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**Nicotinoids: Insecticides for Enhancing Cotton Plants  
against Environmental Stress and Insects Attack**

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# TABLE OF CONTENTS

Subject	Page
<b>INTRODUCTION</b>	1
<b>REVIEW OF LITERATURES</b>	5
<b>Effect of some abiotic factors on cotton plants</b>	5
<b>I-Effect of water-deficit</b>	5
<b>Effect of water-deficit stress and/or some neonicotinoids on some early cotton insects.</b>	5
1-Effect of water-deficit	5
2-Effect of neonicotinoids	9
<b>Effect of water-deficit stress and/or some neonicotinoids on some cotton plant growth criteria (morphological, physiological, biochemical, anatomical and yield)</b>	11
1- Effect of water – deficit	11
2-Effect of neonicotinoids	21
<b>II-Effect of salt stress</b>	26
<b>Effect of salt stress and/or some neonicotinoids on some cotton plant growth criteria (morphological, physiological, biochemical, anatomical and yield)</b>	26
1-Effect of salt stress	26
2-Effect of some inducers	30
<b>Materials and Methods</b>	35
<b>Evaluation of IMI and TMX for enhancing cotton plants against water-deficit stress and early season insects attack</b>	35
Experimental design.	35
Insecticidal treatment	35
Soil analysis	37
Irrigation water management	37
Early-season insect inspection	37
<b>II- Salt stress</b>	37
<b>Pot experiment</b>	37
<b>Field experiment</b>	38
Insecticidal treatment	39
Seawater analysis	39

# CONTENTS

Subject	Page
Soil analysis	40
<b>Evaluation of different treatments on some cotton plant criteria</b>	41
<b>Morphological Criteria</b>	41
<b>Physiological Criteria</b>	41
<b>Anatomical Criteria</b>	42
<b>Biochemical Criteria</b>	43
<b>Samples preparation and extraction</b>	43
<b>Enzymatic assays</b>	43
Antioxidant enzyme assays	43
Poly phenol oxidase	44
<b>Nonenzymatic assays</b>	44
Total protein	44
Quantitative assay	45
Proline content	45
<b>Cotton yield estimation</b>	45
<b>RESULTS AND DISCUSSION</b>	<b>46</b>
<b>Effect of some abiotic factors on cotton plants</b>	46
<b>Effect of water-deficit stress and some neonicotinoids(and/or) their combinations on some early cotton insects.</b>	48
1-Effect of water-deficit	48
2-Effect of neonicotinoids	49
<b>Effect of water-deficit stress and/or some neonicotinoids on some cotton plant growth criteria (morphological, physiological, biochemical, anatomical and yield)</b>	50
<b>Morphological Criteria</b>	50
1-Effect of water-deficit	51
2- Effect of neonicotinoids	53
<b>Physiological Criteria</b>	54
1-Effect of water-deficit	54
2- Effect of neonicotinoids	58
<b>Anatomical Criteria</b>	58
1-Effect of water-deficit	58
2- Effect of neonicotinoids	60
<b>Biochemical Criteria</b>	69
<b>Enzymatic components</b>	69
1-Effect of water- deficit	69

# CONTENTS

<b>Subject</b>	<b>Page</b>
2- Effect of neonicotinoids	72
<b>Nonenzymatic assays</b>	76
<b>Total protein;</b>	76
Quantitative assays	76
1- Effect of water deficit	76
2- Effect of neonicotinoids	76
<b>Qualitative assays</b>	78
1- Effect of water deficit	78
2- Effect of neonicotinoids	79
<b>Proline content</b>	80
1-Effect of water –deficit	80
2- Effect of neonicotinoids)	81
<b>Cotton yield and its parameters.</b>	82
1-Effect of water –deficit	83
2- Effect of neonicotinoids	84
<b>II-Effect of salt stress</b>	86
<b>Effect of salt stress and/or some neonicotinoids on some cotton plant growth criteria (morphological, physiological, biochemical, anatomical and yield)</b>	88
<b>Effect of salt stress (brackish water) on cotton seeds germination</b>	88
<b>Morphological Criteria</b>	91
1-Effect of salt stress	91
2- Effect of neonicotinoids	92
<b>Physiological Criteria;</b>	92
1-Effect of salt stress	92
2- Effect of neonicotinoids	94
<b>Anatomical Criteria:</b>	95
1-Effect of salt stress	95
2- Effect of neonicotinoids	96
<b>Biochemical Criteria</b>	103
<b>Enzymatic components</b>	103
1-Effect of salt stress	103
2- Effect of neonicotinoids	106
<b>Non-enzymatic components:</b>	106
<b>Total protein</b>	106
-Quantitative assay	106
1-Effect of salt stress	106

# CONTENTS

<b>Subject</b>	<b>Page</b>
2- Effect of neonicotinoids	106
<b>Proline Content</b>	108
1-Effect of salt stress	108
2- Effect of neonicotinoids	109
<b>Field Experiment</b>	111
<b>Morphological Criteria</b>	111
1-Effect of salt stress	111
2- Effect of neonicotinoids	113
<b>Physiological Criteria;</b>	113
1-Effect of salt stress	113
2- Effect of neonicotinoids	115
<b>Biochemical Criteria;</b>	116
<b>Enzymatic components</b>	117
1-Effect of salt stress	120
2- Effect of neonicotinoids	120
<b>Non-enzymatic components:</b>	121
<b>Total protein</b>	122
-Quantitative assay	122
1-Effect of salt stress	122
2- Effect of neonicotinoids	122
<b>Proline Content</b>	123
1-Effect of salt stress	123
2- Effect of neonicotinoids	124
<b>Cotton yield and its Criteria.</b>	125
1-Effect of salt stress	125
2- Effect of neonicotinoids	127
<b>Effect of salt stress (brackish water) on the chemical structure of soil</b>	129
<b>Summary</b>	133
<b>Conclusion</b>	147
<b>Recommendations</b>	148
<b>References</b>	149
<b>Arabic summary</b>	

## SUMMARY

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### SUMMARY

One of the most serious obstacles that severely affected agriculture in Egypt is the shortage in fresh water resources that farmers have to use lower volumes of water than necessary or at least to spacing irrigation intervals which resemble a case of water deficit stress on cotton plants. Moreover, the Nile delta aquifer in Egypt severely subjected to seawater intrusion especially at the coastal governorates which means mixing fresh water with a ratio of seawater especially at sea level rise periods, beside the possibility of using a ratio of saline water with fresh water to overcome the shortage of fresh water resources. These cases resemble salt stress on cotton plants

For these reasons the current study was carried out in order to 1<sup>st</sup> determine the effect of both water deficit stress and salt stress on cotton plants. 2<sup>nd</sup> to assay the ability of two neonicotinoids IMI and TMX when used as seed treatment (at their recommended rates) for improving cotton plants performance and characteristics against both of biotic stress (some early cotton insects) and abiotic stress factors (water-deficit and salt stress).

### **Effect of some abiotic factors on cotton plants**

#### **I-Effect of water-deficit**

A field study was conducted to evaluate the potential of IMI and TMX as seed treatment to alleviate the negative effects resulted when cotton plants were exposed to partial environmental stress as represented by water shortage. Two irrigation models were used the first; which is currently used under local conditions (interval between two successive irrigations is 15 days) while, the second resembles environmental stressed in which interval between irrigation was longer than the usual (25 days). The obtained results were as follows;

## SUMMARY

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**Effect of water-deficit stress and/or some neonicotinoids on some early cotton insects.**

### **1-Effect of water-deficit**

Data showed that water shortage suppressed thrips densities along 7 weeks while the opposite was observed with aphid

### **2-Effect of neonicotinoids**

There were no significant differences between the efficiency of any of the used compounds when applied under usual irrigation system or that with a longer interval. Since, the two neonicotinoid insecticides achieved higher efficiency in controlling both thrips and aphid along five weeks with a percentage of reduction exceed 80% for thrips and aphid. Both of IMI and TMX still possess a moderate efficiency around 50% even after 6 weeks

**Effect of water-deficit stress and/or some neonicotinoids on some cotton plant growth criteria (morphological, physiological, biochemical, anatomical and yield)**

**Morphological Criteria; (plant height and leaf area)**

### **1-Effect of water-deficit**

It was found that, cotton plants growth and development was restricted by water deficit as reflected by a significant decrease in both plant height and leaf area along the 100 days. The recorded plant height with untreated check under the usual irrigation system was 18.8, 50.2, 83.7 and 104.2 cm compared to 15.6, 37.1, 68.9 and 89.9 cm with water deficit check after 25, 50, 75 and 100 days of cotton plant age respectively. On the other hand, the recorded values for leaf area were 39.2, 75.8, 112.8 and 113.7 cm<sup>2</sup> under usual irrigation system with respect to 29.3, 48.8, 62.5 and 71.25 cm<sup>2</sup> under stressed conditions.

## SUMMARY

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### **2- Effect of neonicotinoids**

The two tested neonicotinoids had an enhancing effect in cotton plant pertaining plant height and leaf area along the 100, either under stressed or unstressed conditions. The recorded plant height after 100 days of sowing were 114.5, 115.3 and 101.3 cm for IMI, TMX and usual control respectively. The corresponding attained values under water-deficit were 108.7, 109.6 and 89.8 cm with respect to IMI, TMX and stressed control respectively. As for leaf area, the two neonicotinoids didn't achieve significant improvement under usual irrigation system. Their effect under stressed conditions was significant and the corresponding obtained values after 100 days were 99.5, 101.6 and 71.25 cm<sup>2</sup>. with IMI, TMX and stressed check respectively.

### **Physiological Criteria**

#### **1-Effect of water-deficit**

Data Showed that, a decrease with the two parameters (CCI and MQY) was noticed due to water-deficit stress. Water-deficit significantly reduced maximum quantum yield of photosynthesis, as the corresponding values were 0.842, 0.841, 0.847 and 0.486 under usual condition compared to 0.835, 0.829, 0.838 and 0.837 under stressed condition after 25, 50, 75 and 100 days. Chlorophyll content index seems to be stable against water-deficit since, the recorded decrease was insignificant.

#### **2- Effect of neonicotinoids**

The two used neonicotinoids achieved significant increase in the MQY under both stressed and unstressed conditions. The recorded values after 100 days were 0.850, 0.853 and 0.846 for IMI, TMX and usual condition, compared to 0.848, 0.851 and 0.837 for IMI, TMX and water-deficit check. The resulted increase in CCI was not significant either under usual irrigation system or under water-deficit.



## SUMMARY

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### **Anatomical Criteria**

#### **1-Effect of water-deficit stress**

Data indicated a significant reduction of all the recorded anatomical criteria of cotton plant leaves as affected by water deficit (XT, XVD, PTT, VBT, VBW, PLT, STT, LT). As for stem traits, the thickness of xylem and phloem and xylem vessel diameter were significantly reduced, the thickness of cuticle layer was significantly increased while the value of all stem diameters were not significantly affected

#### **2- Effect of neonicotinoids**

Results showed that while XXT, XVD, PTR, VBT, AND VBW, values of cotton leaves restored to the same level attained with untreated plants that received usual irrigation or significantly more, the values of palisade, spongy tissues and the whole plant leaf thickness didn't return to the normal state although it was induced to a level significantly higher than the stressed check, while the presence of the two neonicotinoids overcome the negative effects of drought on stem anatomy that is reaches the normal level attained without stress in some criteria.

### **Biochemical Criteria**

#### **Enzymatic components**

##### **1-Effect of water- deficit**

It can notice that antioxidant enzyme activities were induced to a significant level that reached about two folds with catalase and peroxidase while in case of superoxide dismutase it was doubled more than three folds along the 100 days. With time progress, all the values of antioxidant enzyme activities were increased at all treatments indicating a stressed status induced by water-deficit. While, a slight but significant decrease was recorded with polyphenol oxidase activity due to water-deficit stress.

## SUMMARY

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### **2- Effect of neonicotinoids**

A significant decrease in the oxidative status of stressed and unstressed cotton plant was recorded. The recorded values of the three tested antioxidant enzymes were reduced to about 50 % of those recorded at the same interval. The current data showed that application of the two tested neonicotinoids improved cotton plant oxidative status. The two neonicotinoids significantly increased the activity of polyphenol oxidase of cotton plants after the four intervals under the two applied irrigation system.

### **Non-enzymatic components**

#### **Total protein**

##### **Quantitative assay**

##### **1-Effect of water-deficit**

The current results indicated a gradual but insignificant decrease was observed in total protein content of cotton leaves as affected by water-deficit along 75 days, but after 100 days the effect became significant.

##### **2-Effect of neonicotinoids**

The two neonicotinoids induced protein synthesis to a significant level either under usual or stressed irrigation systems. The resulted protein content after 100 days were 5.75, 5.83 and 4.2 mg/g fresh weight corresponding to IMI, TMX and the check under usual condition. while, under water deficit condition the recorded values were 5.6, 5.75 and 3.54 mg/g fresh weight corresponding to IMI, TMX and check at the same interval.

#### **Qualitative assay**

##### **1-Effect of water-deficit**

Water-deficit alter protein expression to a high extent that three new protein bands were exhibited under water deficit compared to control

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## SUMMARY

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that received usual irrigation those proteins have number of 1, 3 and 4. In addition a protein band of number 11 which presented in plants received usual irrigation but disappeared under water-deficit conditions.

### **2-Effect of neonicotinoids**

The two tested neonicotinoids completely changed the profile of protein either under normal or water deficit stress. An additional 8 protein bands with numbers 1, 2, 4, 5, 6, 7, 12, 13 are presented while maintain protein band number 13 which was disappeared under water-deficit condition.

### **Proline content**

#### **1-Effect of water –deficit**

The present study proved that water-deficit highly induced proline synthesis and accumulation in cotton plants to 4.2, 4.35, 5.2 and 4 folds of proline content after 25, 50, 75 and 100 days of sowing respectively

#### **2-Effect of neonicotinoids**

The two neonicotinoids insecticides had a significant decrease on proline contents of cotton plants survived under both usual and stressed irrigation systems. When neonicotinoids were applied under stress conditions, a significant decrease in proline content along the 100 days was recorded. The percentage of proline compared to its check with this case was 30.68 to 50.94 %.

### **Cotton yield Criteria**

#### **1-Effect of water- deficit**

A severe and deleterious effect of water deficit on cotton plants outcomes was recorded. The corresponding shortage reached 35.8, 37.7, 25 and 44.1% for number of fruiting branches/plant, number of dehiscent bolls /plant, weight of seed cotton/ dehiscent boll and weight of obtained seed cotton/plant respectively.

## SUMMARY

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### **2-Effect of neonicotinoids**

The effect was not significant under usual irrigation system. Under water deficit conditions application of the tested neonicotinoids significantly increased all criteria of cotton yield to reach the same level of significance attained under unstressed conditions. The shortage in all criteria ranged from 1.39 to 6.8 only compared to 25 to 44. 14% in the corresponding check.

### **II-Effect of salt stress**

This part of study was conducted in order to evaluate the adverse effects of using a mixture of seawater and freshwater if it happens in addition to study the possible roles of the tow tested neonicotinoids (IMI and TMX) when used as seed treatment at their recommended rates for protecting cotton plants against salt stress. Also to estimate the optimum mixing ratio(seawater/freshwater) as a partial solution for water shortage problem which maintain cotton plants and yield at their normal state and level.

**Effect of salt stress and/or some neonicotinoids on some cotton plant growth criteria (germination, morphological, physiological, biochemical, anatomical and yield)**

#### **Effect of salt stress of brackish water on cotton seeds germination**

An exploratory pot experiment was conducted in order to choose the proper mixing ratio of (salt/fresh water) in the following research which didn't have impact on cotton seed germination ratio or the emergence time either in the presence or absence of the two tested neonicotinoids. Data reflected that mixing seawater wit freshwater by 33 or 50% can't be used because a reduction in germination percentages ranged from 80 to100% was occurred either in presence or absence of neonicotinoids.

## SUMMARY

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Data indicated that, time consumed for the first emergence of cotyledons leaves took a relatively longer time either on the presence or absence of neonicotinoids was recorded when 33% dilution seawater was used, there was any recorded emergence at 50% dilution. On the other hand, dilution of 25% didn't alter the time of emergence but severely affected germination ratio especially in the presence of the two tested neonicotinoids. So, dilutions of 25,15 and 10% were used for the following pot experiment.

### **The effect of using brackish water for cotton plant irrigation on cotton plants**

Based on the aforementioned results of seed germination under different salinity levels and on personal observation of cotton plant performance that grown in pots irrigated by 25% seawater (SW)/ fresh water (FW) from the beginning of germination, it was decided that, the first stage of plant germination till 30 days of emergence must irrigated by fresh water only. So, brackish water was used after 30 days of emergence either at the pot or field experiment till the end of the experiment to choose the proper ratio of sea water/ fresh water at the field experiment.

### **Pot experiment**

#### **Morphological Criteria**

##### **1-Effect of salt stress**

Plant height and leaf area were significantly reduced as the level of salinity increased. In term of figure plant height was reduced from 74.9 cm to be 67.1, 61.8 and 47.0 corresponding to 10,15 and 25% seawater. The same trend was obtained with leaf area, since it was reduced from 61.1 cm<sup>2</sup> to 54.8, 48.5 and 37.7 respectively.

## SUMMARY

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### **2- Effect of neonicotinoids**

The use of both IMI and TMX resulted in a significant improvement for both criteria either with fresh water or under salt stress at any ratio of dilution. The resulted effect reached the same order of significance of the check only when 10% seawater was used. the two neonicotinoids can protect cotton plants against salinity stress at level of 10% only.

### **Physiological Criteria**

#### **1-Effect of salt stress**

No statistical differences as a result of salt stress in the two recorded criteria.

#### **2- Effect of neonicotinoids**

The values of CCI and or Fv/Fm when the two tested neonicotinoids were used or not were not significantly affected.

### **Anatomical Criteria;**

#### **1- Effect of salt stress**

Data reflecting a general tendency towards a significant reduction of all recorded leaf parameters as affected by salt stress.

#### **2- Effect of neonicotinoids**

Data showed that, all leaf anatomical criteria are induced to a significant higher level in the presence of all two compounds either under stress condition or not.

### **Biochemical Criteria**

#### **Enzymatic**

##### **1-Effect of salt stress**

The activity of the three antioxidant enzymes Superoxide dismutase, Catalase and Peroxidase were induced by salt stress either to a significant or insignificant level but each enzyme had its own pattern. On

## SUMMARY

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the opposite side Poly phenoloxidase activity was significantly decreased at all levels of salt stress.

### **2- Effect of neonicotinoids**

The two neonicotinoids succeeded to reduce the oxidative state of cotton plant by significant values when used under irrigation by fresh or brackish water at level of 10% dilution only. In case of Poly phenoloxidase its activity was significantly increased as a response for using the two insecticides at any level of salinity.

### **Non enzymatic**

#### **Effect on protein and proline contents of cotton plants**

##### **Quantitative**

#### **1-Effect of salt stress**

Salinity at any of the used ratios didn't significantly alter protein levels of cotton plants, while proline contents were increased from 2.67 $\mu$ mole/gm FW of cotton leaves to 9.4, 36.8 and 62.8 in freshwater 10,15 and 25%SW treatments respectively.

#### **2- Effect of neonicotinoids**

Insignificant effect was recorded with the two tested neonicotinoids on cotton protein content either of cotton plants which received fresh or brackish water. The two neonicotinoids succeeded to achieve significant decrease in proline content in all cases, but proline content still above the normal level in all cases with lowest value of reducing about two folds only when the two compounds are applied with ratio of 10% seawater.

## SUMMARY

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### Qualitative

#### 1-Effect of salt stress

The numbers of detected protein bands under fresh water irrigation were 6 bands only where salt stress induced two more bands. salt stress might inhibit or compensate the synthesis of protein of bands number 4, 5 and 8 to a lower level compared to those obtained with plants received fresh water irrigation.

#### 2- Effect of neonicotinoids

Six additional bands that not presented in the check that used fresh water were scanned with numbers 3, 6,7,9,12 and 13 in addition to increase of intensity of protein bands

### Semi-field Experiment

A semi-field experiment was conducted in order to evaluate the previous treatments (pot experiment) under semi-field conditions. Depending on pot experiment data the level of 25%SW was excluded, therefore two levels only were evaluated 10,15% dilutions.

### Morphological Criteria

#### Effect of salt stress

As sea water ratio increased from 0 to 10 and 15%SW plant heights were 108.6,102.3 and 93.7 cm respectively. The significant effect was 15% dilutions. The same trend was recorded with leaf area since it was reduced from 114.2to 97.2 and 84.3 cm<sup>2</sup> respectively.

#### 2- Effect of neonicotinoids

IMI and TMX didn't significantly mitigated the adverse effects of salinity on plant height under the two tested levels of salinity. The two compounds significantly increased the leaf area at level of 15% SW dilution only.



## SUMMARY

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### **Physiological Criteria**

#### **1-Effect of salt stress**

when sea water was diluted to 10% with fresh water CCI didn't significantly affected while, a significant increase in chlorophyll content at 15% level of seawater.

#### **2- Effect of neonicotinoids**

A significant increase in CCI was recorded with the two neonicotinoids when fresh water was used for irrigation only. In addition, the use of the two neonicotinoids at the two levels of salinity induced chlorophyll content to a higher significant values.

### **Biochemical Criteria**

#### **enzymatic**

##### **1-Effect of salt stress**

Significant increase was recorded on all enzyme activity values either at 10% or 15%SW dilution. The noticed increase was significantly increased as the salt increased.

##### **2- Effect of neonicotinoids**

A significant decrease on the activity of the three tested enzymes was recorded either at the check which received fresh water irrigation or the two levels of brackish water irrigation. The two neonicotinoids were succeeded to make recovery in cotton plants that the induced level of the three tested antioxidant enzyme as a response to salt stress (10%) only.

### **Polyphenol oxidase enzyme activity**

#### **Effect of salt stress**

Salt stress wasn't affected poly phenol oxidase activity significantly

#### **2- Effect of neonicotinoids**

Treatment with the tested neonicotinoids resulted in a significant increase in poly phenoloxidase activity when compared to the corresponding check.

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### **Non enzymatic**

#### **Effect on protein and proline contents of cotton plants**

##### **Quantitative assay**

###### **1-Effect of salt stress**

Protein content was increased from 4.85 to 5.63 and 6.4 mg/g F.W. corresponding to fresh water 10 and 15%SW.

###### **2- Effect of neonicotinoids**

At any level of salinity there were no significant differences in protein content either in the absence or presence of the tested neonicotinoids but the two compounds induce protein level to significant level when data compared with that of the fresh water check.

#### **Effect on proline**

##### **1-Effect of salt stress**

The use of brackish water 10%SW induced proline content by 2.99 folds while salt stress while salt stress increase to 15%SW the resulted induction effect reached to 5.26 folds.

##### **2- Effect of neonicotinoids**

Neonicotinoids treatment significantly reduced the up rise of proline content to less significant level with a value around 0.6 ratio of the corresponding control.

#### **Cotton yield Criteria**

##### **1-Effect of salt stress**

Data reflected that salt stress adversely affected all the recorded parameters and this harmful effect was increased as salt stress was increased. Number of bolls/ plant significantly decreased by 19.2 and 31.52% as a result of using 10 and 15%SW dilutions respectively. The obtained seed cotton/ plant was reduced by 14.58 and 27.51% respectively.

##### **2- Effect of neonicotinoids**

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## SUMMARY

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It can conclude that the two tested neonicotinoids improved cotton plant performance and growth which resulted in a noticeable increase in all cotton yield criteria in all treatments. In term of figures, whereas the number of bolls/plant reduced by 19.2% as affected by salt stress, the two neonicotinoids attained 7.6 and 7.8% reduction only corresponding to IMI and TMX respectively. In addition, the obtained seed cotton/plant was reduced by 14.56% as affected by 10%SW, while this reduction was 6.64 and 7.68 only when the two neonicotinoids were used. The obtained values of number of bolls/ plant under 15%SW was 31-25% reduction compared to 23.09 and 23.91% when IMI and TMX were used. The shortage in seed cotton/ plant under salt stress15% was 27.15% which became 23.05 and 23.4when the neonicotinoids were used.

### **Effect of salt stress on the chemical structure of soil**

Data revealed that the use of 10 and15%SW increased soil EC from 8.16 to 10.6 and 13.7 ds/m at the end of the season. Sodium ions (the most critical ions) was increased from 30.9 to 90.5 and 122.2mg/ L. chloride ions were increased from 37.8 to be 65.4 and 116.4 mg/L. finally potassium ion was increased from 2.4to 3.54 and 4.1 mg/L respectively.