol (250) µg/l | 3.396±0.576a | 7.246±0.245b,a | 9.913±0.279c,a |
| Mannitol 250 µg/l and -0.01 $\Psi_w MPa$ | 3.063±0.313a | 6.913±0.357b,a | 9.580±0.308c,a |
| Mannitol 250 µg/l and -0.02 $\Psi_w MPa$ | 2.996±0.271a | 6.579±0.646b,a | 9.246±0.578c,a |
| Mannitol 250 µg/l and -0.03 $\Psi_w MPa$ | 2.896±0.371a | 6.246±0.437b,a | 8.913±0.361c,a |

**Table 5:** Effect of mannitol and water stress on potassium content (mg/g) of *Lepidium sativum* L. Data are mean ± SD, of three replicates (n=3) were analyzed using graph pad prism 5.2 by Two way Anova followed by Bonferroni multiple comparison post – test P<0.05*, P<0.01**, P<0.001*** significance level. Different lower case letters in a table indicate significant difference between control and treatments.

| Treatments | 35 Days | 75 Days | 110 Days |
|---|---|---|---|
| Control | 28.521±0.514a | 58.647±0.653b,a | 69.695±0.574c,a |
| Control Mannitol (50) µg/l | 30.480±0.333a | 60.537±0.696b,a | 70.495±0.342c,a |
| Mannitol 50 µg/l and -0.01 $\Psi_w MPa$ | 30.147±0.587a | 60.203±0.591b,a | 70.161±0.587c,a |
| Mannitol 50 µg/l and -0.02 $\Psi_w MPa$ | 29.814±0.334a | 59.870±0.660b,a | 69.828±0.891c,a |
| Mannitol 50 µg/l and -0.03 $\Psi_w MPa$ | 29.480±0.667a | 59.537±0.328b,a | 69.468±0.661c,a |
| Control Mannitol (100) µg/l | 31.814±0.334a | 61.870±0.297b,a | 71.461±0.360c,a |
| Mannitol 100 µg/l and -0.01 $\Psi_w MPa$ | 31.480±0.350a | 61.537±0.305b,a | 71.128±0.616c,a |
| Mannitol 100 µg/l and -0.02 $\Psi_w MPa$ | 31.147±0.596a | 61.203±0.565b,a | 70.795±0.923c,a |
| Mannitol 100 µg/l and -0.03 $\Psi_w MPa$ | 30.814±0.650a | 60.870±0.342b,a | 70.461±0.708c,a |
| Control Mannitol (250) µg/l | 33.480±0.888a | 62.537±0.328b,a | 72.161±0.571c,a |
| Mannitol 250 µg/l and -0.01 $\Psi_w MPa$ | 32.147±0.587a | 62.203±0.553b,a | 71.828±0.324c,a |
| Mannitol 250 µg/l and -0.02 $\Psi_w MPa$ | 32.814±0.350a | 61.870±0.852b,a | 71.495±0.325c,a |
| Mannitol 250 µg/l and -0.03 $\Psi_w MPa$ | 32.480±0.683a | 61.203±0.539b,a | 71.161±0.581c,a |

and 250µg/l applied with water potential -0.01, -0.02 and -0.03 $\Psi_w MPa$ the saponin content is significantly increased as compare to their respective control at 35, 75 and 110 days.

## ABTS

The effects of mannitol and water stress treatments on antioxidant activity by ABTS assay of *Lepidium sativum* L. illustrated in Fig. 8. and table 8. The antioxidant activity enhanced in concentration dependent manner. While mannitol 50, 100 and 250µg/l applied with water potential -0.01, -0.02 and -0.03 $\Psi_w MPa$ the antioxidant activity is significantly increased as compare to their respective control at 35, 75 and 110 days.

## DPPH

The effects of mannitol and water stress treatments on antioxidant activity by DPPH assay of *Lepidium sativum* L. illustrated in Fig. 9. and table 9. The

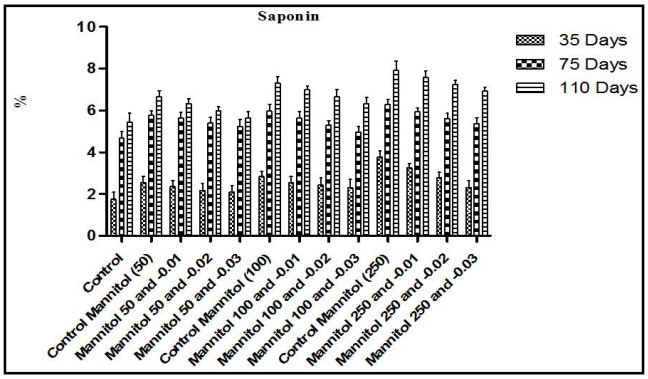


**Fig. 7:** Effect of mannitol and water stress on saponin content.

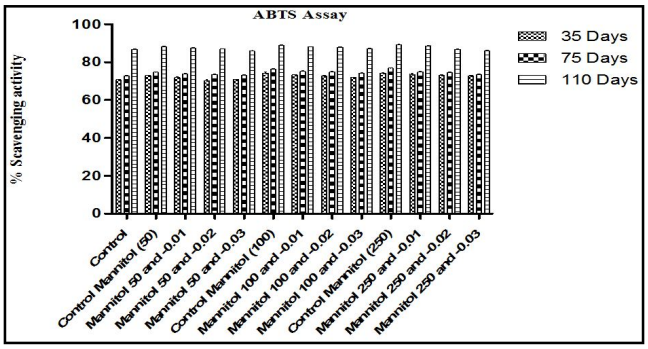


**Fig. 8:** Effect of mannitol and water stress on antioxidant ABTS assay.

**Table 6:** Effect of mannitol and water stress on alkaloid content (%) of *Lepidium sativum* L. Data are mean ± SD, of three replicates (n=3) were analyzed using graph pad prism 5.2 by Two way Anova followed by Bonferroni multiple comparison post – test P<0.05*, P<0.01**, P<0.001*** significance level. Different lower case letters in a table indicate significant difference between control and treatments.

| Treatments | 35 Days | 75 Days | 110 Days |
|---|---|---|---|
| Control | 1.842±0.650a | 4.734±0.527b,a | 6.667±0.461c,a |
| Control Mannitol (50) µg/l | 2.500±0.556a | 5.833±0.317b,a | 7.763±0.485c,a |
| Mannitol 50 µg/l and -0.01 $\Psi_w MPa$ | 2.366±0.484a | 5.493±0.344b,a | 6.633±0.305c,a |
| Mannitol 50 µg/l and -0.02 $\Psi_w MPa$ | 2.166±0.536a | 5.146±0.626b,a | 6.533±0.365c,a |
| Mannitol 50 µg/l and -0.03 $\Psi_w MPa$ | 1.933±0.392a | 5.066±0.488b,a | 6.466±0.660c,a |
| Control Mannitol (100) µg/l | 3.533±0.317a | 6.20±0.563b,a | 7.896±0.381c,a |
| Mannitol 100 µg/l and -0.01 $\Psi_w MPa$ | 3.400±0.115a | 5.823±0.354b,a | 7.563±0.548c,a |
| Mannitol 100 µg/l and -0.02 $\Psi_w MPa$ | 3.200±0.519a | 5.650±0.526b,a | 7.363±0.621c,a |
| Mannitol 100 µg/l and -0.03 $\Psi_w MPa$ | 3.113±0.420a | 5.350±0.482b,a | 7.030±0.216c,a |
| Control Mannitol (250) µg/l | 4.233±0.520a | 6.123±0.365b,a | 8.163±0.584c,a |
| Mannitol 250 µg/l and -0.01 $\Psi_w MPa$ | 3.866±0.402a | 5.786±0.333b,a | 7.830±0.320c,a |
| Mannitol 250 µg/l and -0.02 $\Psi_w MPa$ | 3.566±0.233a | 5.450±0.335b,a | 7.496±0.654c,a |
| Mannitol 250 µg/l and -0.03 $\Psi_w MPa$ | 3.366±0.317a | 5.110±0.452b,a | 7.296±0.557c,a |

**Table 7:** Effect of mannitol and water stress on saponin content (%) of *Lepidium sativum* L. Data are mean ± SD, of three replicates (n=3) were analyzed using graph pad prism 5.2 by Two way Anova followed by Bonferroni multiple comparison post – test P<0.05*, P<0.01**, P<0.001*** significance level. Different lower case letters in a table indicate significant difference between control and treatments.

| Treatments | 35 Days | 75 Days | 110 Days |
|---|---|---|---|
| Control | 1.754±0.568a | 4.667±0.580b,a | 5.440±0.735c,a |
| Control Mannitol (50) µg/l | 2.550±0.498a | 5.766±0.384b,a | 6.643±0.509c,a |
| Mannitol 50 µg/l and -0.01 $\Psi_w MPa$ | 2.350±0.524a | 5.633±0.491b,a | 6.310±0.428c,a |
| Mannitol 50 µg/l and -0.02 $\Psi_w MPa$ | 2.176±0.598a | 5.400±0.452b,a | 5.976±0.331c,a |
| Mannitol 50 µg/l and -0.03 $\Psi_w MPa$ | 2.113±0.512a | 5.233±0.578b,a | 5.643±0.509c,a |
| Control Mannitol (100) µg/l | 2.833±0.425a | 5.966±0.572b,a | 7.310±0.528c,a |
| Mannitol 100 µg/l and -0.01 $\Psi_w MPa$ | 2.533±0.504a | 5.633±0.554b,a | 6.976±0.331c,a |
| Mannitol 100 µg/l and -0.02 $\Psi_w MPa$ | 2.420±0.615a | 5.300±0.360b,a | 6.643±0.614c,a |
| Mannitol 100 µg/l and -0.03 $\Psi_w MPa$ | 2.296±0.707a | 4.966±0.472b,a | 6.310±0.528c,a |
| Control Mannitol (250) µg/l | 3.766±0.533a | 6.266±0.448b,a | 7.910±0.780c,a |
| Mannitol 250 µg/l and -0.01 $\Psi_w MPa$ | 3.264±0.328a | 5.933±0.317b,a | 7.576±0.541c,a |
| Mannitol 250 µg/l and -0.02 $\Psi_w MPa$ | 2.766±0.488a | 5.600±0.452b,a | 7.243±0.326c,a |
| Mannitol 250 µg/l and -0.03 $\Psi_w MPa$ | 2.306±0.604a | 5.366±0.491b,a | 6.910±0.349c,a |

antioxidant activity enhanced in concentration dependent manner. While mannitol 50, 100 and 250µg/l applied with water potential -0.01, -0.02 and -0.03 $\Psi_w MPa$ the antioxidant activity is significantly increased as compare to their respective control at 35, 75 and 110 days.

## Discussion

Tolerance to abiotic stresses is very complex at the whole plant and cellular levels (Foolad *et al.,* 2003; Ashraf and Harris, 2004). This is in part due to the complication of interactions between stress factors and numerous molecular, biochemical and physiological phenomena affecting plant growth and development (Zhu, 2002). Currently, there are no economically viable technological means to facilitate crop production under stress environments. However, development of crop plants tolerant to environmental stresses reflected an auspicious approach, which may help satisfy growing food demands of the developing and under-developed countries. Development of crop plants with stress tolerance, however, requires, amongst knowledge of the physiological mechanisms and genetic controls of the subsidizing traits at different plant developmental stages. In the past 2 decades, biotechnology research has providing considerable insights into the mechanism of abiotic stress tolerance in plants at the molecular level (Hasegawa *et al.,* 2000; Zhu, 2001; Prabhavathi *et al.,* 2002; Rontein *et al.,* 2002). For example, though stress forbearance mechanisms may vary from species to species and at different developmental stages (Foolad and Lin, 2001);

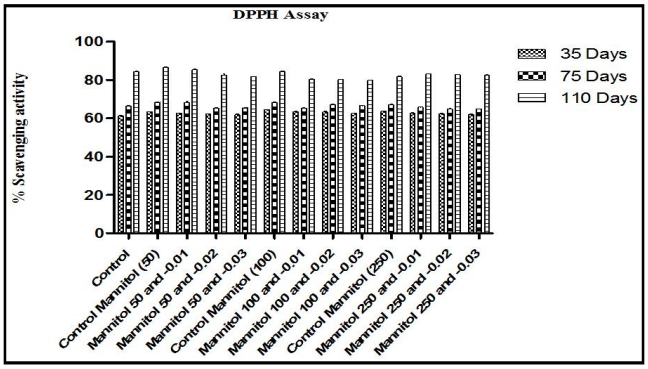


**Fig. 9:** Effect of mannitol and water stress on antioxidant DPPH assay.

**Table 8:** Effect of mannitol and water stress on antioxidant activity by ABTS assay of *Lepidium sativum* L. Data are mean ± SD, of three replicates (n=3) were analyzed using graph pad prism 5.2 by Two way Anova followed by Bonferroni multiple comparison post – test P<0.05*, P<0.01**, P<0.001*** significance level. Different lower case letters in a table indicate significant difference between control and treatments.

| Treatments | 35 Days | 75 Days | 110 Days |
|---|---|---|---|
| Control | 70.720±0.642a | 72.843±0.550b,a | 86.780±0.471c,a |
| Control Mannitol (50) µg/l | 72.926±0.353a | 74.613±0.353b,a | 88.286±0.375c,a |
| Mannitol 50 µg/l and -0.01 $\Psi_w MPa$ | 71.823±0.609a | 73.736±0.407b,a | 87.496±0.585c,a |
| Mannitol 50 µg/l and -0.02 $\Psi_w MPa$ | 70.220±0.925a | 73.413±0.351b,a | 86.830±0.656c,a |
| Mannitol 50 µg/l and -0.03 $\Psi_w MPa$ | 70.820±0.215a | 73.156±0.361b,a | 85.820±0.496c,a |
| Control Mannitol (100) µg/l | 74.180±0.860a | 76.366±0.683b,a | 88.910±0.432c,a |
| Mannitol 100 µg/l and -0.01 $\Psi_w MPa$ | 73.303±0.260a | 75.180±0.393b,a | 88.120±0.285c,a |
| Mannitol 100 µg/l and -0.02 $\Psi_w MPa$ | 72.596±0.663a | 74.810±0.489b,a | 87.886±0.425c,a |
| Mannitol 100 µg/l and -0.03 $\Psi_w MPa$ | 71.896±0.303a | 73.996±0.532b,a | 87.106±0.440c,a |
| Control Mannitol (250) µg/l | 74.133±0.303a | 76.836±0.548b,a | 89.076±0.410c,a |
| Mannitol 250 µg/l and -0.01 $\Psi_w MPa$ | 73.563±0.719a | 74.840±0.433b,a | 88.613±0.614c,a |
| Mannitol 250 µg/l and -0.02 $\Psi_w MPa$ | 73.103±0.470a | 74.510±0.482b,a | 86.680±0.475c,a |
| Mannitol 250 µg/l and -0.03 $\Psi_w MPa$ | 72.780±0.409a | 73.363±0.441b | 85.946±0.427c,a |

**Table 9:** Effect of mannitol and water stress on antioxidant activity by DPPH assay of *Lepidium sativum* L. Data are mean ± SD, of three replicates (n=3) were analyzed using graph pad prism 5.2 by Two way Anova followed by Bonferroni multiple comparison post – test P<0.05*, P<0.01**, P<0.001*** significance level. Different lower case letters in a table indicate significant difference between control and treatments.

| Treatments | 35 Days | 75 Days | 110 Days |
|---|---|---|---|
| Control | 61.239±0.450a | 66.450±0.473b,a | 84.514±0.584c,a |
| Control Mannitol (50) µg/l | 63.433±0.152a | 68.354±0.653b,a | 86.600±0.668c,a |
| Mannitol 50 µg/l and -0.01 $\Psi_w MPa$ | 62.660±0.115a | 68.406±0.733b,a | 85.451±0.550c,a |
| Mannitol 50 µg/l and -0.02 $\Psi_w MPa$ | 62.223±0.410a | 65.392±0.594b,a | 82.694±0.631c,a |
| Mannitol 50 µg/l and -0.03 $\Psi_w MPa$ | 61.880±0.814a | 65.432±0.330b,a | 81.716±0.403c,a |
| Control Mannitol (100) µg/l | 64.630±0.138a | 68.210±0.366b,a | 84.465±0.462c,a |
| Mannitol 100 µg/l and -0.01 $\Psi_w MPa$ | 63.630±0.604a | 65.303±0.366b,a | 80.441±0.552c,a |
| Mannitol 100 µg/l and -0.02 $\Psi_w MPa$ | 63.310±0.672a | 67.173±0.493b,a | 80.221±0.459c,a |
| Mannitol 100 µg/l and -0.03 $\Psi_w MPa$ | 62.693±0.295a | 66.590±0.268b,a | 79.826±0.313c,a |
| Control Mannitol (250) µg/l | 63.660±0.212a | 67.250±0.719b,a | 81.771±0.586c,a |
| Mannitol 250 µg/l and -0.01 $\Psi_w MPa$ | 62.690±0.434a | 65.906±0.276b,a | 83.126±0.338c,a |
| Mannitol 250 µg/l and -0.02 $\Psi_w MPa$ | 62.326±0.767a | 64.813±0.585b,a | 82.793±0.288c,a |
| Mannitol 250 µg/l and -0.03 $\Psi_w MPa$ | 61.986±0.716a | 64.930±0.233b,a | 82.593±0.336c,a |

basic cellular comebacks to abiotic stresses conserved among most plant species (Zhu, 2002). During the process of adaptation to a water-deficit condition, the plant experiences osmotic stress due to production of toxic reactive oxygen species (ROS), which affects the plant's homeostasis. Tolerance to water-deficit condition is a complex trait achieved by the plants through synchronized action of physiological, biochemical and molecular adaptations. The syntheses of organic osmolytes (proline, glycine betaine and soluble sugars) and enzymatic (superoxide dismutase, SOD; catalase, CAT; ascorbate peroxidase, APX; and guaiacol peroxidase, GPX) (Mittler, 2002; Jaleel *et al.,* 2009) and non-enzymatic antioxidants such as ascorbate, glutathione, tocopherols and carotenoid (Jaleel *et al.,* 2009). Measurement of such processes in comeback to water stress may deliver valuable information on the numerous approaches of the plant intended to remove or to reduce the detrimental effects of water-deficit in soil or plant tissues. Furthermore, different abiotic stress factors may provoke osmotic stress, oxidative stress and protein denaturation in plants, which lead to similar cellular adaptive comebacks such as accumulation of compatible solutes, induction of stress proteins, and acceleration of reactive oxygen species scavenging systems (Zhu, 2002). One of the utmost common stress responses in plants is overproduction of different types of compatible organic solutes (Serraj and Sinclair, 2002). Compatible solutes are low molecular weight, extremely soluble compounds that are frequently nontoxic at high cellular concentrations. Generally, they defend plants from stress through different courses, including contribution to cellular osmotic adjustment, detoxification of reactive oxygen species, protection of membrane integrity, and stabilization of enzymes/proteins (Yancey *et al.,* 1982; Bohnert and Jensen, 1996). Furthermore, because some of these solutes also defend cellular components from dehydration damage, they frequently referred to as osmoprotectants. These solutes include proline, sucrose, polyols, mannitol, trehalose and quaternary ammonium compounds (QACs) such as glycine betaine, alanine betaine, proline betaine, choline O-sulfate, hydroxyl proline betaine, and pipecolate betaine (Rhodes and Hanson, 1993). Alternatively, in some plants augmented resistance to abiotic stresses has accomplished by exogenous application of various organic solutes this approach, which may significantly subsidize to increased crop production in stress environments. Mannitol, an important osmolyte, normally synthesized in large amount in many plant species (Su *et al.,* 1999; Mitoi *et al.,* 2009). Its proportion is about 50% of the total translocated photo assimilates (Loester *et al.,* 1992). Although mannitol plays an important role in osmotic adjustment, it acts as an antioxidant to scavenge of hydroxyl radicals (OH-) (Shen *et al.,* 1997; Srivastava *et al.,* 2010). However, there is little information available in the literature on the role of mannitol in stress forbearance in plants of agronomic importance. Mannitol found more effective in improving the plants growth, development and stabilization of membrane integrity (Ashraf & Ali, 2008). However, in a number of plant species the fabrication of these organic compounds is not adequate to fulfill the plant requirements. In the present study, foliar-applied mannitol significantly enhanced the carbohydrate, protein, proline, sodium, potassium, alkaloid, saponin, ABTS and DPPH responses in *Lepidium sativum* L. under water stress condition as compared to their respective control. This enhancement due to foliar application of mannitol, which utilized in the leaves, where they might have acted as a source of C, as has been previous reported in different studies (Mitoi *et al.,* 2009; Anjum *et al.,* 2011).

## Conclusion

Water stress causes osmotic stress in plants, which causes reduction in growth, imbalance ion transport, and a decrease in transpiration rate and an increase in membrane permeability. Such effects consequence in less water-absorbing capacity of crop plants, and diverse plant species and genotypes within a species respond differently to adverse environmental conditions. In mandate to counteract unfavorable environmental circumstances, plants accumulate different types of organic and inorganic solutes in cytosol to decrease osmotic potential by which they can maintain cell turgor. The safety and survival of the plants depends on the coordination of these vital osmoprotectants with antioxidant enzymes. The present study summarized that the *Lepidium sativum* L. maintained physio-biochemical responses during water stress, which may be due to efficient osmotic regulation maintained through exogenous application of mannitol. Generally, application of mannitol under stress circumstances, which not only assistances in maintaining cell turgor is also involved in quenching free radicals, upholding sub-cellular structures, and shielding cellular redox potential. Exogenous solicitation of mannitol were enhanced the carbohydrate, protein, proline sodium, potassium, alkaloids, saponins as well as antioxidant, ABTS AND DPPH capacity in *Lepidium sativum* L. plant as compared to their respective control.

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