# EFFECTS OF EXOGENEOUS APPLICATION OF PUTRESCINE ON DIFFERENT BIOCHEMICAL PARAMETERS OF *Zea mays* L. UNDER SALINITY STRESS

**HUMAIRA GUL[*1], LAILA ANJUM, MAMOONA ARIF[1], LUBNA[1], MOHIB SHAH[1] AND ADAM KHAN[2]**

*[1]Department of Botany, Abdul Wali Khan University, Mardan, Pakistan.*
*[2]Dr. Moinuddin Ahmed Research laboratory of Dendrochronology and plant Ecology, Department of Botany, Federal Urdu University of Arts, Science and Technology Karachi, Pakistan*
**Corresponding author: gulhumaira@awkum.edu.pk*

خلاصہ

اس پراجیکٹ میں پیوٹرسن اور نمک کے اثرات مکئی کے مختلف کیمیائی خصوصیات ضیائی تالیفی فگمنٹ ( کلوروفل بی ،کلوروفل اے اور کروٹینائیڈ ) معیاری اور غیر میعاری شوگر ،نشاستہ ،فیلونائیڈ ،کل پروٹین ،کل انٹی اکسائیڈنٹ کا مطالعہ کیا گیا ۔ایک مکمل ترتیب ڈیزائن میں تجربہ کیا گیا جس میں نمک کے مختلف حراستی (۴۰ ملی میٹر ،۸۰ ملی میٹر ) اور مختلف پیوٹرسن (۵ ملی میٹر ،۱ ملی میٹر اور ۲ ملی میٹر ) کو سپرے کے طور پر لاگو کیا گیا ۔معیاری اور غیر معیاری شوگر ،کل پروٹین ،مختلف ضیاء تالیفی فگمنٹ اور نشاستہ میں نمک کی وجہ سے نمایا کمی واقع ہوگئی جبکہ کل انٹی اکسائیڈنٹ فینول اور فیلونائیڈ میں اضافہ ہوگیا مندرجہ ذیل عناصر میں پیٹرسن کی وجہ سے نمکین اور غیر نمکین حالت میں نمایا بہتری دیکھی گئی ۔اس سے ظاہر ہوا کہ پیوٹرسن مکئی پودے کے اوپر نمک کے اثرات کو کم کر سکتے ہیں ۔

**Abstract**

This project was design to test the impact of putrescine and salt on different biochemical attributes [photosynthetic pigments (chlorophyll b, chlorophyll a and carotenoids), reducing and non-reducing sugars, carbohydrates, total flavonoids, total proteins, total antioxidants and total phenols] of *Zea mays* L (corn). Experiment was arranged in a complete randomized design with different concentration of salt (40mM, 80mM,) and putrescine with different concentrations (0.5mM, 1mM and 2mM) was applied as a foliar spray. Reducing and non-reducing sugars, total proteins, different photosynthetic pigments and total carbohydrates showed significant decrease due to salinity while total antioxidant, phenols, and flavonoids increased with increased salinity. After the application of putrescine above mentioned parameters exhibited significant improvement under non-saline as well as saline condition. Foliar application of putrescine overcomes the harmful impacts of salt stress in *Zea mays* L.

## Introduction

Salt stress is a type of common and major abiotic stress factor which severely affected growth and productivity of crops (Munns and Tester, 2008). Salinity commonly hindering germination of seeds and severely affects plant's growth and its development (Dash and Panda, 2001), enzymatic activities (Seckin *et al.,* 2009), emergence of seedling and growth (Ashraf *et al.,* 2002). Presence of salt in soil becomes a huge problem in world's agriculture that badly disturbs crop. FAO survey (2008) reveals that in near future over 800 million hector will be affected by salt stress. The total salt effected soils in Pakistan are 11.5 million hectares. Two main reasons for salt stress are different processes induced by human or natural reason. Salinity creates ionic and osmotic stress which affects physiology of plant at entire plant level or at cellular level (Murphy and Durako, 2003). *Zea mays* (Maize) is belonging to the family Poaceae and it is the most important cereal crop that is cultivated worldwide. *Zea mays* considered as basic ingredient for animal's feed, good raw material for manufacturing products in different industries and also serve as important human nutrient. This plant also serves as good emollient for ulcer, wound, and swellings. Kidney stones and urinary tract infections also cured after corn silk use (Lans, 2006).

Polyamines are present in all living organisms except some Archaea and structurally they are amines with different aliphatic groups. In plants, spermidine, spermine and putrescine are major and important types which performed different cellular processes. Foliar application of polyamines application delays the physiological processes and fruit senescence leading to the fruit ripening. Putrescine ($C_4H_{12}N_2$) present in living cells and it cause delay in fruit ripening when endogenously present at high level (Dibble *et al.,* 1988). Polyamines generally in plants associated with regulation of different physiological processes such as organogenesis, embryogenesis, leaf senescence, floral initiation and development, plant abiotic and biotic stress response and

development of fruit and its ripening (Alcázar *et al.,* 2010; Kusano *et al.,* 2008). When plant exposes to different stress conditions, changes occur in polyamines concentrations within cells in response of that stress (Alcázar *et al.,* 2010). Present project was performed to examine the effect of exogenous application of putrescine on some biochemical parameters (total phenols, flavonoids, total chlorophyll, chlorophyll b, chlorophyll a, carotenoids, antioxidants, protein, reducing and non- reducing sugars, total carbohydrates) of *Zea mays* L. raised under different concentrations of salt.

## Material and Methods

*Zea mays L.* seeds were purchased from the local market of Mardan, Khyber Pukhtunkhuwa, Pakistan. This project was designed with total 36 pots and these pots were divided into 4 sets which irrigated with different concentrations of salt and plants were sprayed with different concentrations of putricine. Details of all sets were as follows:

Set 1: Foliar application of distilled water and irrigation of plants performed with different salt concentrations.

Set 2: Foliar application of 0.5 mM putrescine solution and irrigation of plants performed with different salt concentrations.

Set 3: Foliar application of 1 mM putrescine solution and irrigation of plants performed with different salt concentrations.

Set 4: Foliar application of 2 mM putrescine solution and irrigation of plants performed with different salt concentrations.

The size of plastic pots was 10 cm in diameter and 15 cm in depth and having basal outlet for drainage. Each set consisted of 9 out of 36 pots and 3 replicates were maintained for each treatment while three treatments were control (non-saline), ii) 40 mM, and iii) 80 mM. All pots were filled with 1KG of thoroughly washed sandy loam soil. Approximately uniform sized seeds were selected and surface sterilization was performed, for this purpose mercuric chloride was used. Immersed the seeds in 0.1% $HgCl_2$ for single minute and then rinse with distilled water two to three time. Put five seeds in each pot. Hoagland's solution was then applied to each pot and later on tap water was given daily in equal quantity. When the plantlets achieve three leaves stage the experiment was discarded and separated as one seedling per pot. Experiment was designed in completely randomized (CRD) manner in Botanical Garden of Botany Department, Abdul Wali Khan University, Mardan. Salt treatment has been started and different concentrations of salt solution and tap water in case of control was applied twice a week. Different concentrations of putrescine were applied foliarly after four weeks of germination in different sets.

***Biochemical Estimations***

At grand period of growth leaves were collected and biochemical estimations were performed as follows.

***Chlorophyll Extraction and Estimation***

Estimation of chlorophyll and carotenoids was performed by a method described by Maclachlam and Zalik (1963).

***Determination of Reducing and non-reducing sugars***

Determination of reducing sugars was performed by method described by Nelson-Somogy method (Nelson, 1944; Somogyi, 1952).

***Total Carbohydrates Determination***

The amount of total carbohydrates was analyzed using the method of Yemm and Willis (1956).

***Determination of Proteins***

The method is rapid and sensitive for the quantification of microgram quantities of proteins utilizing the principle of protein-dye binding (Bradford, 1976).

***Estimation of Total Flavonoids***

Aluminum chloride method was applied to determine the total flavonoid content of the sample (Mervet *et al.,* 2009).

***Total Phenol determination***

The amount of total phenolics was analyzed using the Folin–Ciocalteu (FC) colorimetric method described previously by Malik and Singh (1980).

***Antioxidants Status***

The ferric ion reducing power capability of samples was determined by using modified method Yen and Chen (1995).

***Statistical analysis and experimental design***

Experiment was completely randomized Design (CRD) with two concentrations of salt while putricine have three levels and experiment had three replicates. SPSS Ver. 21 was used for statistical analysis of collected data. ANOVA (analysis of variance) was applied on collected data while Duncan's multiple range test ($P < 0.05$) was done for different mean's comparison.

## Results and Discussion

### *Chlorophyll*

In plants photosynthetic pigments are good indicator for the determination of physiological status. In present investigation plants irrigated with different doses of salt showed significant (P<0.001) reduction in total chlorophyll of maize plant (Table 1). Doganlar *et al.* (2010) observed reduction in chlorophyll contents as a result of inhibition/reduction in the synthesis level of different photosynthetic pigments. Many investigators reported that during early exposure to salinity cause reduction in the process of photosynthesis after stomatal conductance reduction which results in lower $CO_2$ availability and then limitations in biochemical processes after long term exposure to salt stress (Silva *et al.,* 2011). Srivastava *et al.,* (1988) stated that for the determination of salt stress in different crops total chlorophyll content of plant serves as leading parameter. When plant exposed to salt stress chlorophyll molecules destruction occurs after production of ROS and increase of free radicles synthesis occur in chloroplast which in turn decrease photosynthesis process and growth (Dolatabadian and Saleh Jouneghani, 2009). Plant foliarly applied putricine with different doses showed significant (P<0.001) decrease in chlorophyll content of maize plant at different concentration of salt. In present study result clearly reveals an increase in chlorophyll content at high salinity after the application of putrescine. Gupta *et al.* (2003) observed in wheat plants that polyamines application increases the total chlorophyll contents. According to Besford *et al.* (1993) different molecular complexes that are present in thylakoid membrane stabilized after application of putrescine. Duan *et al.,* (2008) suggested foliar application of different polyamines on plants which results in alleviation in salt induced reduction upto certain extent in photosynthetic efficiency. They further commented that this phenomenon strongly depend on polyamine type, concentration and salt stress level.

### *Carotenoids*

Plant irrigated with different doses of salt showed a significant (P<0.001) decrease in carotenoids (Table 2). Several reports confirmed that in number of glycophytes exhibited reduction in carotenoids and chlorophyll contents of leaves after application of salt (Agastian *et al.,* 2000). Chlorophyll degradation occur by enzyme chlorophyllase and its activity increased under salinity stress which in turn reduce total carotenoids in plant (Mishra and Sharma, 1994). Plants treated with 0.5mM, 1mM and 2mM of putrescine showed non-significant decrease except 0.5mM of putrescine in which control plant showed increase.

### *Reducing and Non-Reducing Sugars*

Plants irrigated with different doses of salt (40mM, 80mM) showed significant (P <0.001) reduction in non-reducing sugars as compared to untreated plants (Table 2). Nasser (2011) observed that when wheat plant organs raised under salt stress exhibit significant decrease in reducing sugars. When wheat plant studied under NaCl stress significant reduction in reducing sugars was observed which is the result of decrease in photosynthetic efficiency which in turn retard carbohydrate biosynthesis (Patricia *et al*., 1992). Singh and Dubey (1995) stated that when plants subjected to salt stress it enhanced partial utilization of total carbohydrates of plants into other metabolic pathways. Application of different doses of putricine exhibited improvement in this parameter under stressed and non-stressed conditions. After application polyamines on plant leaves during drought stress enhancement in reducing sugar level, peroxidase activity and proline while reduction in total soluble proteins were observed (Saruhan *et al.,* 2006).

### *Carbohydrates*

Plant irrigated with different doses of salt showed significant (P<0.001) decrease in total carbohydrate as compared to untreated plant (Table 2). Mostafa (2004) observed reduction in total carbohydrates, reducing sugars and non-reducing sugars at low and moderate salt levels. Rejeskova *et al.,* (2007) worked on *Olea europaea* L. shoots under salt conditions and observed reduction in total carbohydrates due to osmotic stress and presence of sodium and chloride ions at toxic levels. Plants foliarly applied with different concentration (0.5mM ,1mM ,2mM ) of putrescine showed non-significant decrease and increase at different salinity level (40mM,80mM). Importance of polyamines in carbohydrate metabolism was proved by different investigators. It has been studied that application of different polyamines had positive and important role in synthesis and accumulation of carbohydrates in leaves (El- Bassiouny *et al.,* 2008). Exogenous application of different concentrations of polyamines on leaves causes synthesis of high carbohydrate content as compared to the untreated control. Application of polyamine under stress conditions alleviate its adverse effect after promotion in total soluble sugars which is the activity of amylase at high rate and presence of higher concentration of chlorophyll (Sood and Nagar, 2003).

### *Proteins*

Plants irrigated with different concentrations of salt (40mM, 80mM) exhibited significant (P <0.001) reduction in total proteins as compared to their control plants (Table 3). Sultana *et al.,* (2000) studied rice plant under salt stress and observed reduction in total protein of plant. Amirjani also studied the seedlings of same plant in 2010 and observed reduction in total proteins at higher salinity level (200mM). Plants foliarly applied

with different concentration of putrescine (0.5mM,1mM ,2mM) showed non-significant increase in total protein at different salinity levels. After studying *Phyllanthus amarus* it was observed that foliar application of different polyamine cause improvement in protein content of plant (Jaleel *et al.,* 2008). Foliar application of polyamines on wheat plant gave highest values of crude proteins in plants (Sood and Nagar, 2003).

***Flavonoids***

Present study showed significant (P<0.001) reduction in flavonoids in plants when treated with different concentrations of salt (Table 3). Miladinova *et al.,* (2013) observed leaves of the Paulownia clones ($TF_{01}$ and $EF_{02}$) and noticed increased in total flavonoid content with increasing salt stress. Many reports also confirmed the phenomenon in various plant species that after applying salt stress enhanced levels of anthocyanins and flavonoids were observed (Treutter, 2006). Ali and Abbas (2003) studied barley plants under salt stress and observed significant enhancement in flavonoid concentrations in roots and shoots. Plants treated with 0.5mM, 1mM, 2mM of putrescine showed significant (P<0.001) increase in this parameter in non-saline as well as in salinity treated plants.

***Phenols***

Many plants in response to environmental factors as well as genetically have variations in phenolic compound's kind, composition and its level in plants (Awika and Rooney, 2004). In the present investigation it is also observed that salinity induced significant (P<0.05) promotion in total polyphenol in leaves of *Zea mays* (Table 3). Increase in phenolic content is clear indication of secondary metabolism stimulation under salt stress and possible role of phenolic compounds against oxidative stress under salinity as defense mechanism. Agastian *et al.,* (2000) studied many plants and observed polyphenol content increase under increasing salinity levels. Navarro *et al.,* (2006) studied red peppers and observed enhancement effect on total phenolics when plants treated with moderate salinity levels. In salt tolerant plants phenols accumulation prevent cell membrane damage during stress and also provides defense mechanism for scavenging the oxygen free radicals (Singh, 2004). Present investigation results exhibited that after application of putricine on leaves showed significant (P<0.001) promotion in total phenols. Agastian *et al.* (2000) studied cowpea plants and observed that application of polyamines shoots exhibited significant promotion in total phenol content as well as IAA content. It is evident that most of the phenolic compounds are polyphenols and diphenols that are involved in IAA oxidase activity inhibition and as a result IAA accumulate in plant which reflected stimulated growth and yield of treated plants.

**Table 1. Effect of Putrescine treatment on Chlorophyll a, chlorophyll b, total chlorophyll and ab ratio of *Zea mays* grown under different salinity regimes.**

| Putrescine Treatment | NaCl Treatment | Chlorophyll a *(mg/g fr.wt)* | Chlorophyll b *(mg/g fr.wt)* | Total Chlorophyll *(mg/g fr.wt)* | a/b |
|---|---|---|---|---|---|
| **Control** | ***Control*** | 5.666 ± 1.538 b | 4.656 ± 1.705 c | 10.323 ± 0.494 c | 2.717 ± 2.066 a |
| | ***40 mM NaCl*** | 2.6 + 0.230 a | 2.833 ± 0.166 b | 5.433 ± 0.284 b | 0.924 ± 0.096 a |
| | ***80 mM NaCl*** | 1.3 ± 0.251 a | 0.666 ± 0.24 a | 1.966 ± 0.409 a | 2.783 ± 1.156 a |
| **0.5 mM** | ***Control*** | 5.351 ± 0.548 b | 4.566 ± 0.683 c | 9.918 ± 1.232 c | 1.192 ± 0.068 a |
| | ***40 mM NaCl*** | 1.751 ± 0.075 a | 1.675 ± 0.156 b | 3.427 ± 0.142 b | 1.065 ± 0.118 a |
| | ***80 mM NaCl*** | 1.766 ± 0.41 a | 1.332 ± 0.241 a | 3.098 ± 0.636 a | 1.305 ± 0.168 a |
| **1 mM** | ***Control*** | 3.033 ± 0.366 b | 3.266 ± 0.233 c | 6.3 ± 0.6 c | 0.921 ± 0.05 a |
| | ***40 mM NaCl*** | 1.266 ± 0.088 a | 1.6 ± 0.288 b | 2.866 ± 0.202 b | 0.869 ± 0.218 a |
| | ***80 mM NaCl*** | 1.303 ± 0.547 a | 1.066 ± 0.033 a | 2.37 ± 0.533 a | 1.239 ± 0.524 a |
| **2 mM** | ***Control*** | 3.55 ± 0.275 b | 3.116 ± 0.372 c | 6.666 ± 0.292 c | 1.192 ± 0.237 a |
| | ***40 mM NaCl*** | 1.32 ± 0.091 a | 1.4 ± 0.251 b | 2.72 ± 0.166 b | 1.052 ± 0.307 a |
| | ***80 mM NaCl*** | 0.858 ± 0.129 a | 0.715 ± 0.236 a | 1.573 ± 0.221 a | 1.894 ± 1.071 a |
| **$LSD_{0.05}$** | | 0.876 | 1.01 | 0.796 | 0.315 |
| **Probability Level** | ***Salinity*** | P<0.0001 | P<0.0001 | P<0.0001 | Non-Significant |
| | ***Putrescine*** | P<0.001 | Non-Significant | P<0.0001 | Non-Significant |

**Table 2. Effect of Putrescine treatment on total carotenoids, reducing sugars, non-reducing sugars and total carbohydrates of *Zea mays* grown under different salinity regimes.**

| Putrescine Treatment | NaCl Treatment | Total carotenoids *(mg/g fr.wt)* | Reducing Sugars *(mg/g fr.wt)* | Non-Reducing Sugars *(mg/g fr.wt)* | Total Carbohydrates *(mg/g fr.wt)* |
|---|---|---|---|---|---|
| **Control** | ***Control*** | 3.166 ± 0.327 b | 1.476 ± 0.327 c | 1.31 ± 0.215 c | 2.786 ± 0.111 c |
| | ***40 mM NaCl*** | 1.5 ± 0.251 a | 0.936 ± 0.159 b | 0.477 ± 0.17 b | 1.413 ± 0.318 b |
| | ***80 mM NaCl*** | 1.133 ± 0.233 a | 0.27 ± 0.14 a | 0.246 ± 0.095 a | 0.516 ± 0.233 a |
| **0.5 mM** | ***Control*** | 3.466 ± 0.066 b | 1.466 ± 0.327 c | 1.333 ± 0.259 c | 2.8 ± 0.134 c |
| | ***40 mM NaCl*** | 1.433 ± 0.185 a | 0.706 ± 0.298 b | 0.85 ± 0.11 b | 1.556 ± 0.324 b |
| | ***80 mM NaCl*** | 0.943 ± 0.407 a | 0.203 ± 0.046 a | 0.269 ± 0.067 a | 0.472 ± 0.097 a |
| **1 mM** | ***Control*** | 2.3 ± 0.611 b | 1.866 ± 0.352 c | 0.965 ± 0.11 c | 2.831 ± 0.242 c |
| | ***40 mM NaCl*** | 1.2 ± 0.378 a | 0.87 ± 0.231 b | 0.547 ± 0.157 b | 1.417 ± 0.256 b |
| | ***80 mM NaCl*** | 0.9 ± 0.115 a | 0.146 ± 0.081 a | 0.161 ± 0.028 a | 0.308 ± 0.057 a |
| **2 mM** | ***Control*** | 1.303 ± 0.072 b | 2.163 ± 0.018 c | 0.866 ± 0.317 c | 3.03 ± 0.308 c |
| | ***40 mM NaCl*** | 1.433 ± 0.491 a | 0.967 ± 0.175 b | 0.406 ± 0.157 b | 1.373 ± 0.313 b |
| | ***80 mM NaCl*** | 1.3 ± 0.321 a | 0.106 ± 0.032 a | 0.546 ± 0.164 a | 0.653 ± 0.172 a |
| **$LSD_{0.05}$** | | 0.363 | 0.142 | 0.090 | 0.163 |
| **Probability Level** | ***Salinity*** | P<0.0001 | P<0.0001 | P<0.0001 | P<0.0001 |
| | ***Putrescine*** | P<0.01 | Non-Significant | Non-Significant | Non-Significant |

***Antioxidants***

Plants treated with different concentration of salt (40mM, 80mM) exhibited significant (P <0.01) increase in total antioxidants as compared to non-stressed plants (Table 3). Under stress condition promotion in activities of different anti-oxidative enzymes such as glutathione reductase, peroxidase, superoxide dismutase and catalase enhance plants defense against reactive oxygen species by scavenge reactive oxygen species (Mittova *et al.,* 2003). Lechno *et al.* (1997) studied cucumber plant under NaCl stress and observed significant promotion in antioxidative enzymes activities such as glutathione reductase and catalase, ascorbic acid content which swrves as antioxidant and reduced glutathione but does not affect the activity of superoxide dismutase. It has been reported that antioxidant enzymes activity such as peroxidase has increased under salt stress in cucumber (Lechno *et al.,* 1997). Present study reveals that plants foliarly applied with different doses of putrescine (0.5mM, 1mM and 2mM ) exhibited non-significant reduction in total antioxidant at different salinity level. It has been reported that putrescine increase plant resistance to different abiotic and biotic stresses (Capell *et al.,* 2004). Research has shown that polyamine compounds such as antioxidant compounds can eliminate free radicals. Due to the reduction of free radicals, enzyme activity is decreased in the presence of the polyamine compound (Velikova *et al.,* 2000).

**Table 3. Effect of putrescine treatment on total proteins, flavonoids, phenols and antioxidants of *Zea mays* grown under different salinity regimes.**

| Putrescine Treatment | NaCl Treatment | Total Proteins *(mg/g fr.wt)* | Flavonoids *(mg/g fr.wt)* | Total Phenols *(mg/g fr.wt)* | Total antioxidants *(mg/g fr.wt)* |
|---|---|---|---|---|---|
| **Control** | ***Control*** | 3.053 ± 0.381 b | 0.682 ± 0.027 a | 1.397 ± 0.311 a | 0.473 ± 0.193 a |
| | ***40 mM NaCl*** | 1.4 ± 0.548 b | 2.235 ± 0.234 b | 3.736 ± 0.329 ab | 15.736 ± 2.275 a |
| | ***80 mM NaCl*** | 0.273 ± 0.033 a | 8.656 ± 1.489 c | 7.713 ± 0.668 b | 38.986 ± 24.707 b |
| **0.5 mM** | ***Control*** | 10.53 ± 7.637 b | 0.682 ± 0.027 a | 6.72 ± 0.882 a | 0.353 ± 0.041 a |
| | ***40 mM NaCl*** | 1.82 ± 0.355 b | 2.235 ± 0.234 b | 8.374 ± 0.333 ab | 6.326 ± 3.073 a |
| | ***80 mM NaCl*** | 0.536 ± 0.254 a | 13.11 ± 2.759 c | 9.044 ± 0.017 b | 18.633 ± 0.808 b |
| **1 mM** | ***Control*** | 6.043 ± 0.848 b | 0.544 ± 0.119 a | 5.07 ± 1.975 a | 0.246 ± 0.092 a |
| | ***40 mM NaCl*** | 1.561 ± 0.695 b | 65.333 ± 6.861 b | 15.738 ± 2.546 ab | 5.146 ± 3.436 a |
| | ***80 mM NaCl*** | 0.442 ± 0.211 a | 122.861 ± 11.684 c | 63.854 ± 57.323 b | 11.646 ± 4.559 b |
| **2 mM** | ***Control*** | 2.81 ± 0.289 b | 0.941 ± 0.071 a | 10.11 ± 0.032 a | 0.493 ± 0.185 a |
| | ***40 mM NaCl*** | 1.113 ± 0.348 b | 112.05 ± 38.496 b | 372.936 ± 368.886 ab | 10.293 ± 6.873 a |
| | ***80 mM NaCl*** | 0.453 ± 0.038 a | 181.19 ± 3.361 c | 996.603 ± 305.051 b | 23.293 ± 9.118 b |
| **$LSD_{0.05}$** | | 0.516 | 0.879 | 0.347 | 0.130 |
| **Probability Level** | ***Salinity*** | P<0.001 | P<0.0001 | P<0.05 | P<0.001 |
| | ***Putrescine*** | Non-Significant | P<0.0001 | P<0.001 | Non-Significant |

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