PHYSIOLOGICAL STUDIES ON THE EFFECT OF CITRINE ON GROWTH, YIELD AND SOME CHEMICAL CONSTITUENTS OF MAIZE (*Zea mays* L.) PLANTS

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ABSTRACT

Two field experiments were carried out during the two successive seasons of 2005 and 2006 to investigate the effect of foliar application of citrine (a compound fertilizer containing citric acid with some micronutrients) on growth, yield and some chemical constituents of maize (*Zea mays* L.) plants. The plants were grown in clay soil, and foliar sprayed with eleven treatments (0, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45 and 0.5%) of combined fertilizer (citrine) which contains (15% citric acid, 2% Fe, 2% Mn and 2% Zn). Generally, it was found that all studied vegetative growth parameters (i.e. plant height, stem diameter, number of leaves /plant, dry weight of leaves) as well as grain yield /fed. and some of their components (i.e. ear length, ear diameter, number of rows/ear, number of grains/row, grain weight/ear, weight of 100 grain and ear weight/plant) and some chemical constituents of leaves **(**chlorophyll a, b, total caroteniods, anthocyanin, total carbohydrates, total and reducing sugars, total free amino acids, total indoles, nitrogen, phosphorous and potassium) and grain protein %, were increased with application of the different treatments. The maximum values were obtained from the treatment of 0.3%. On the contrary citrine treatments decreased reducing sugars and free phenol in leaves as compared to the control. The best results were obtained by the application of citrine treatment at 0.3%. Hence, it can recommend to use citrine fertilizers as foliar application at the rate of 0.3% for improving growth, yield and chemical constituents of maize plants.

Key Words: Maize plants, (*Zea mays* L.) citric acid, micronutrients, Fe, Mn and Zn fertilizers, growth, yield, chemical constituents.

INTRODUCTION

Maize plants, (*Zea mays* L.) is one of the most important cereal crops principally during the summer season in Egypt. Maize grain is used for human consumption, animal and poultry feeding as well as for industrial purposes. In Egypt, great attention has been paid to increase its total production. Therefore, efforts are being focused on increasing the productivity of maize crop by growing high yielding varieties and /or improving the agronomic practice such as fertilization. Antioxidants such as citrine have an auxinic action due to their own citric acid. They have a synergistic effect on growth, yield and some chemical constituents of many crops as well as controlling the incidence of most fungi on many crops **(Ahmed *et al.*, 1997a and Ahmed *et al.*, 1998a** on apple; **Ahmed *et al.*, 1997b; Ahmed *et al.*, 1998b; Ahmed *et al.*, 2002 and Ahmed** and **Abd El-Hameed, 2004** on grapevine; **Shehata *et al.*, 2000** on cotton**; Shehata *et al.*, 2001** on maize; **Zaghlool *et al.*, 2001 on** dry bean; **Ahmed *et al.*, 2003** on banana; **Zaghlool *et al.*, 2006** on wheat**, Rady, 2006** on sunflower **and Shalaby, 2006** and **El Yazal, 2007** on onion plants. The beneficial effect of micronutrients on growth, yield and some chemical constituents of maize plants

as reported by several workers (**Mohamed, 1998; Abd El-Maksoud *et al*., 2000; Osman, *et al*., 2001; El-Mahdy, 2001; Yakout *et al*., 2001** and **Allam *et al*., 2001** on maize; **Rehan *et al*., 2003; Seaf El-Yazal** and **Sayim, 2004** and **Mabrouk** and **Zayed, 2004** on faba bean)**.** However in Egypt, soils suffering from some deficiencies of micronutrients especially Fe, Zn and Mn **(Fawzi, 1991)**. Accordingly, the aim of this work was to study the effect of exogenous application of citrine (citric acid, Fe, Zn and Mn) at different rates on growth, yield, as well as some chemical constituents of maize plants during 2005 and 2006 seasons by the aim of increasing maize productivity.

MATERIALS AND METHODS

This investigation was carried out during the two successive seasons of 2005 and 2006 at the Experimental area in clay soil at Faculty of Agriculture, Fayoum, University, Egypt. Before planting the most important physical and chemical characters of the selected soil was determined according to **Wilde *et al*., (1985)** as shown in Table (1).

Table (1): The physical and chemical analysis of the used soil before planting in both seasons.

Properties		2005	2006
Physical			
Sand %		26.78	27.05
Silt %		26.70	26.10
Clay %		46.52	46.85
Texture grade		Clay	Clay
Chemical			
Organic matter%		1.78	1.76
pH(1:2.5)		7.35	7.27
Ec (ds m^{-1})		0.63	0.67
$CaCO_3$ %		5.62	5.47
Available macro and microelements (ppm)	N	475.00	482.00
	P	419.00	426.00
	K	21.20	24.10
	Fe	85.20	83.50
	Zn	27.99	27.09
	Mn	3.40	3.25
	Cu	0.78	0.74

In all experiments, the different treatments were arranged in randomized complete design with three replications. The experimental plot was 4 x 3.5 m ($14m^2$) contained 5 rows each of 4 m long and 70 cm width, each plot contain 66 plants.. Maize triple hybrid 310 was hand sown at the rate of 15 kg seeds/fed. in hills 30 cm apart in June 26^{th} in the two seasons. After 21 days from sowing plants were thinned to a single plant per hill on one side of the ridge. Phosphorous as calcium super phosphate (15.5% P_2O_5) at the rate of 200 kg/fed., was added to the field before sowing. Nitrogen at the rate of 150 kg N /fed. as urea (46% N) and potassium sulphate (48% K_2O) at the rate of 100 kg/fed., were given in two equal doses. The first dose was added after thinning (21 days from sown), the second dose given after 30 days from the first one. The

other cultural practices of growing maize at Fayoum Governorate were followed as normal. The plants were sprayed with tap water (as a control) or citrine (containing 15% citric acid, 2% Fe, 2% Zn, 2% Mn, 3% adhesive sugar substances and 76% water) at two times; the first was sprayed after thinning with a volume 150 L /fed. and the second was applied three wakes later with a volume 150 L /fed. Triton B as a wetting agent at 0.1% was added to the antioxidant solutions. Spraying was carried out till runoff.

The experiment involved the following treatments

1- Control (spraying with tap water) 2- Spraying with citrine at rate 0.05 %
3- Spraying with citrine at rate 0.1 % 4- Spraying with citrine at rate 0.15 %
5- Spraying with citrine at rate 0.2 % 6- Spraying with citrine at rate 0.25%
7- Spraying with citrine at rate 0.3% 8- Spraying with citrine at rate 0.35%
9- Spraying with citrine at rate 0.4% 10- Spraying with citrine at rate 0.45%
11- Spraying with citrine at rate 0.5 %

Data recorded

1- Growth characters:

Two plant samples were taken from each experimental plot. The first (ten plants from each treatment in the three replications) was taken 75 days after planting to study the following traits (i.e. plant height (m), stem diameter (cm), number of leaves /plant and dry weight of leaves per plant (g). The second sample was taken at harvesting time four months from planting (ten plants were chosen randomly from each plot) to estimate ear length (cm), ear diameter (cm), number of rows/ear, number of grains/ row, grain weight/ear (g), weight of 100 grain (g), ear weight/plnt (g) and grain yield/ fed (ton).

Chemical analysis

Fresh and dried leaves and seeds (75 days of old plants for leaves and at harvest, after 120 days from planting for seeds) were used for determination of the following constituents:**.** Photosynthetic pigments: chlorophyll a, b and total caroteniods were extracted from fresh leaves by acetone (80%) then, their concentrations were determined as mg/100g fresh weight according to **Welburn and Lichtenthaler (1984),** Total carbohydrates mg/g dry weight were determined colorimetrically by using phenol-sulphuric acid reagent according to the method described by **Herbert *et al.*, (1971)**. Total and reducing sugars were determined using phosphomolybdic acid reagent as described by **A.O.A.C. (1995),** and recorded as mg/g dry weight. Anthocyanin concentration (mg/100g dry weight) was determined according to the method described by **Hoagland (1980).** Total free amino acids in fresh leaves were determined colorimetrically using ninhydrin reagent according to the method described by **Jayarman (1981)** and recorded as mg/g dry weight**.** Total indoles mg/g fresh weight were determined by using 4-dimethylaminobenzaldehyde reagent as described by **Larson *et al*. (1962),** Free phenols in fresh leaves were determined using Folin-Denis reagent as described by **A.O.A.C. (1995**) and recorded as mg/g dry weight**.** Nitrogen %, in leaves and crude protein percentage in seeds was determined according to **A.O.A.C. (1995),** phosphorus % was determined according to **A.O.A.C. (1995)**, potassium was determined by Flame Photometer, Parkin–Elmer model 52 according to the method described by **Page *et al*., (1982).**

Statistical analysis:

The experiment was in a complete randomized block design with 11 treatment and 3 replicates for each treatment. Results were statistically analyzed using the L.S.D. at probability level of 5% for comparisons according to (**Gomez and Gomez, 1983).**

RESULTS:

A- Growth characters:

Data presented in Table (2) clearly indicate that sparing maize plants with the antioxidant and micronutrients namely citrine at the rates from 0.05 up to 0.5% improved the studied growth parameters of maize plant, i.e. plant height, stem diameter, number of leaves/plant and dry weight of leaves /plant as compared to the control plants. Such trend was true during the two studied seasons. The highest increase were recorded with citrine foliar spray at the rate 0.3% which surpassed the control by 22.39 and 22.66% for plant height, 32.06 and 32.38% for stem diameter, 5.63 and 7.09% for number of leaves/plant and 8.20 and 8.31% for dry weight of leaves/ plant respectively in both seasons over the control plants

B- Grain yields and their components:

Data in Tables (2 and 3) indicate that foliar application of citrine at the concentrations from 0.05 up to 0.5% increased grain yields and its components (ear length, ear diameter, number of rows/ear, number of grains/row, grain weight/ear, weight of 100 grain, ear weight/plant and grain yield/ fed.) as compared to the control plants. Such trend was true during the two studied seasons. The data also show that the promotion occurred in such yield and its components under this study were associated with the increase in citrine levels from 0.05 to 0.3% and thereafter, a slight decreased were generally occurred by using concentration more than 0.3%, but the values were still over the control one treatment. The maximum increase were recorded with citrine foliar application at the rate 0.3% which recorded 14.04 and 15.64% for ear length, 12.94 and 13.35% for ear diameter, 6.92 and 6.11% for number of rows/ear, 4.55 and 4.81% for number of grains/row, 26.91 and 27.26% for grain weight/ear, 14.44 and 13.53% for weight of 100 grain, 44.32 and 43.38 % for ear weight/plant and 26.89 and 27.26% for grain yield/ fed in the first and second seasons respectively over the control plants.

C- Chemical constituents of plants:

1- Leaf pigments concentration:

Data presented in Table (4) clearly show in two successive seasons that, the concentration of leaf pigments (chlorophyll a, b, total caroteniods and anthocyanin) were significantly increased when maize plants were sprayed with citrine (citric acid, Zn, Fe and Mn) at the rates from 0.05 up to 0.5 % as compared to the control. The best results were observed when maize plants were sprayed with citrine at the rate of 0.3% which recorded 23.72 and 23.55% for chlorophyll a, 17.35 and 17.17% for chlorophyll b, 28.83 and 25.75% for total caroteniods and 14.60 and 15.06% for anthocyanin in the first and second seasons respectively over the control plants.

2-Total soluble carbohydrates, total and reducing sugars concentration

Data recorded in Table (4) clearly revealed in the two successive seasons that, all used concentration of citrine increased total soluble carbohydrates and total sugars as compared to the control plants. The highest increase were recorded with citrine foliar application at the rate of 0.3% which recorded 45.37

and 46.78% for total carbohydrates and 14.60 and 14.60 % for total sugars in the first and second seasons respectively over the control plants. On the

Table 2

Table 3

contrary, reducing sugars content were decreased by using the same concentrations used in the study as compared to the control plants.

3- Total free amino acids, total indoles and free phenols.

Data recorded in Table (5) clearly indicate in the two successive seasons that, the total free amino acids, total indoles and free phenols were affected by the application of citrine at all used rates compared with the control plants. The best result were obtained when maize plants were sprayed with citrine at the rate of 0.3% which recorded 89.53 and 88.75% for total free amino acids and 10.44 and 10.53% for total indoles in the first and second seasons respectively over the control plants. On the contrary a marked decrease in free phenols concentration in leaves was recorded by the application of citrine at all used rates comparing with the control plants.

4- Nitrogen, phosphorus and potassium concentrations:

Data in both seasons of the study are presented in Table (5) revealed that, leaves of maize plants contained high concentration of nitrogen, phosphorus and potassium under foliar spray with citrine comparing to control plants. The maximum increase were obtained when citrine was application at the rate of 0.3 % which recorded 21.45 and 21.49% for nitrogen, 6.53 and 6.58% for phosphorous and 19.92 and 20.08% for potassium in both seasons respectively as compared to the control plants.

5- Grains crude protein%:

Data presented in Table (5) clearly show that spraying maize plants with the citrine (antioxidant) at the rates from 0.05 up to 0.5% significantly improved grain crude protein content as compared to the control plants. Such trend was true during the two studied seasons. The highest increase was recorded when citrine was sprayed at the rate of 0.3% which surpassed the control by 24.51 and 24.75 % in the first and second seasons respectively, as compared to the control plants.

DISCUSSION

Spraying maize plants with citric acid combined with some micronutrients (Fe, Mn and Zn) namely citrine resulted in vigorous growth as well as highly productivity of seeds with good quality. An increasing the measured growth characters (plant height etc…) was due to that these fertilizers resulting in more availability of micronutrients (Fe, Mn and Zn) and antioxidant such as citric acid to be absorbed by the recorded plants. The positive effect of the antioxidants on growth might be attributed to their positive action on enhancing cell divisions and protecting plant cells from free radicals that is responsible for plant senescence, also to be attributed to their effect on counteracting drought, salinity and diseases stresses as well as they have an auxinic action, consequently enhancing plant growth characters **(Raskin, 1992 and Elade, 1992).** Moreover, micronutrients used in this study (Zn, Fe and Mn) also have an improving affect on vegetative growth parameters. This may be attributed to the essential role of Zn in synthesis of tryptophan amino acid and consequently formation of auxin, i.e. IAA which act as growth regulator especially in prolonging height of plants **(Devendra, *et al.*, 1999).** Also, the

favorable effect of the used nutrients on stem diameter may be due to their stimulation effect on cell division and expansion. Moreover, the increase in dry weight of leaves/plant might be attributed to its stimulating effect on vegetative growth and physiological processes, i.e. increasing number of cells through cell division and meristematic activity of tissues. Increasing number of leaves/plant (Table 2) by micronutrients application may be attributed to the increment in cell division and cell elongation. The stimulating effect of the used micronutrients on plant growth may be due to their role in transmission of the electron from water to chlorophyll and producing oxygen gas in the photosynthesis, in addition to their role in the nitrogen metabolism through activated nitrite reductase enzyme **(Baza, 1984).** The improving effect of citric acid and micronutrients on yield and its components was mainly attributed to its positive action on enhancing growth parameters (Table 2) and photosynthetic pigments of plants leaves (Table 4). In this respect, **Al-Qubaie (2002)** stated that antioxidant especially ascorbic acid has an auxinic action and also synergistic effect on the biosyntheses of carbohydrate and controlling the incidence of most fungi on plants makes them in vigorous states which consequently is reflected on seed yield. Moreover, the increase in yield and its components may be attributed to the metabolic role of Zn, Fe and Mn in plant. In this respect**, Wilson and Allison (1978**) suggested that grain yield may sometimes be limited by photosynthesis (source) and grain (sink) simultaneously and it is possible to increase grain yield by keeping safe the balance between them. The favorable effect of Zn, Fe and Mn on grain yield and its components might be attributed to the increase in photosynthetic pigments concentration **(Price *et al.*, 1972)** as well as enzyme activity, which consequently enhancing plant metabolism **(Boardman, 1975)**. Also, zinc has essential role in carbohydrate metabolism, protein synthesis, tryptophan and IAA synthesis, since it activates number of enzymes for photosynthesis **(Gardner *et al.*, 1985** and **Marschner, 1986).** The promotive effect of citrine on leaf pigments concentration might be attributed to the enhancing effect of antioxidants and micronutrients on the nutritional status of maize plants .In this respect, **Elade (1992)** and **Farag (1996)** stated that most antioxidants were responsible for accelerating the biosynthesis of various pigments leading to the increase in biosynthesis of sugars. Moreover, **Tsuda *et al.* (1994)** show that anthocyanins may play an important role in the prevention of lipid peroxidation of cell membranes induced by active oxygen radicals. Moreover, the stimulating effect of micronutrients on chlorophyll formation, total carbohydrates and total sugars concentration were reported by **Mohr and Schopfer (1995)** who stated that this increase may be due to the enhancing effect of Fe, Mn and Zn on chlorophyll formation and consequently photosynthesis. In addition, the decrease in reducing sugars by citrine (citric acid, Zn, Fe and Mn) application may be attributed to the high capacity of the plant sprayed with this substance in building up non reducing sugars (sucrose) from the simple sugars, which decreased. In this respect, **Price *et al.*, (1972)** reported that the basic function of zinc in plant was related to its role in carbohydrates metabolism. The increase in total indoles and total free amino acids may be attributed to the role of Zn in synthesis of tryptophan (amino acid) and consequently the formation of natural auxin in plants, i.e. indole 3-acetic acid (IAA). In addition, the reduction in free phenols contrasted with the increase in total indoles, i.e. endogenous promoters increased and consequently endogenous inhibitors decreased in the leaves leading to an increase in plant growth parameters such as plant height and leaves

number/plant as shown in Table (2). In this respect, **Sagi and Garay (1961)** showed that the effect of phenolic compounds on plant growth was contributed to either the antagonism with IAA activity. The increase in macronutrients (N,P, and K) were supported by the results of **Ahmed and Abd El-Hameed (2004)** who reported that the

Table 4

Table 5

effect of antioxidants on producing healthy plants leads to enhancing the plants to have a great ability for uptake mineral elements. Moreover, **Devlin and Withman (1985)** reported that, the increase in leaves N, P and K may be due to the effect of Zn on biosynthesis of auxin (IAA) which promote rooting process and root distribution and consequently the amounts of mineral elements absorbed by roots and translocated into the different parts of the plant. Also, **El-Fouly and Fawzi (1996)** recorded that the use of micronutrients as foliar fertilizers led to an increase in root growth and thereby higher uptake of macro and micronutrients. The increase in protein content of grain may be attributed to the increment in total nitrogen percent of leaves and grains. In this respect, Zn has an essential role in protein syntheses (**Gardner *et al*., 1985** and **Marschner, 1986).**

Finally, from the present results, it could be concluded that the application of citrine (citric acid, Fe, Zn, and Mn) greatly increased growth and grain yield as well as improved grain quality and its chemical constituents. These elements participate in the different metabolic processes which increased syntheses of chlorophyll, carbohydrates, total free amino acids, IAA and absorption of essential nutrients, so that the used micronutrients with antioxidants could be increase maize productivity with high quality seeds.

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دراسات فسيولوجية على تأثيرمركب السترين على النمو والمحصول والمحتوى الكيماوي لنباتات الذرة

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أجريت تجربتين حقليتين خلال موسمين متتاليين ٢٠٠٥ و ٢٠٠٦م فى تربة طينية بمزرعة التجارب بكلية الزراعة بالفيوم لدراسة تأثير رش النباتات بمركب السترين (حامض الستريك متحدا مع بعض العناصر المغذية الصغرى) على النمو والمحصول والمحتوى الكيماوي لنباتات الذرة. تم رش النباتات بأحد عشرة معاملة وهى ٠ و٠.٠٥ و٠.١ و ٠.١٥و ٠.٢و ٠.٢٥ و ٠.٣ و ٠.٣٥ و ٠.٤ و ٠.٤٥ و٠.٥ % من السماد المركب (سترين) المحتوى على حامض الستريك بنسبة ١٥% وكل من الحديد بنسبة ٢% والزنك بنسبة ٢% والمنجنيز بنسبة ٢%.

أوضحت النتائج المتحصل عليها بصفة عامة أن كل الصفات الخضرية (طول النبات – عدد الأوراق للنبات – قطر الساق – الوزن الجاف للأوراق لكل نبات) وايضا صفات المحصول (طول الكوز – قطر الكوز – عدد الصفوف للكوز- عدد الحبوب لكل صف – وزن حبوب الكوز- وزن ١٠٠ حبه – وزن الكوز – محصول الفدان من الحبوب) والمكونات الكيماوية للأوراق (الكلوروفيل أ ، ب والكاروتينويدات الكلية والأنثوسيانين والكربوهيدرات الكلية والسكريات الكلية والأحماض الأمينية الحرة الكلية والاندولات الكلية وبعض العناصر محل الدراسة (نتروجين – فسفور – بوتاسيوم) وأيضا محتوى الحبوب من البروتين الخام قد زادت نتيجة الرش بالمعدلات المختلفة من السترين، وتم الحصول على أعلى القيم للقياسات السابقة عند استخدام المعدل ٠.٣% من السترين.

وعلى العكس من ذلك أدى الرش باستخدام السترين إلى تناقص محتوى أوراق النبات من السكريات المختزلة والفينولات الحرة مقارنة بالكنترول.

وهكذا نوصى باستخدام مخصب السترين رشا على النباتات بمعدل ٠.٣% لتحسين النمو الخضري والمحصول والمكونات الكيماوية لنباتات الذرة.