

## **EFFECT OF SPRAYING SOME ORGANIC, AMINO ACIDS AND POTASSIUM CITRATE ON ALLEVIATION OF DROUGHT STRESS IN COTTON PLANT**

**Gebaly, Sanaa G. ; Fatma M. M. Ahmed and Alia A. M. Namich  
Cotton Res. Institute, Agric. Res. center, Giza.**

### **ABSTRACT**

Two field experiments were carried out to investigate effects of spraying organic acids and amino acids and combinations between organic acid and potassium citrate or amino acid and potassium citrate under drought stress. The objectives of these studies were aimed to investigate the effect of organic and amino acids application and potassium citrate on plant growth characters, yield and yield components, leaf chemical constituents, enzyme activity as well as seed quality and fiber properties of Giza 86 cotton cultivar. The experiments were conducted at Sakha Agricultural Research station, during 2009 and 2010 seasons.

Plant irrigation was carried out regularly at plant needs using tap water up to the start of flowering stage then, some of plants subjected to drought conditions by preventing water supply till the appearance of sign of wilting and spraying with organic acids or amino acids and potassium citrate, leaving the control plants without treatments.

Drought conditions significantly decreased plant height, number of fruiting branches, chloroplast pigments i.e., chlorophyll a, b, a + b, carotein and catalase activity in cotton leaves, while the contrary was obtained in proline content in leaf and peroxidase activity in comparison with the control. Also, reduction in yield and yield components was observed as results of water stress.

The results clearly showed that spraying cotton plants with organic acid, amino acid and combinations between them and potassium citrate under drought conditions tended to increase growth parameters i.e., plant height, number of fruiting branches, number of open bolls per plant, seed index, boll weight, lint percentage, seed cotton yield per feddan and some chemical content in cotton leaves i.e., chlorophyll a, b, total chlorophyll and carotein, proline and antioxidant enzyme activities.

On the other hand, no significant effects with application of organic and amino acids on fiber properties.

**Keywords:** Cotton, organic acid, amino acid, potassium citrate, growth characters, yield components, leaf chemical composition and fiber properties.

### **INTRODUCTION**

Water stress is considered the most agronomic variable affecting crop production worldwide. In Egypt, the forthcoming water shortage, though it is currently not well recognized by the agro-public, is a true challenge facing agricultural development and crop production in particular. Under such pressing threat of water limitations, irrigation water should be efficiently utilized so that water savings could be used in other agricultural activities. Water is generally considered the most limiting factor in higher plants than any other single environmental factor. Exposing cotton plant to water stress especially during the flowering stage, adversely affected plant growth and

productivity, (Meek *et al.*, 2003 and Ahmed and Kassem, 2008). Therefore, it seems imperative to work for improving water use efficiency for major crops including cotton which could be approached by searching for means helping in promoting drought tolerance of cotton plants attempt to tolerate or resist stresses due to decreased water availability, by making osmotic adjustments to cells through increase in organic ions or organic solutes (Ashraf and Foolad, 2007).

Citric acid is one of the organic acids presented in tricarboxylic acid cycle or malic acid conversion to citric acid. Growth of cotton, corn, bean, pea and sunflower increased by organic acids external treatment especially succinic, citric and malic acids (Nofal *et al.*, 1990). Ghourab (2000), reported that the application of citric acid increased significantly plant height, No. of fruiting branches, seed index, lint %, earliness and seed cotton yield/fed. Ascorbic acid is a common antioxidant component in the apoplast and it affects plant growth and many physiological processes. With regard to cotton crop, Namich (2006) found that ascorbic acid application increased plant height, No. of fruiting branches, No. of open bolls, boll weight and seed cotton yield/fed. Salicylic acid (SA) is an endogenous regulator of phenolic nature, which is involved in the regulation of various physiological processes in plants (Shakirova *et al.*, 2003) and in alleviating the deleterious effect of drought stress (Bideshki and Arvin, 2010). It improves plant growth, enzyme activities and ion uptake and transport (Khan *et al.*, 2010).

Proline plays an adaptive role in the tolerance of plant cells to salinity and water stresses by increasing the concentration of cultural osmotic components in order to equalize the osmotic potential of the cytoplasm. (Ashraf and Foolad, 2007). Proline has been exogenously applied to a variety of crops in an effort to improve salt stress tolerance and yield. It also may cause cytoplasmic acidosis and maintaining appropriate NADP<sup>+</sup>/NADPH ratio compatible with metabolism. Hossain and Fujita (2010), found that proline plays role as an osmolytes for osmotic adjustment, buffering cellular redox potential (under stress conditions). In the case of osmo regulation the compatible solute glycinebetaine (GLybet), a small organic metabolite soluble that can potentially play a crucial role in effective protection against salt, drought and extreme temperature stress, (Ashraf and foolad, 2007 and Chen and Murata, 2008).

The naturally occurring quaternary ammonium compound and glycinebetaine has received attention as compatible solute that may aid in drought tolerance by allowing maintenance of turgor pressure (Meek *et al.*, 2003). Glycinebetaine also protects physiological processes such as photosynthesis and protein synthesis from the result of water deficit and other stresses (Meek *et al.*, 2003). Glycinebetaine has been exogenously applied to a variety of crops in effort to improve stress tolerance and yield. Some of crops include cotton (Gorhan and Jokinen, 1998), Maiz and Sorghum (Agboma *et al.*, 1997). Namich and Emara (2008) found that spraying cotton plants with glycinebetaine under normal and drought conditions increase physiological processes i.e., stomatal conductance, photosynthetic rate and this tended to a significant increase in growth parameters and some chemical contents in cotton leaves. Potassium (K) plays a particular role in stress

physiology. It is the nutrient that contributed most to the tolerance of plants to various environmental stresses (Cakmak, 2005), also K increases water uptake and transport in plants and its role on the control of osmotic pressure is vital under conditions of water stress (Marchand, 2007). It has been shown that adequate K supply enhances cotton growth and productivity (Hamed, 2007).

The present study was aimed to investigate the influence of organic acid, amino acid and potassium citrate and their combinations on growth characteristics, yield and chemical constituents of cotton plant under drought stress.

## **MATERIALS AND METHODS**

Two field experiments were carried out at Sakha Agricultural Research Station, during two successive seasons of 2009 and 2010 to study the effect of spraying the bio-nutrients (Ascorbic acid, salicylic acid and citric acid). Amino acids (Glycine and proline) and potassium citrate on growth, earliness, seed cotton yield and its components and some of leaf chemical composition of the Egyptian cotton Giza 86 cultivar under drought conditions. The experimental design was randomized complete blocks with four replicates. The preceding crop was rice in the two seasons. The size of each plot was (4 x 3.5) m<sup>2</sup> i.e. 14 m<sup>2</sup> including seven rows which were 60 cm apart, the seed were planted in the last week of April in both seasons. Nitrogen fertilizer was added in bands and divided in two equal portions; the first one was applied after thinning just before the second irrigation and the second part before the third irrigation. Irrigation treatments were carried out as regularly after 15 days between (control) until the second dose of nitrogen fertilizer was added and so drought treatments were done monthly. Other cultural practices were done as recommended in cotton production that is involved a basic dose of 150 kg calcium superphosphate (15.5 % P<sub>2</sub>O<sub>5</sub>) during land preparation besides 50 kg potassium sulphate (48 % K<sub>2</sub>O) per feddan before the fourth irrigation for all plots.

Six bio-nutrients treatments were applied as follows:

1. Control treatment
2. Control stress.
3. Spraying organic acids (Citric acid at 500 PPM, Ascorbic acid at 300 PPM, Salselic acid at 200 PPM).
4. Spraying amino acids (GLycin at 600 PPM, Proline at 200 PPM).
5. Spraying organic acids (CA, ASA, SAA) + spraying potassium citrate at 2.5 g/L.
6. Spraying amino acids (GL, Pro) + spraying potassium citrate at 2.5 g/L.

Potassium, organic and amino acids were sprayed twice, i.e., after the second dose of nitrogen fertilizer and 30 days after.

Soil samples were taken in the two seasons before planting cotton to estimate the soil characters as described by Chapman and Parker (1981). The results of soil chemical analysis presented in Table 1.

**Table 1: Chemical analysis of the experimental soil in 2009 and 2010 seasons.**

Soil characteristics	2009	2010
Texture	Clay loam	Clay loam
Organic mater %	1.89	1.95
E.C. (soil paste at 25°C)	8.50	8.75
PH	8.00	8.20
Available N (K sulphate ext.)	16.90 PPm	17.20 PPm
Available P (olsen ext.)	23.90 PPm	24.10 PPm
Available (Amm. Acetate ext.)	213.60 PPm	215.00 PPm

Soil and water Res. Inst., Agric. Res. Center, Giza, Egypt.  
At the picking time, the following characters were recorded:

1. Plant height (cm).
2. Number of fruiting branches per plant.
3. Number of open bolls per plant.
4. Boll weight gm.
5. Seed index gm.
6. Lint percentage (%)
7. Earliness percentage (%)
8. Seed cotton yield/feddan in kentars.
9. Fiber properties:

Micronaire value and Pressley index were measured at the Laboratories of Cotton Research Institute, under the stander conditions of test ( $65 \pm 2$  relative humidity and  $76^{\circ}\text{f}$  temperature) according to (A.S.T.M., 1975).

#### **Chemical analysis of leaves:**

The fourth leaf from stem apex was collected after 15 days from the second spraying and used for determination the chemical constituents.

##### **1. Determination of Pigment content:**

The chlorophyll a, b and total chlorophyll were determined according to the method describes by (Arnon, 1949) and carotenoids using the method of (Rolbelen, 1957).

##### **2. Determination of proline content:**

Proline content of cotton leaves were determined according to method of (Bates *et al.*, 1973).

##### **3- Determination of enzyme activity:**

Catalase (EC 1.11.1.6) was measured according to the method described by (Jaleel *et al.*, 2007). The activity of peroxidase (EC 1.11.1.7) was assayed as described by (Jaleel *et al.*, 2007).

The differences between means of treatments were statistically tested as described by (Snedecor and Cochran, 1981) using L.S.D. values at 5 %.

## RESULTS AND DISCUSSION

### Reproductive characters:

The results presented in Table 2 clearly indicated that drought stress exerted a significant influence on all growth characters in both seasons. It could be noticed that plants subjected to water stress conditions (control) decreased plant height, number of fruiting branches per plant, number of open bolls per plant, boll weight and seed cotton yield per feddan. On the contrary, foliar application of organic acids, amino acids and combinations between organic acid and potassium citrate or amino acid and potassium citrate increased all studied growth characters as compared with the drought stress (control) in both seasons.

**Table 2: Effect of foliar spraying of organic, amino acids, organic acids + potassium citrate and amino acids + potassium citrate under drought conditions on plant height and yield and yield components of Egyptian cotton Giza 86 cultivar during 2009 and 2010 seasons.**

Treatments	Plant height (cm)		No. of fruiting branches / plant		No. of open bolls / plant		Boll weight (g)		Seed cotton yield Kentar/fed.	
	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
1. Control (Normal).	113.8	128.8	13.2	12.6	12.9	13.0	3.01	2.72	9.70	10.72
2. Control (Stress)	93.3	126.1	11.5	12.0	10.98	12.50	2.80	2.75	8.33	9.15
3. Organic acids	99.0	128.6	12.3	12.5	13.41	14.60	2.89	2.81	10.22	10.86
4. Amino acids	97.0	130.3	11.7	12.4	13.14	13.80	2.79	2.74	10.01	10.61
5. Organic acids + P. citrate	100.5	129.8	12.1	12.5	14.31	17.70	2.91	2.92	10.29	12.82
6. Amino acids + P. citrate	94.0	132.0	12.4	12.3	11.43	14.40	2.81	2.691	8.68	9.94
LSD at 5 %	NS	NS	NS	NS	1.74	1.16	NS	NS	1.41	1.28

### 1- Growth and reproductive characters:

The results given in Table 2 could be shown that plant height and number of fruiting branches were insignificantly affected by organic acids and amino acids applications of all treatments.

In general organic acids (CA, ASA and SA) application lead to increase in plant height and number of fruiting branches per plant. This result may be due to the stimulating effect of ASA on plant growth involved in the regulation of cell-wall extensibility. The previous results could be interpreted that the low PH which occurred as a result of spraying ascorbic acid (ASA) activated enzymes in cell-wall that mediate cell extension e.g., glycosyltransferase.

The results obtained go along with these of El-Shazly and Morsi (2003) and Namich (2006).

It is clear from results in Table 2 that foliar application of amino acids had significant increase in all studied growth characters as compared with

the control in both seasons. The pronounced increase in vegetative growth due to spraying glycine betaine may be a result of a corresponding increase in photosynthesis pigment, photosynthesis rate and carbohydrates content. Glycine betaine as a source of carbon (acetyl Co-A) the availability of carbon in the vicinity of the leaf enhances the photosynthesis rate. These results are in agreement with findings of Ashraf and Foolad (2007) and Namich and Emara (2008).

## 2- Yield and Yield components:

Results in Tables 2 and 3, clearly showed that the effect of foliar application of organic acid, amino acid and potassium citrate on yield characters of cotton plants under drought conditions. As shown previously, many differences in yield characters of cotton plants in response to different treatments. Application of organic acids, amino acids and the combination between organic acid and potassium citrate or amino acid and potassium citrate were significantly increased number of open bolls per plant, seed index and seed cotton yield per feddan as compared with the control in both seasons.

**Table 3: Effect of foliar spraying of organic, amino acids, organic acids + potassium citrate and amino acids + potassium citrate under drought conditions earliness, yield components and fiber properties of Egyptian cotton Giza 86 cultivar during 2009 and 2010 seasons.**

Treatments	Earliness (%)		Lint %		Seed index (g)		Micronaire reading		Pressley	
	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
1. Control (Normal).	62.30	62.37	39.80	39.50	10.04	9.96	4.70	4.50	9.90	10.40
2. Control (Stress)	60.60	64.90	39.73	39.50	9.33	9.96	4.70	4.50	9.90	10.30
3. Organic acids	60.70	65.28	41.39	40.10	9.89	9.99	4.70	4.50	9.80	10.10
4. Amino acids	61.80	65.17	40.58	40.19	9.89	10.15	4.80	4.40	9.90	10.30
5. Organic acids + P. citrate	60.90	65.50	40.59	40.26	10.18	10.32	4.80	4.50	10.00	10.30
6. Amino acids + P. citrate	66.40	65.10	41.59	39.98	9.69	9.60	4.80	4.40	9.90	10.10
LSD at 5 %	NS	NS	NS	NS	0.28	0.18	NS	NS	NS	NS

The highest number of open bolls, boll weight, seed index and seed cotton yield was obtained from using organic acids and potassium citrate in both seasons. In this respect, El-Shazly and Morsi (2003) and Namich (2006) found that ascorbic acid application led to significant increase in number of open bolls/plant boll weight and seed cotton yield/feddan. Concerning boll weight, earliness and lint percentage during the two seasons, there were insignificant effects with the application of organic and amino acids and potassium citrate on these characters.

## 3- Fiber properties:

Micronaire reading and Pressley index were not significantly affected by foliar application of organic acids, amino acids or by the combinations between organic, amino acids and potassium citrate.

#### **4- Chemical constituents of cotton leaves:**

Cotton leaves obtained from this experiment were employed to determine their contents of chlorophyll a, b, total chlorophyll, carotenoids, proline and determine the antioxidant enzyme activities (catalase and peroxidase). The obtained results are illustrated graphically in Figs. (1-4) showed that the contents of chlorophyll a, b, total chlorophyll and carotenoids of stressed cotton plants were decreased in comparison with control plants. Spraying of cotton plants with application of organic acid, amino acid and the combination between organic acid or amino acid and potassium citrate under drought conditions increased chlorophyll a, b, total chlorophyll and carotenoids contents of cotton plants to be near control plants under normal conditions. This increasing in pigments content of cotton plants is varied between treatments. The results generally indicated that chlorophyll a, b, total chlorophyll and carotenoids contents were significantly increased as a result of foliar application of organic acid and potassium citrate in comparison with amino acid and potassium citrate. Similar results were obtained by Namich and Emara (2008).

Results presented in Fig. 5 indicate that proline content in leaves was significantly increased by drought treatment in cotton plant, free proline content in control plants was very low, while it rapidly rises in plants exposing to drought. The foliar application of organic acid or amino acid and potassium citrate on cotton plants under drought conditions increased the contents of proline in comparison with untreated stressed plants. In this study higher level of proline accumulation in cotton plants enabled the water stressed plants to maintain low water potentials. By decreasing water potentials, proline accumulation involved in osmoregulation appeared to allow additional water to be taken up from the environment.

Catalase and peroxidase are antioxidant enzymes that protect cells from oxidative stress of highly reactive free radicals. The results graphically illustrated in Figs. (6-7) showed that foliar application of organic acid, amino acid and the combination between organic acid or amino acid and potassium citrate to cotton plants under drought conditions increased the activities of catalase and peroxidase in comparison with control plants. The results showed that the foliar application of organic acid or amino acid have induced all antioxidant enzyme activities in cotton plants under drought conditions, which may be related to the induction of antioxidant responses enzymatic and non-enzymatic that protect the plant from oxidative damage. This finding was in agreement with those reported by Khalil *et al.* (2009) they found that the activities of the antioxidant enzymes (peroxidase) in wheat shoots exposed to the high temperature stress for 4 or 8 hrs. were significantly increased, while superoxide dismutase and catalase were decreased as compared with those of control plants. These results were in good conformity with that reported by Magdy *et al.* (2012) they studied the effect of the exogenous application of SNP, PUT or GB to cotton plant resulted in enhancement of growth and yield and increasing of pigments content, proline and antioxidant enzyme activities during water stress as compared to untreated plants.

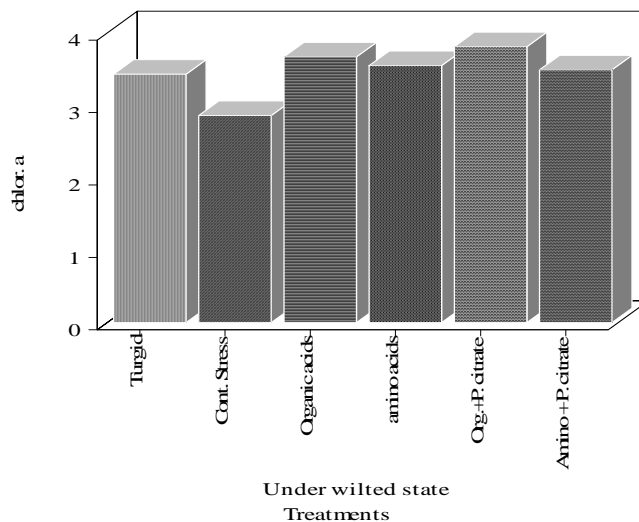


Fig. 1: Effect of organic acids, amino acids, (org. + P. citrate) and (amino + P. citrate) on Chlorophyll a.

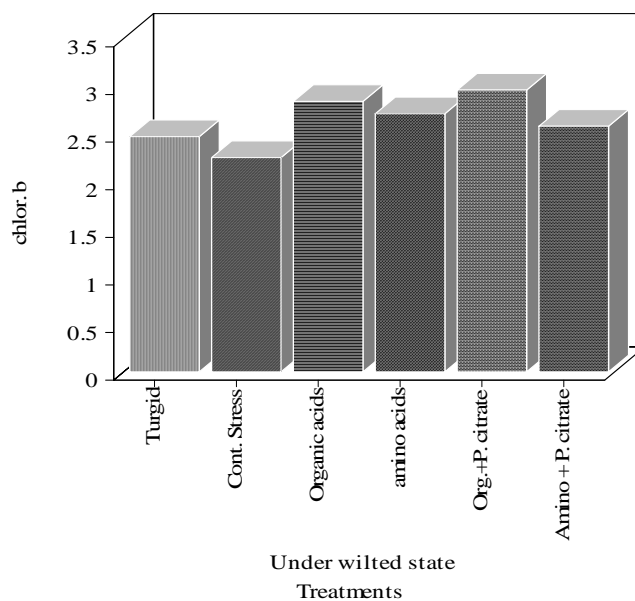


Fig. 2: Effect of organic acids, amino acids, (org. + P. citrate) and (amino + P. citrate) on Chlorophyll b.



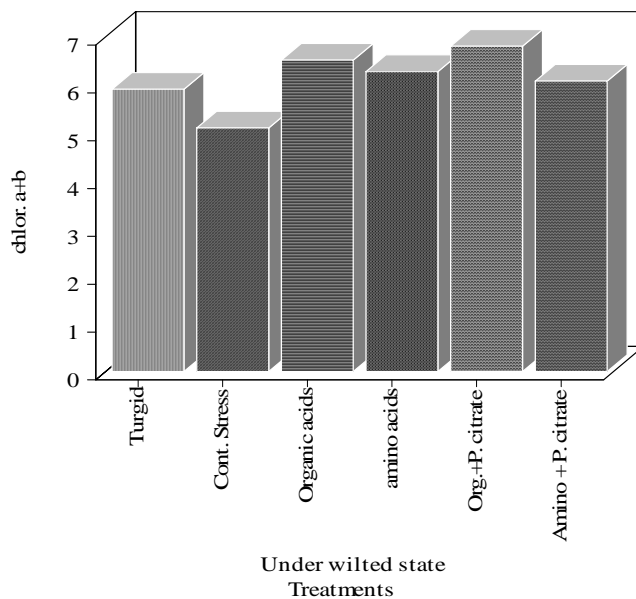


Fig. 3: Effect of organic acids, amino acids, (org. + P. citrate) and (amino + P. citrate) on Chlorophyll (a+b).

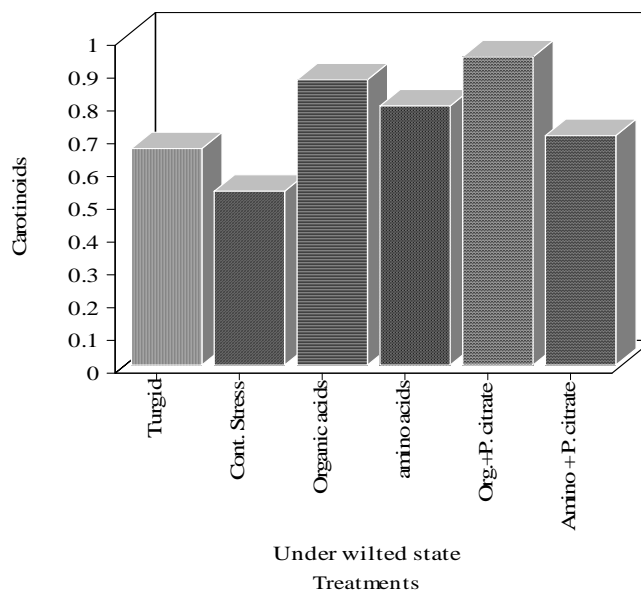


Fig.4: Effect of organic acids, amino acids, (org. + P. citrate) and (amino + P. citrate) on Carotenoids.

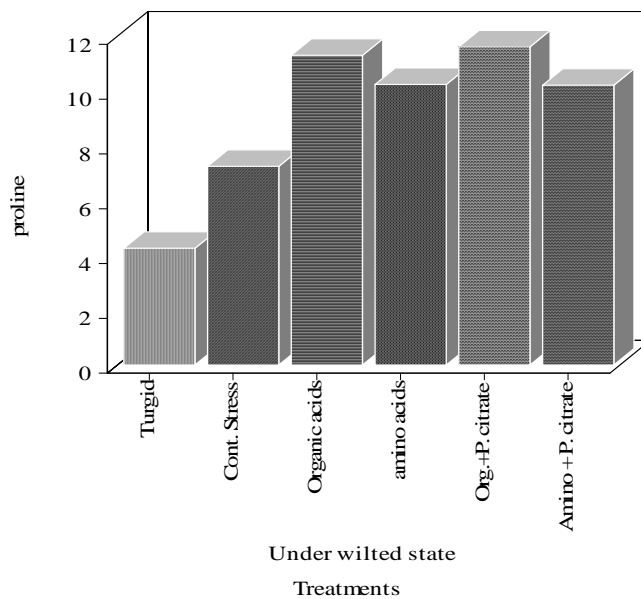


Fig. 5: Effect of organic acids, amino acids, (org.+ P. citrate) and (amino+ P. citrate) Proline.

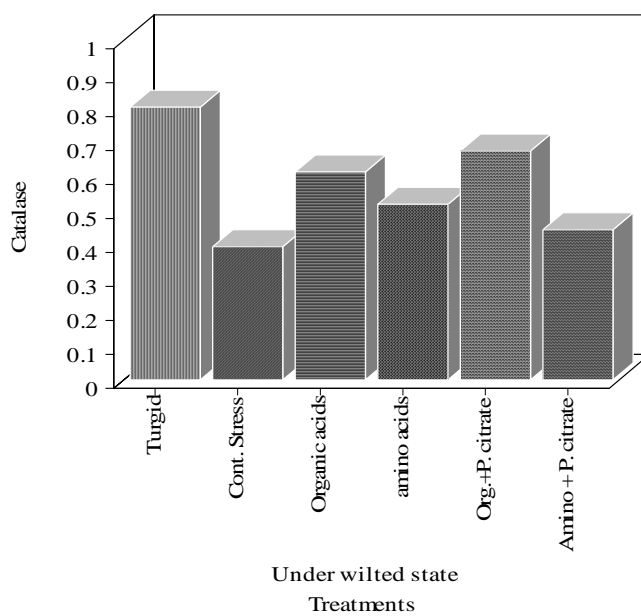
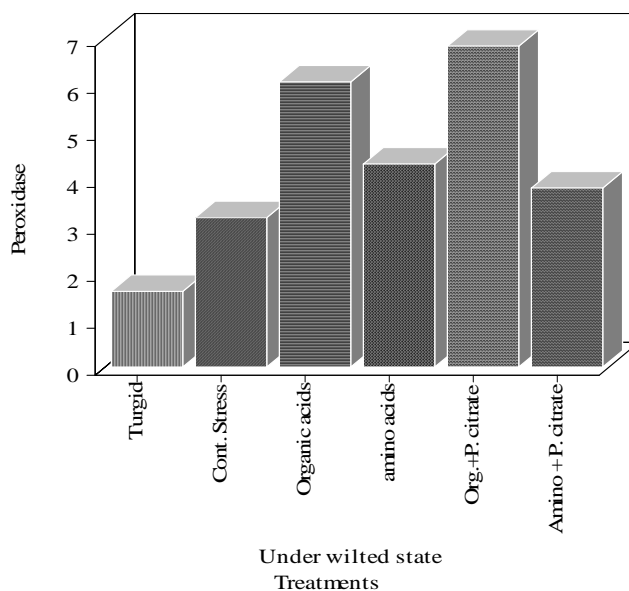


Fig. 6: Effect of organic acids, amino acids, (org.+ P. citrate) and (amino+ P. citrate) on catalase.



**Fig. 7: Effect of organic acids, amino acids, (org.+ P. citrate) and (amino+ P. citrate) on peroxidase.**

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### تأثير الرش ببعض الأحماض العضوية والأمينية والبوتاسيوم في تخفيف الأثر الضار للتعطيش في نبات القطن

سناء جمعه جبالى ، فاطمه محمد محمد أحمد و عالية عوض محمود ناميش  
معهد بحوث القطن – مركز البحوث الزراعية – جيزة - مصر

أقيمت تجربتان حقليتان لدراسة تأثير الرش بالأحماض العضوية والأحماض الأمينية وكذلك الأحماض العضوية مع سترات البوتاسيوم والأحماض الأمينية مع سترات البوتاسيوم تحت ظروف الجفاف. وتهدف الدراسة إلى معرفة تأثير الرش بالأحماض العضوية والأحماض الأمينية على صفات النمو والمحصول ومكوناته ومحتوى الأوراق من المكونات الكيميائية والنشاط الإنزيمى لصف القطن جيزة ٨٦ المنزرع بمحطة البحوث الزراعية بسخا (محافظة كفر الشيخ) خلال الموسمين ٢٠٠٩ – ٢٠١٠. حيث تم رى النباتات بانتظام باستخدام مياة الصنبور حتى بداية التزهير ثم تم تعريض بعض النباتات للتعطيش لحين ظهور علامات الجفاف على النباتات ثم رشت النباتات بالأحماض العضوية وكذلك بالأحماض الأمينية ومخلوط الأحماض العضوية مع سترات البوتاسيوم ومخلوط الأحماض الأمينية مع سترات البوتاسيوم مع ترك جزء بدون معاملة (كنترول). وكانت النتائج المتحصل عليها كالاتى:

١. أظهرت النتائج أن التعطيش أدى إلى نقص معنوى فى طول النبات وعدد الأفرع الثمرية ونقص محتوى الأوراق من الصبغات النباتية مثل كلورفيل أ ، ب والكلورفيل الكلى والكاروتين ونقص بعض الإنزيمات المضادة للأكسدة (بيروكسيداز و الكتاليز). بينما حدث العكس بالنسبة لمحتوى الأوراق من البرولين ونقص فى المحصول ومكوناته نتيجة التعطيش.
٢. أوضحت النتائج أن الرش بالأحماض العضوية أو الأمينية أو كلاهما مع سترات البوتاسيوم تحت ظروف التعطيش أدى إلى زيادة معنوية فى عدد الفروع الثمرية وعدد اللوز المتفتح على النبات ومعامل البذرة (وزن ١٠٠ بذرة) ومتوسط وزن اللوزة ومحصول القطن الزهر للقدان.
٣. أشارت نتائج إلى أنه لم يكن للرش بالأحماض العضوية والأمينية تأثيراً معنوياً على النسبة المئوية للتبكير والنسبة المئوية للشعر كذلك الصفات التكنولوجية للألياف.
٤. أظهرت النتائج أن الرش بالأحماض العضوية والأمينية وكلاهما مع سترات البوتاسيوم إلى زيادة معنوية فى كل صور الكلورفيل أ ، ب والكلورفيل الكلى والكاروتين والبرولين ، وأيضاً زيادة نشاط الإنزيمات المضادة للأكسدة (بيروكسيداز و الكتاليز).
٥. أوضحت النتائج أن رش الأحماض العضوية مع سترات البوتاسيوم إلى زيادة معنوية فى صفات المحصول وكذلك الصفات الكيميائية فى الورقة.

يمكن أن نستخلص من هذه الدراسة أن الرش بالأحماض العضوية (ستريك ، أسكوريك ، سالسيلك) أو الأحماض الأمينية (برولين ، جليسين بتاين) أو كلاهما مع سترات البوتاسيوم يؤدي إلى بعض التحورات المورفولوجية والبيوكيميائية بالنبات والتي تحسن من صفات النبات تحت ظروف العطش مما يعنى إمكانية إستخدامها فى زيادة مقاومة القطن للعطش.

قام بتحكيم البحث

كلية الزراعة – جامعة المنصورة  
مركز البحوث الزراعيه

أ.د / على السعيد الشريف  
أ.د / محمد حامد عبد العال