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### **Application of hexaconazole to ameliorate salinity stress by inducing some antioxidant enzymes in mung bean, *Vigna radiata* L. plant**

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**Abstract:** The ability of hexaconazole (HEX) to ameliorate salinity stress was studied in mung bean, *Vigna radiata* L. plants subjected to salinity stress as sodium chloride (NaCl) treatments. The obtained results showed that application of NaCl at different concentrations (2000, 4000, 6000 mg/L and tap water as a control) reduced all the studied growth parameters, chlorophyll content as well as increased proline (Pro) content in mung bean plants. In addition, NaCl stress increased the activity of antioxidant enzymes, such as peroxidase (POX), super-oxide dismutase (SOD) and catalase (CAT). Application of hexaconazole (HEX) solutions at 25 or 50 mg/L on the plants subjected to irrigation with saline solutions at different concentrations ameliorating the inhibitory effects of salinity. These effects might be attributed to the physiological role of (HEX) on increasing growth parameters such as shoot growth, number of branches, dry weight, photosynthetic pigments and activity of some antioxidant enzyme. Our results suggested that hexaconazole has an important role in the enhancement of plant antioxidant enzymes and will be able to overcome the toxic effects of NaCl stress on mung bean seedlings.

**Keywords:** salinity stress, mung bean plant, *Vigna radiata*, growth parameters, yield, chlorophyll, antioxidant (hexaconazole).

### **Introduction**

Mung bean (*Vigna radiata* L. Wilkzek) is a summer crop with short duration (70-90 days) and high nutritive value. The seeds contain 22-28% protein, 60-65% carbohydrates, 1.0-1.5% fat, 3.5-4.5% fibers and 4.5-5.5% ash. They have many effective uses, green pods in cooking as peas, sprout rich in vitamins and amino acids. This crop can be used for both seeds and forage since it can produce a large amount of biomass and then recover after grazing to yield abundant seeds<sup>1,2</sup>. For these facts and to increase the demand for increased productivity of mung bean plant is going for the eyes of agriculture scientists about the expansion of cultivation in the newly reclaimed land suffering from an increase in the proportion of soil salts and a lack of fresh water for irrigation.

Salinity is considered as sever problem in agriculture as it results in a noticeable reduction in the productivity of economic crops. Lack of fresh water for irrigation together with the poor drainage of water from the cultivated soils resulted in the accumulation of salts. In Egypt the cultivated regions restricted to the Nile valley depending on fresh water of the River Nile for irrigation does not exceed 4% of the total land area of

Egypt. Most of the newly reclaimed lands depend on underground water of various degrees of salinity for irrigation. In addition progressive accumulation of salts became a serious problem in many cultivated areas of the Delta as a result of high ground water table, especially when accompanied by poor drainage.

Salinity is known to interfere with multiple physiological processes like photosynthesis by decreasing the chlorophyll content<sup>3</sup> and inducing the closure of stomata, thereby decreasing partial CO<sub>2</sub> pressure within the leaf. However, the degree of salt-induced reduction in photosynthetic capacity depends upon the area of photosynthesizing tissue, the amount of photosynthetic pigments, and stomatal and nonstomatal factors that affect the CO<sub>2</sub> assimilation (gas exchange and metabolism)<sup>4</sup>. Salt induced osmotic stress triggers the formation of reactive oxygen species which can cause damage to the mitochondria and chloroplasts by cellular structures<sup>5</sup>.

The inhibitory effects of salinity on plant growth are also to specific ion cytotoxicity, low external osmotic potential, and nutrients deficiencies<sup>2</sup>. Ion cyto-toxicity is caused by the replacement of K<sup>+</sup> by Na<sup>+</sup> in biochemical hydration shells and interfere with the non-covalent interaction among the amino acids, hence, the ability of plants to maintain a high cytosolic K<sup>+</sup>/Na<sup>+</sup> ratio is a key feature of plant salt tolerance<sup>6</sup>. Some plant growth regulators (PGRs) can increase the plant tolerance against salinity<sup>5</sup>.

Proper application of plant growth regulators is being increasingly used to manipulate plant growth and yield, and to enhance the tolerance of plants to many environmental stresses<sup>5,7,8</sup>. Hexaconazole (HEX; chemical name of (RS)-2-(2,4-dichlorophenyl)-1-(1H-1,2,4-triazol-1-yl) hexan-2-ol) is an active member of the triazole family with plant growth-regulating properties<sup>9</sup>. It can cause many morphological changes, such as reducing shoot growth, stimulating root growth<sup>9,10</sup>, inhibiting gibberellin biosynthesis, increasing chlorophyll content, altering carbohydrate status, increasing cytokinin biosynthesis and temporarily enhancing the (abscisic acid) ABA content. Triazole compounds can also increase the photosynthetic rate (Pn), phosphoenol pyruvate carboxylase activity and root oxidizability<sup>7,11</sup>. Recent studies have shown that the application of ketoconazole and uniconazole, a closely related triazole, enhances tolerance to stress induced by salinity and water logging<sup>5,12</sup>, heat<sup>13</sup> and freezing<sup>14</sup> in canola seedlings. Uniconazole also enhances drought tolerance in soybeans<sup>15</sup>. Triazole compounds improve the tolerance of stressed plants, which may be due to improved antioxidant defense mechanisms with higher enzyme activities of superoxide dismutase (SOD) and peroxidase (POX) decreasing lipid peroxidation and membrane deterioration<sup>7,9,16</sup>.

For all the above mentioned reasons, this study investigated the effect of hexaconazole (HEX) on non-stressed and NaCl-stressed mung bean plants. The concentrations of NaCl and hexaconazole were selected based on previous studies showing mung bean tolerance to NaCl up to 200 mM and a positive effect of triazole compounds on plant metabolism<sup>17,18,19</sup>. This investigation aimed to study the ability of growth retardant "hexaconazole" to ameliorate the harmful effects of salinity stress with an emphasis on several growth and yield parameters, photosynthetic pigments content, proline content, and activity of some antioxidant enzymes (POX, SOD and CAT).

## Material and Methods

Pot experiments were carried out during two successive growth seasons (2011 and 2012) in the screen of the National Research Centre, Dokki, Giza, Egypt. Seeds of mung bean plant, *Vigna radiata* were obtained from Field Crops Research Institute, Agricultural Research Centre, Ministry of Agriculture, A.R.E.

### **The seeds were presoaked for 10 h in 500 mL of the following solutions:**

Distilled water (control), 25 or 50 mg L<sup>-1</sup> of growth retardant hexaconazole "HEX". After soaking, five seeds were sown on 17<sup>th</sup> of February, 2011 and 2012, respectively in plastic pots 30cm in diameter that had been filled with 13Kg of a soil mixture containing air-dried loamy clay soil and sand in a ratio of 1:1 (V:V). After two weeks from sowing the seedlings were thinned to two seedlings per pot. Before sowing the seeds, the pots were irrigated with the different treatment solutions and the soil electrical conductivity (EC) was then measured. The pots were then divided into four groups according to the seed pretreatments, and irrigated accordingly with tap water (control), 2000, 4000 or 6000 mM NaCl. The plants were irrigated with saline solutions one time every week. To avoid the toxicity resulted from the accumulation of salts ions around the root system the plants irrigated three times with equal amounts of saline solutions followed by one time with tap water.

### Fertilization:

Every pot was received 3.0g super-mono-phosphate (15.5% P<sub>2</sub>O<sub>5</sub>) and 1.0g potassium sulphate (48-52% K<sub>2</sub>O) mixed with the soil before sowing. Nitrogen fertilizer was added in two equal doses (one during sowing and the other after thinning) in the rate of 0.6g N for pot as ammonium sulphate (20.6% N).

**Sampling:** the plants were collected randomly at 75 DAS and divided as follows:

**Group A:** used for measuring growth parameters: including shoot length (cm), number of branches per plant and dry weights of shoots (g).

**Group B:** The plant shoot system and new leaves were immediately weighed, frozen with liquid nitrogen and stored in deep freezer at -20°C until used for extraction and estimation of enzymatic antioxidants (superoxide dismutase, peroxidase and catalase).

**Group C:** fresh new leaves were used for estimation of photosynthetic pigments and proline content.

### Biochemical analysis

**Photosynthetic pigments:** An accurate weight (0.5g) of fresh young leaves of mung bean, *Vigna radiata* seedlings were homogenized in 85% acetone and used for estimation of photosynthetic pigments (chl."a", chl."b" and total carotenoids) using spectrophotometric method developed by Gilley and Fletcher <sup>20</sup>.

**Proline content:** Proline (Pro) content was determined according to the method of **Bates *et al.*** <sup>21</sup>, with some modifications. Fresh new leaves of mung bean plants (0.2g) were homogenized in a mortar and pestle with 3 mL sulfosalicylic acid (3% W/V), and the homogenate was then centrifuged at 18,000 rpm for 15 min. The supernatants were placed into test tubes, and 2 mL glacial acetic acid and 2 mL of an acid-ninhydrin solution (1.25g of ninhydrin dissolved in 30mL glacial acetic acid and 20 mL 6M orthophosphoric acid) were added to the tubes. The tubes were incubated, and they were then allowed to become cool at room temperature. Toluene (4 mL) was then added, and the samples were mixed on a vortex mixer for 20 s. The test tubes were permitted to stand for at least 10 min to allow the separation of the toluene and aqueous phases. The toluene phase was carefully removed by a pipette and placed into a glass test tube, and the absorbance of the toluene phase was measured at 520 nm by a spectrophotometer.

### Assays of some antioxidant enzymes

#### Superoxide dismutase (SOD)

The activity of SOD enzyme was assayed according to the method of **Giannopolitis and Ries** <sup>22</sup>. The reaction mixture contained 100 µL of 1 µM riboflavin, 100 µL of 12 mM l-methionine, 100 µL of 0.1 mM EDTA (pH 7.8), 100 µL of 50 mM Na<sub>2</sub>CO<sub>3</sub> (pH 10.2), 100 µL of 75 µM nitroblue tetrazolium (NBT), 2300 µL of 25 mM sodium phosphate buffer (pH 6.8) and 200 µL of crude enzyme extract with a final volume of 3 mL. SOD activity was assayed by measuring the ability of the enzyme extract to inhibit the photoreduction of NBT. Glass test tubes containing the mixture were illuminated with a fluorescent lamp (120 W), and identical tubes that were not illuminated served as blanks. After illumination for 15 min, the absorbance was measured at 560 nm. One unit of SOD was defined as the amount of enzyme activity that was able to inhibit the photoreduction of NBT to blue formazan by 50%. The SOD activity of the extract was expressed as SOD units min<sup>-1</sup> mg<sup>-1</sup> protein.

#### Peroxidase (POX)

The activity of POX enzyme was assayed by the oxidation of guaiacol in the presence of H<sub>2</sub>O<sub>2</sub>. The increase in absorbance was recorded at 470 nm <sup>23</sup>. The reaction mixture contained 100 µL of crude enzyme, 500 µL of 5 mM H<sub>2</sub>O<sub>2</sub>, 500 µL of 28 mM guaiacol and 1900 µL of 60 mM potassium phosphate buffer (pH 6.1). POX activity of the extract was expressed as POX units min<sup>-1</sup> mg<sup>-1</sup> protein.

#### Catalase (CAT)

Catalase enzyme activity was assayed by the method of **Cakmak and Horst** <sup>24</sup>. Frozen leaf tissues (0.5

g) were homogenized in a mortar and pestle with 3 mL of ice-cold extraction buffer (25 mM sodium phosphate; pH 7.8). The homogenate was centrifuged at 18,000 rpm for 30 min at 4°C, and the supernatant was then used for the enzyme assay. The reaction mixture contained 100 µL of crude enzyme extract, 500 µL of 10 mM H<sub>2</sub>O<sub>2</sub> and 1400 µL of 25 mM sodium phosphate buffer. The decrease in the absorbance at 240 nm was recorded for 1 min by a spectrophotometer. The CAT activity of the extract was expressed as CAT units min<sup>-1</sup> mg<sup>-1</sup> protein.

### Statistical analysis

The experiment was carried out with factorial randomized complete block (RCB) design with three replicates <sup>25</sup>. All data were subjected to analysis, and the significance of the differences among treatment means was tested using a least significant differences (LSD) test at a 5% probability level.

## Results and Discussion

### Vegetative growth parameter:

**Table (1): Effect of different concentrations of hexaconazole (HEX) on shoot length, number of branches and dry weight of mung bean plants (at 75 DAS) grown under different levels of salinity**

Dry weight as (g)/plant	Number of branches / plant	Shoot length (cm)	Hexaconazole (mg/L <sup>-1</sup> )	Treatments Salinity
4.47	7.00	34.00	0	Tap water
4.05	8.33	31.67	25	
3.90	7.87	30.01	50	
5.24	6.33	39.80	0	2000 mg/L <sup>-1</sup>
4.98	8.00	37.90	25	
4.71	7.67	35.67	50	
4.13	6.00	31.50	0	4000 mg/L <sup>-1</sup>
3.67	7.77	28.00	25	
3.43	7.00	25.09	50	
2.63	5.67	21.00	0	6000 mg /L <sup>-1</sup>
2.97	7.33	23.67	25	
2.45	6.17	19.00	50	
0.07	0.14	1.38		LSD at 5% for: Salinity HEX Interaction
0.09	0.11	1.19		
0.16	N S.	1.12		

As shown in Table (1) irrigating mung bean plants with three levels of saline solutions; tap water, 2000, 4000 or 6000 mg /L<sup>-1</sup> mostly lead to significant reduction in all the studied growth criteria e.g. shoot length (in cm), number of branches/plant and dry weights of the shoots (g) of the produced plants all over the experimental period as compared with that obtained from the plants irrigated with tap water. The decrements were directly proportion with the salinity levels used. These results are in agreement with those obtained recently<sup>3,26</sup>, found that treating bean seedlings (*Phaseolus vulgaris* L.) with different concentrations of saline solutions led to reduction in vegetative growth parameters.

Soaking seeds of mung bean plants in solutions of hexaconazole (HEX) at different concentration (25 or 50 mg/L<sup>-1</sup>) caused significant reduction in all the studied vegetative growth parameters such as shoot length (in cm), number of branches/plant and dry weights of the shoots (g), fresh and dry weights of the shoots of the produced seedlings as compared with their corresponding control produced from soaking their seeds in distilled water.

Concerning, the interaction of salinity and growth retardant (hexaconazole) the data reveal that treating the seeds with solutions of hexaconazole at three used concentrations namely: distilled water, 25 or 50 mg/L<sup>-1</sup> and irrigating the produced seedlings with different levels of salinity (tap water, 2000, 4000 or 6000 mg/L<sup>-1</sup>) induced significantly reduction in all the above vegetative growth parameters all over the experiment period in comparison with that obtained from the control (untreated seeds) as well as their respective salinity controls.

The results presented in this work are in agreement with those obtained by many investigators using different growth retardants (antigibberellins) compounds at different concentrations<sup>27,28,29,30</sup>.

The reduction in most vegetative growth parameters of plants treated with growth retardant and grown under stress condition could be due to reduction in the cell size which might be attributed to changes in osmotic cell enlargement dependent on solute accumulation *i.e.*, due to drastic changes in ion relationship<sup>2,16,31</sup>.

### Photosynthetic pigments

It is well known that physiological processes in plants pass through many metabolic pathways and lead to metabolic products. Stress in general and salinity in particular resulted in the occurrence of physiological disorders in crop plants.

**Table (2): Effect of different concentrations of hexaconazole (HEX) on photosynthetic pigments and free proline content of mung bean plant leaves (at 75 DAS) grown under different levels of salinity**

Free Proline $\mu$ mole/g fresh weight	Total carotenoids mg/g fresh weight	Chlorophyll "b" mg/g fresh weight	Chlorophyll "a" mg/g fresh weight	Hexaconazole (mg/L <sup>-1</sup> )	Treatments	
					Salinity	
Tap water	0	3.50	1.37	0.85	10.01	
	25	4.02	1.50	1.03	12.10	
	50	4.26	1.87	1.31	13.68	
2000 mg/L <sup>-1</sup>	0	3.03	1.30	0.75	11.37	
	25	3.25	1.36	0.83	12.09	
	50	3.81	1.76	0.92	13.91	
4000 mg/L <sup>-1</sup>	0	2.75	1.23	0.63	11.55	
	25	2.80	1.33	0.69	12.35	
	50	3.00	1.65	0.77	14.55	
6000 mg/L <sup>-1</sup>	0	2.14	1.14	0.51	12.01	
	25	2.45	1.26	0.57	12.36	
	50	2.87	1.41	0.63	14.89	
LSD at 1 % for: Salinity HEX Interaction		0.22 0.18 0.52	0.13 0.18 0.25	0.12 0.14 0.21	1.56 1.35 2.07	

It is clear from Table (2) that irrigating mung bean plants with saline solutions at different levels; tap water, 2000, 4000 or 6000 mg/L<sup>-1</sup> decreased significantly the amounts of photosynthetic pigments (chl."a", chl. "b" and carotenoids) of mung bean leaves. The highest value of decrements (38.86 %) was obtained from the application of 6000 mg/ L<sup>-1</sup> relative to the control plants.

These results are in agreement with those obtained by Starck and Karwowska<sup>32</sup>, concluded that inhibition of photosynthetic pigments of bean leaves irrigated with NaCl may be attributed to the inhibition of assimilates translocation. **Patil *et al.***<sup>33</sup> who postulated that salinity stress affected the photosynthetic rate of carbon assimilation. **Reddy and Vora**<sup>34</sup>, attributed the reduction in photosynthetic pigments of wheat leaves grown under salt stress to increase in the activity of chlorophyllase enzyme. Recently, both<sup>3,26</sup> reported that

treating bean seedlings (*Phaseolus vulgaris* L.) and (*Vigna radiata* L) respectively with different concentrations of saline solutions led to reduction in the photosynthetic pigments.

Spraying mung bean plants with any used concentration of hexaconazole (25 or 50 mg/L<sup>-1</sup>) increased significantly the amount of photosynthetic pigments (chl."a", chl. "b" and carotenoids) content of mung bean leaves as compared with those obtained from the untreated plants. The value of increment was estimated by 21.71, 36.50 and 54.12%, respectively over their respective control (untreated plants).

These results are in agreement with those obtained by<sup>35</sup> who reported that using growth retardant uniconazole resulted in several modifications of bean plants such as increase in chlorophyll levels and enlarged chloroplasts<sup>2</sup>. Regarding the combined effect of salinity and hexaconazole, it is clear from the results of the present investigation that treating the plants with hexaconazole solution at 50 mg/L<sup>-1</sup> and irrigated with saline solutions resulted in significant increases in chl."a", chl."b" and carotenoids as compared with that obtained from their corresponding salinity control. These results are in agreement with those obtained by<sup>35,36</sup>.

In this connection, it may be mentioned that carotenoids provide photosynthetic systems with a method of photo-protection. Active oxygen 1/2 O<sub>2</sub> has been detected in chloroplast of water stressed wheat, i.e. Singlet oxygen is an extremely powerful oxidant, which is very harmful to plant cell. Carotenoids prevent the formation of singlet oxygen (1/2 O<sub>2</sub>) by quenching the triplet states of the chlorophyll molecules as they arise<sup>16,37</sup>.

### **Proline content**

The data recorded in Table (2) show that irrigating mung bean plants with saline solutions resulted in significant increases in the proline content of recently leaves. Proline is believed to protect plant tissue against stress by acting as a nitrogen-storage compound, osmoregulate-solute and hydrophobic protectant for enzymes and cellular structures<sup>38</sup>. Recently, many scientists<sup>1,2,3,26</sup> indicated that treating *Phaseolus vulgaris* seedlings with different concentrations of saline solutions led to increase in the proline content.

Treating the plants with different concentrations of hexaconazole (25 or 50 mg/L<sup>-1</sup>) led to significant increases in the proline content of newly leaves of mung bean plants. These increments were concentration dependent i.e., the highest value of proline was obtained from the plants response to highest dose of hexaconazole (50 mg / L<sup>-1</sup>).

The combined treatments mostly increased significantly the proline content in mung bean leaves as compared with the corresponding control, thus spraying the plants with hexaconazole at the two used concentrations may add more protection of the plant tissues against harmful effects of salinity stress. In this concern, the author<sup>16</sup> proposed a specific role for proline in osmoregulation and suggested that proline accumulation in the cytoplasm allows maintenance of a balance across the tonoplast. In addition, the authors<sup>2,35</sup> observed that application of growth retardants led to secretion of proline-rich protein by salt adapted bean cells may be specific for the adaptation of the cell wall to salt stress and may cause changes in the cell wall that allow cells to tolerate salt stress.

### **Activity of some oxidative enzymes**

#### **Activity of Super oxide dismutase enzyme (SOD)**

Table (3) show that irrigating mung bean plants with different concentrations of saline solutions (tap water, 2000, 4000 or 6000 mg/L<sup>-1</sup>) increased significantly enzyme activity of SOD by 6.26, 11.81 and 26.04%, respectively relative to the control (plants irrigated with tap water).

Spraying mung bean plants with any used concentration of hexaconazole (25 or 50 mg/L<sup>-1</sup>) increased significantly the amount of enzyme activity of SOD in comparison with that obtained from the control plants. The highest value of increment (22.19%) was obtained from the application of HEX at 50 mg/L<sup>-1</sup> relative to the control.

Concerning the interaction of salinity and hexaconazole it is clear from the data that all the combined treatments induced significantly increases in the activity of SOD enzyme as compared with those obtained from their corresponding control. The greatest increase (13.67%) was detected in plants received the highest dosage of hexaconazole (50 mg/L<sup>-1</sup>) and irrigated with salinity at 6000 mg/ L<sup>-1</sup> relative to their respective control.

The obtained results are in agreement with that recorded by<sup>1,2</sup>.

### Activity of peroxidase enzyme (POX)

It is clear from the data recorded in Table (2) that salinity treatments had stimulatory effect on the activity of peroxidase enzyme (POX) in comparison with that obtained from their corresponding control. The maximum value of activity (47.38 %) was observed in the plants subjected to irrigation with saline solution at 6000 mg/ L<sup>-1</sup> relative to their respective control (plants irrigated with tap water).

Application of hexaconazole at any used concentration (25 or 50 mg/L<sup>-1</sup>) increased significantly the amount of enzyme activity peroxidase (POX) as compared with control plants. The value of increment was estimated by 19.15 and 41.49%, respectively over their respective control (untreated plants).

It is obvious from the data that all the combined treatments of salinity and hexaconazole induced significantly increases in the activity of POX enzyme as compared with those obtained from their corresponding control. The greatest increase (30.48%) was detected in plants received the highest dosage of HEX (50 mg/L<sup>-1</sup>) and irrigated with salinity at 6000 mg/ L<sup>-1</sup> relative to their respective salinity control.

The obtained results are in agreement with those obtained by<sup>39</sup> who reported that water stress could increase the accumulation of peroxidase substances such as glutathione, ascorbate and phenolic compounds which in turn act as scavengers of cell harmful reactive oxygen species (1/2 O<sub>2</sub>). In addition, many authors<sup>1,2,28,40</sup> all indicated that soaking pea and faba bean seeds in different concentrations of growth retardant (uniconazole) and irrigating the seeds with different levels of salinity led to significant increases in the activity of peroxidase enzyme.

### Activity of catalase enzyme (CAT)

**Table (3): Effect of different concentrations of hexaconazole (HEX) on the activity of some antioxidant enzymes of mung bean leaves (extracted at 75 DAS) grown under different levels of salinity**

Treatments Salinity levels	Hexaconazole (mg/L <sup>-1</sup> )	Super oxide Dismutase (SOD)	Peroxidase (POX)	Catalase (Cat.)
Tap water	0	40.55	12.22	85.08
	25	44.09	14.56	80.09
	50	49.55	17.29	77.44
2000 mg/L <sup>-1</sup>	0	38.01	14.45	80.99
	25	40.77	16.17	77.87
	50	42.45	19.00	71.99
4000 mg/L <sup>-1</sup>	0	35.76	16.55	68.99
	25	37.09	18.19	64.66
	50	39.89	21.06	60.11
6000 mg /L <sup>-1</sup>	0	29.99	18.01	53.47
	25	32.55	21.07	48.99
	50	34.09	23.50	42.01
LSD at 1 % for: Salinity HEX Interaction		2.02 2.65 4.11	1.35 1.67 3.06	3.06 3.56 5.77

Table (3) show that irrigating mung bean plants with different concentrations of saline solutions (tap water, 2000, 4000 or 6000 mg/L<sup>-1</sup>) decreased significantly enzyme activity of catalase compared to their corresponding control. The value of decrement was estimated by 4.81, 18.91 and 37.15%, respectively relative to that obtained from the plants irrigated with tap water.

Spraying the plants with any used concentration of hexaconazole (25 or 50 mg/L<sup>-1</sup>) induced significantly decrease in the enzyme activity of catalase as compared with the control plants. The value of decrement was estimated by 5.87 and 8.98%, respectively over their respective control (untreated plants).

The interaction of salinity and hexaconazole showed that application of HEX at any used concentration under three levels of salinity up to 6000 mg/L<sup>-1</sup> decreased the enzyme activity of catalase as compared with corresponding control. The highest value of decrements (21.43%) was obtained from the application of HEX at 50 mg/L<sup>-1</sup> and irrigated with salinity at 6000 mg/L<sup>-1</sup> relative to their corresponding salinity control.

The results obtained in this investigation are in agreement with those recorded by<sup>28,16</sup> all reported that soaking bean seeds in different concentrations of uniconazole (10, 20 or 30 ppm) and irrigated with different levels of salinity up to 400 ppm led to reduction in the catalase activity. The reduction in catalase activity resulted in accumulation of toxic amounts of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) which might restrict the growth of seedlings. Elevated (H<sub>2</sub>O<sub>2</sub>) concentrations could release peroxidase from cell membrane structure<sup>41,42</sup>.

### **Yield and its components**

**Table (4): of different concentrations of hexaconazole (HEX) on yield and its components of mung bean plants grown under different levels of salinity**

Yield (g/plant)		Seeds number/pod	Pods wt. (g/plant)	Pods number/plant	Plant height at harvest (cm)	Hexaconazole (mg/L <sup>-1</sup> )	Treatments
straw	seeds						Salinity
1.58	1.55	3.90	2.10	6.11	58.67	0	Tap water
8.08	2.19		2.82	7.14	53.00	25	
7.83	1.80		2.67	6.80	50.00	50	
7.40	1.69	3.80	2.29	5.67	52.67	0	2000 mg/L <sup>-1</sup>
7.33	2.86	5.42	3.08	7.85	50.00	25	
7.06	2.33	4.90	2.85	7.17	46.04	50	
6.67	1.09	3.39	2.05	4.12	43.00	0	4000 mg/L <sup>-1</sup>
6.31	1.80	4.67	2.65	5.67	40.33	25	
6.00	1.33	3.85	2.26	5.09	37.00	50	
6.00	0.95	2.13	1.63	2.80	35.33	0	6000 mg/L <sup>-1</sup>
5.67	1.25	3.67	2.12	4.10	32.67	25	
5.00	1.10	2.33	2.00	3.32	29.82	50	
0.13	0.08	0.22	0.06	0.21	1.53		LSD at 5% for:
0.08	0.06	0.20	0.05	0.19	1.00		Salinity
0.24	0.20	0.52	0.11	0.50	2.16		HEX
							Interaction

Table (4) show that irrigated mung bean plants with saline solutions up to 6000 mg/L<sup>-1</sup> decreased significantly plant height (cm) at harvest stage and yield and it's component (number of pods, weight of pods, number of seeds, weight of seeds and straw weight all /plant) as compared with that obtained from the control plants (plants irrigated with tap water). These results are in agreement with those recorded by<sup>1,2</sup>.

Spraying the plants with any used concentration of hexaconazole (25 or 50 mg/L<sup>-1</sup>) caused significant increases in all the above mentioned characters of yield and it's component as compared with that obtained from the control plants (plants sprayed with distilled water). On the other hand, application of hexaconazole at two concentrations decreased significantly plant height of mung bean plants at harvest stage as compared with control plants.

Concerning the interaction between salinity and hexaconazole, it is clear from the recorded data that all the combined treatments increased significantly the amounts of the above mentioned characters of yield and it's components as compared with their corresponding salinity controls. The effect was more pronounced with the

concentrations used of hexaconazole and salinity. Our results are in agreement with those reported by<sup>16</sup>.

## Conclusion and recommendation

The results recorded in this investigation suggested that application of hexaconazole at 25 mg/L led to improvement in growth, yield and it's component of mung bean plants grown under salinity stress up to 6000 mM. These effects might be attributed to the important role of HEX in the enhancement of plant antioxidant enzymes and resistance to salinity stress.

## References

1. Saha P, Chatterjee P, Biswas AK. "NaCl pretreatment alleviates salt stress by enhancement of antioxidant defense system and osmolyte accumulation in mung bean (*Vigna radiata* L. Wilczek)," Indian Journal of Experimental Biology, 2010, 48(6): 593–600.
2. Iqbal N, Umar S, Khan NA, Khan MIR. "A new perspective of phytohormones in salinity tolerance: regulation of proline metabolism," Environmental and Experimental Botany, 2014, 100: 34–42.
3. Roychoudhury A, Ghosh S. "Physiological and biochemical responses of mung bean (*Vigna radiata* L. Wilczek) to varying concentrations of cadmium chloride or sodium chloride," Unique Journal of Pharmaceutical and Biological Sciences, 2013, 1(3): 11–21.
4. Amirjani MR. Effect of Salinity Stress on Growth, Sugar Content, Pigments and Enzyme Activity of Rice. International J. of Botany, 2011, 7: 73-81.
5. Akbari GA, Hojati M, Modarres-Sanavy SAM, Ghanati F. Aminolevulinic acid improves photosynthetic gas exchange capacity and ion uptake under salinity stress in oilseed rape (*Brassica napus* L.) plants. Pesticide Biochem. and Physio., 2011, 100: 244–250.
6. Zhu JK. Salt and drought stress signal transduction in plants. Ann. J. Plant Biol., 2002, 53: 247-273.
7. Abdul Jaleel C, Gopi R, Manivannan P, Kishorekumar A, Sankar B, Panneerselvam R. Paclobutrazol Influences on vegetative growth and floral characteristics of *Catharanthus roseus* (L.) G. Don, Indian J. Appl. Pure Biol., 2006, 21: 369–372.
8. Abdul Jaleel CA, Gopi R, Manivannan P, Kishorekumar A, Gomathin-ayagam M, Panneerselvam R: Changes in biochemical constituents and induction of early sprouting by triadimefon treatment in white yam (*Dioscorea rotundata* Poir.) tubers during storage, J. Zhejiang Univ. Sci. B, 2007a, 8: 283–288.
9. Abdul Jaleel CA, Gopi R, Manivannan P, Panneerselvam R. Responses of antioxidant defense system of *Catharanthus roseus* (L.) G. Don. to paclobutrazol treatment under salinity, Acta Physiol. Plant., 2007b, 29: 205–220.
10. Kishorekumar A, Abdul Jaleel C, Manivannan P, Sankar B, Sridharan R, Panneerselvam RR. Differential effects of hexaconazole and paclobutrazol on the foliage characteristics of Chinese potato (*Solenostemon rotundifolius* Poir. J.K. Morton). Acta Biol. Szegediensis, 2006, 5: 127–129.
11. Kopyra M, Gwozdz EA. Antioxidant enzymes in paraquat and cadmium resistant cell lines of horseradish, Biol. Lett., 2003, 40: 61–69.
12. Tan Y, Zongsuo L, Hongbo S, Feng D. Effect of water deficits on the activity of anti-oxidative enzymes and osmoregulation among three different genotypes of Radix astragali at seeding stage, Colloids Surf. B: Biointerfaces, 2006, 49: 60–65.
13. Reddy AR, Chaitanya KV, Vivekanandan M. Drought induced responses of photosynthesis and antioxidant metabolism in higher plants. J. Plant Physiol., 2004, 161: 1189–1202.
14. Manivannan P, Jaleel CA, Sankar B, Kishorekumar A, Somasundaram R, Lakshmanan GMA, Panneerselvam R. Growth, biochemical modifications and proline metabolism in *Helianthus annuus* L. as induced by drought stress, Colloids Surf. B: Biointerfaces, 2007, 59: 141–149.
15. Sankar B, Jaleel CA, Manivannan P, Kishorekumar A, Somasundaram R, Panneerselvam R. Drought induced biochemical modifications and proline metabolism in *Abelmoschus esculentus* (L.) Moench, Acta Bot. Croat., 2007, 66: 43–56.
16. Wu X, He J, Chen J, Yang S Zha D. "Alleviation of exogenous 6-benzyladenine on two genotypes of eggplant (*Solanum melongena* Mill.) growth under salt stress," Protoplasma, 2014, 251(1): 169–176.
17. Dolatabadian A, Sanavy SAMM, Chashmi NA. The effects of foliar application of ascorbic acid (Vitamin C) on antioxidant enzymes activities, lipid and proline accumulation of canola (*Brassica napus* L.) under conditions of salt stress, J. Agron. Crop Sci., 2008, 194: 206–213.

18. Mohamed AM, Raldugina GN, Kholodova VP, Kuznetsov VIV. Osmolyte accumulation in different rape genotypes under sodium chloride salinity, Russ. J. Plant Physiol., 2006, 53: 649–655.
19. Navarro A, Shnchez-Blanco MJ, Morte A, Ban S. The influence of mycorrhizal inoculation and paclobutrazol on water and nutritional status of *Arbutus unedo* L., Environ. Exp. Bot., 2009, 66: 362–371.
20. Gilley A, Fletcher RA. Gibberellin antagonizes paclobutrozol-induced stress protection in wheat seedlings. J. Plant Physiol., 1998, 153: 200-207.
21. Bates LS, Waldern RP, Teave ID. Rapid determination of free proline for water stress studies. Plant Soil, 1973, 39: 205–207.
22. Giannopolitis C, Ries S. Superoxid desmutase. I: Occurrence in higher plant. Plant Physiol., 1997, 59: 309–314.
23. Ghanatib F.; A. Morita and H. Yokota. Induction of suberin and increase of lignin content by excess boron in tobacco cell. Soil Sci. Plant Nutr., 2002, 48: 357–364.
24. Cakmak L, Horst W. Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tip of soybean (*Glycine max*). Plant Physiol., 1991, 83: 463-468.
25. Steel RGD, Torrie JH. Principles and procedures of statistics. P. 107 Mc. Craw-Ni: Bood Co. Inc. New York, Toronto, London, 1990.
26. Stoeva N, Kaymakanova M. Effect of salt stress on the growth, photosynthetic rate of bean plants (*Phaseolus vulgaris* L). J. Central Eur. Agric., 2008, 9 (3): 385-392.
27. Gent MPN. Persistence of triazole growth retardants on stem elongation of rhododendron and kalmia. J. Plant Growth Regul. 1997, 16: 197-203.
28. Bekheta MA. Physiological studies on the effect of uniconazol on the growth and productivity of Vicia faba plants grown under different levels of salinity stress. Ph.D. Thesis, Bot. Dept. Faculty of Science, Cairo Univ., 2000.
29. Bekheta MA. Combined effect of gibberellic acid and paclobutrazole on wheat plants grown in newly reclaimed lands. J. Agric., Sci., Mansoura Univ., 2004, 29 (8): 4499-4512.
30. sin L, Alegre S, Montserrat R. Effect of paclobutrazol, prohexadione-ca, deficit irrigation, summer pruning and root pruning on shoot growth, yield and return bloom in "Blanquilla"pear orchard. Scientia Horti., 2007, 113 (2): 135-142.
31. Grossmann K, Konig-Karnz S, Kwiatkowski J. Phytohormonal changes in intact shoots of wheat and oilseed rape treated with the acylcyclohexanone. Physiologia. Plantarium, 2006, 90: 139-143.
32. Starck Z, Karwowska R. Effect of salt stress on the hormonal relation of growth, photosynthesis and distribution of C<sub>14</sub> assimilate in bean plants. Acta Soc. Bot. Pol., 1978, 47:245-267.
33. Patil TM, Marijakor PB, Hegade BA, Joshi GV. Influence of soil salinity on morphology, rate of CO<sub>2</sub> assimilation, photosynthetic products and enzyme activities in sorghum hybrid CSH-S. Indian J. Plant Physiol., 1983, 26: 153.
34. Reddy MP, Vora AB. Effect of salinity on germination and free proline content of bajra (*Pennisetum typhoides* S & H) seedlings. Proc. Indian. Nat. Sci. Acad., B, 1983, 49, p.702-705.
35. Bekheta MA, Shabaz R, Lieberei R. Uniconazole induced changes of stress responses of *Vicia faba* L. polyphenol oxidase activation pattern serves as an indicator for membrane stability. Journal of Applied Botany and Food Quality, 2006, 80: 129-134.
36. Jaleel CA, Gopi R, Kishorekumar A, Manivannan P, Sankar B, Panneerselvam R. Interactive effects of triadimefon and salt stress on antioxidative status and ajmalicine accumulation in *Catharanthus roseus*. Acta Physiol. Plant., 2008, 30: 287–292.
37. Fyfe P, Cogdell RJ, Hunter CN, Jones Mr. Study of the carotenoids binding pocket of photosynthetic reaction center from purple bacterium *Rhodobacter sphaeroides* P. Mathis (Ed), photosynthesis from light to Biosphere Vol., IV: 47-50 of X<sup>th</sup> International Photosynthesis Congress. Montpellier, France, 20-25 Augst, 1995.
38. Greenway H, Munns R. Mechanism of salt tolerance of non halophytes. Ann. Revi. Plant Physiol., 1980, 31: 149-190.
39. Winston GW. Physiochemical basis for free radical formation in cells: Production and defenses. In stress responses in plants adaptation and acclimation mechanism. Edited by Alsche, R.G. and Cumming, J. R. 57-86. Wley-Liss, Inc. New York, 1990.
40. Hathout TA. Diverse effects of Uniconazole and nicotinamide on germination, growth endogenous hormones and some enzymatic activities of pea plants. Egypt. J. of Physiol. Sci., 1995, 19 (1, 2): 77-95.

41. Gorden D, Beck E. H<sub>2</sub>O<sub>2</sub> destruction by ascorbate dependent systems from chloroplasts. Biochem. Biophys. Acta, 1997, 546: 426-435.
42. Nguyen HT, Shim IS, Kobayashi K, Usui K. Effects of salt stress on ion accumulation and antioxidative enzymes activities of *Oryza sativa* L. and *Echinochola oryzicola* Vasing. Weed biology and management, 2005, 5 (1): 1-7.

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