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BIOCHEMICAL STUDIES ON THE INFLUENCE OF FOLIAR APPLICATION OF SOME MICRONUTRIENTS AND ASCORBIC ACID ON THE PROTEIN AND LIPID CONTENTS OF MAIZE SEEDS

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ABSTRACT: *Field experiments were conducted to evaluate the effect of foliar application of some micronutrients (Mn + Fe + Zn), ascorbic acid and their interaction with or without usual fertilization (N/P) on biochemical constituents of maize seeds.*

All applied treatments caused significant increase in crude protein percent in both seeds germ and endosperm. Different effect on the protein fractions percentage was observed, whereas in most of cases albumin and globulin were significantly increased.

These treatments also improved the maize seeds oil quality as they increased essential fatty acids, unsaturated fatty acids, campesterol esters and β-sitosterol esters relative percentage, while squalene and some other hydrocarbons were significantly decreased.

Key words: *Maize, Protein fractions, Fatly acids, Sterols.*

INTRODUCTION

Maize is one of the most important cereal crops all over the world. Many efforts are made in egypt to improve its nutritional value and to increase its yield. Recently, interest has been manifested to micro nutrients, which cause various physiological and biochemical effects on the growth and development of plants. In this connection there were many attempts by several scientists to study the effects of micro nutrients Zn, Fe and Mn on maize and wheat plants. Belokobyi (1976) reported that Mn alone and in combination with P or N showed an increase in production of maize hybrid seeds as well as P and protein contents of the seeds while zein content decreases. Soaking of maize seeds in 0.02% solution of $MnSO_4$ increased their protein content, the water and salt – soluble zein fractions, while alcohol soluble zein fraction decreased (Belokobyi, 1972). Maize seeds treated with Mo, Zn and Mn lead to an increase in grain yields and grain protein contents, also a change in the proportion of the different protein fractions was showed, (Vlasyuk *et al.*, 1974). Asthana and Srivastava (1978) indicated that soaking of maize seeds in ascorbic acid enhanced the ethanol-soluble protein content. Zn and Mn caused a decrease in soluble nitrogen and an increase in insoluble and total nitrogen in the different stages of wheat and maize growth. As reported by Kitagishi and Obata (1986), Dhillon

et al. (1987), Kvyatkovskii (1988), Moussa *et al.* (1993) and Fecenko and Lozek (1998) Zn and Mn showed an increase in crude protein, albumine, globuline and insoluble protein wheat seeds while the prolamine content showed an opposite trend.

Eissa *et al.*, (1992). Mentioned that foliar application of Fe, Mn and Zn individually or together increased seeds yield wheat and protein percentage, while oil percentage showed no change. Also unsaturated fatty acids, essential fatty acids, total sterols and β-sitosterol relative concentrations were increased while hydrocarbons were decreased in soybean seed oil under the same conditions (El-Fiki 1994).

Addition of Zn or Mo individually and a combination of them led to a decrease in protein and oil contents in maize, whereas Zn and Mo combined with some macronutrients (K and P) caused an increase in total hydrocarbons, total sterols and β- sitosterol in maize seed oil (Bayoumi *et al.* 2002).

The aim of this investigation was to study the effect of foliar application of some micronutrients (Fe, Zn, Mn) and ascorbic acid on protein and lipid contents in zea maize seed.

MATERIALS AND METHODS

The farm experiment was carried out in Agriculture Research Center Giza. Variety of maize seeds (Cutivar Giza 2). The seeds were planted in winter (2000-2001). The plants were foliar sprayed with sulphate salts of Zn, Fe and Mn mixture with ratio of 1:2:1 respectively, and the conentration of that mixture was ½ g/L. Ascorbic acid was added in the conc. of 1 g/L. The above salts and ascorbic acid were used as follow:

A- With usual fertilization (Nitrogen and phosphate).

B- Without fertilization.

The fertilization with nitrogen and phosphate were performed according to the recommendations of Ministry of Agriculture for this crop.

As.A,M.N. and As.A + M.N. mixture as follows:

1- Control: spray with distilled water.

2- Spray with Ascorbic acid (1 g/L.) (As. A)

3- Spray with Zn + Fe + Mn mixture (½ g/L) (M. N.)

4- Spray with Ascorbic acid (1 g/L) + Zn, Fe and Mn mixture (½ g/L.) (As. A + M.N.)

At the harvest the grains were collected and washed by tap water, then germs were separated from indosperm mechanically and both dried at 40 °C for 24hr.

-Chemical analysis:

-Total nitrogen was determined in defatted samples in both germ and indosperm by micro- Kjeldah method (A. O. A. C. 1986). The N content was multiplied by the factor 6.25 to obtain the protein content.

-Protein fractions were determined as stated by Van Etten *et al.* (1967).

-Oil was extracted from seed germs by hexane according to AOAC (1990). The fatty acid methyl esters and unsaponifiable matters were analysed by gas liquid chromatography as reported by Ibrahim *et al.* (1964).

Statistical analysis:

The obtained results were computed and analyzed according to Selvin, 1996. Moreover variability between the two treated groups was done by analysis of variance (ANOVA test) and Least Significant Difference (LSD) was conducted to compare between means on a computer program (SPSS 2000).

Results and Discussion:

Table (1) demonstrated the effect of ascorbic acid (As.A.) and micronutrients (M.N.), which include Mn + Fe + Zn, individually or combined on the crude protein percentage and protein fractions relative percent (relative to crude protein) in maize seed germs. It was noticed that the application of M.N. and As.A. combined or solely showed a significantly increase in the crude protein percentage with or without fertilization as they compared with control. Gab Alla *et al.* (1986) observed that spraying wheat plant with Mn SO_4 or Zn So_4 increases significantly the protein percentage in the grains. This result are in agreement with those obtained by Abido *et at.* (1995) and Hassan (1996), they found that N content of wheat grains was significantly increased when Zn was added. This effect may be attributed to the effect of these nutrients on plant growth and enzymes activity (Moussa and Barsoum, 1995 and Fecenko and Lozek, 1998). As for protein fractions, data in Table (1) revealed that the relative percentage of the protein fractions albumin, globulin and prolamin were increased significantly as ascorbic acid was applied with or without fertilization, while glutelin percentage was increased only when fertilization was applied. The application of (M.N.) with fertilization led to significant increase of the realtive percentage of albumin and globuline, while the other protein fractions were decreased. On the other hand only globulin and non soluble protein showed significant increase while the other fractions were decreased if the above treatment was applied without fertilization. These results are partially in agreement with those obtained by Vlasyk *et al.* (1974). Spraying with (As. A.) and (M.N.) combined with fertilization increased significantly the ratio of prolamin, glutelin and non-soluble protein, while albumin and globulin decreased. Without fertilization the application of the previous mixture lead to significant increase in albumin, globulin and non-soluble protein percentage whereas the other fractions decreased .

Table (1): Effect of asorbic acid (As. A.) and some micronutrients (M. N.) on the relative percentage of protein fractions in maize seed germs.

Treatment	Crude protein (%)	Protein fraction relative %				
		Albumin	Globulin	Prolamin	Glutelin	Residue
With Fertilization						
Control	11.51 e	13.38 e	11.94 b	14.59 g	16.96 c	43.01 c
As. A.	14.12 a	17.00 b	13.95 c	15.22 f	17.63 c	36.19 d
M. N.	13.41 b	14.02 d	13.42 d	11.56 h	14.69 d	42.20 c
As. A. + M.N.	13.70 b	11.31 g	13.72 g	15.69 f	21.24 a	43.86 b
Without Fertilization						
Control	10.21 g	16.75 c	5.88 f	19.29 d	20.18 b	32.81 e
As. A.	11.22 e	17.56 b	6.06 e	22.90 a	3.83 h	45.90 a
M. N.	13.50 b	11.48 g	17.19 a	15.26 g	9.40 f	42.89 c
As. A. + M.N.	13.60 b	18.90 a	6.32 e	18.89 d	10.74 e	43.82 b

(M. N.) = Micronutrients : Zn + Fe + Mn
As. A. = Ascorbic acid
Values in the same column with different letters are significantly different ($P \geq 0.01$).

Table (2): Effect of asorbic acid (As. A.) and some micronutrients (M. N.) on the relative percentage of protein fractions in maize seeds endosperm.

Treatment	Crude protein (%)	Protein fraction relative %				
		Albumin	Globulin	Prolamin	Glutelin	Residue
With Fertilization						
Control	7.12 g	4.21 g	10.81 e	25.00 c	30.2 a	29.21 e
As. A	9.83 c	4.37 f	8.75 f	21.77 d	25.33 b	34.08 a
M. N.	9.12 e	7.56 c	24.45 a	31.03 a	7.89 g	25.77 f
As.A. + M.N.	12.64 a	12.90 a	13.61 d	17.00 f	23.02 c	31.25 c
Without Fertilization						
Control	8.32 f	8.29 b	21.64 c	31.01 a	5.17 h	30.89 c
As. A	10.36 b	7.43 c	21.53 c	19.11 e	19.88 d	30.21 d
M. N.	9.41 d	6.38 d	22.85 b	21.89 d	14.67 f	31.03 c
As.A. + M.N.	10.35 b	5.02 e	21.55 c	24.93 c	14.11 f	32.08 b

(M. N.) = Micronutrients : Zn + Fe + Mn
As. A. = Ascorbic acid
Values in the same column with different letters are significantly different ($P \geq 0.01$).

In Table (2) the application of As.A. and M.N. individually or as a combination of them caused significant increase in crude protein in maize seeds endosperm with or without fertilization. For protein fractions the application of (As.A.) with fertilization caused significant increase in albumin and non soluble protein relative percentage but the other fractions were decreased, whereas the spray with (M.N.) increased albumin, globulin, prolamin relative percentage significantly while the other fractions were decreased.The combined(As.A.) and (M.N.) with fertilization showed a significant increase in albumin, globulin and non-soluble protein relative percentage, while the other fractions were decreased. Without fertilization, the application of (As. A.) and (M. N.) solely or combined caused significant increase in glutelin relative percentage while albumine and prolamin were decreased and the other fractions showed no great change.

Table(3) illustrates the effect of (As. A.) and (M. N.)on the fatty acids content in maize seed oil. It is clear that (As.A.) and (M. N.) solely and combined increased the ratio of essential fatty acids (linoleic and linolenic) as well as the total unsaturated fatty acids with and without fertilization significantly. It is worthy to indicate that linolenic acid exist only when (As. A. and M.N.) were applied solely with fertilization.

Araschidic acid was decreased clearly as (As. A.) and (M. N.) applied solely with fertilization and disappeared in all other cases. El-Fiki (1994) indicated that foliar application of Fe + Mn increased the unsaturation degree and essential fatty acids of soybean seed oil.

From (Table 4) the application of (As. A.) and (M. N.) individually or combined together caused significant increase in campsterol relative percent, while a negative effect on the β- sitosterol was observed not only with fertilization but also without it, Bayoumi *et al.* (2002) indicated that the addition of Zn + Mo + Macronutrients increased the relative conc. of β-sitosterol in maize seeds oil.

Table (5) indicated the effect of (As. A.) and (M. N.) individually or combined on the composition of less polar compounds in maize seeds oil. Dodecane, tetradecane, octadecane, campsterol ester and β-sitosterol ester significantly increased as (As. A.) was applied with fertilization, while tetradecane, tetracosane, pentacosane, hexacosane, tricontane and squalene showed significant decrease. Treatment withont fertilization caused significant increase in dodecane, tetradecane, hexadecane, eicosane, tetracosane, hexacosane and β-sitosterol ester when ascorbic acid was added whereas octadecane, squalene and tricontane was markedly decreased. The application of (M. N.) increased significantly the relative concentration of the major components tricontane, campesterol ester and β-sitosterol ester with or without fertilization while squalene and other hydrocarbons decreased. Bayoumi *et al.* (2002) were demonstrated that the application of Zn + Mo + K caused an increase in total hydrocarbons.

Table (3): Effect of asorbic acid (As. A.) and some micronutrients (M. N.) on the fatty acids percentage in the maize seed oil.

Fatty acids	With fertilization				Without fertilization			
	Control	As. A.	M. N.	As.A. + M.N.	Control	As. A.	M. N.	As.A. + M.N.
$C_{16:0}$	16.75 b	12.61 h	15.17 e	16.68 b	16.33 c	14.21 f	17.73 a	15.56 d
$C_{18:0}$	0.98 b	4.80 a	-- b	-- b	-- b	-- b	-- b	-- b
$C_{18:1}$	37.89 f	37.69 d	39.59	37.38 e	38.52 c	39.68 a	36.44 g	35.76 h
$C_{18:2}$	43.01 g	43.12 h	44.04	45.94	45.15 e	46.11 b	45.83 d	48.59 a
$C_{18:3}$	-- c	0.79 a	0.64 b	-- c	-- c	-- c	-- c	-- c
$C_{20:0}$	1.78 e	0.95 a	0.56 b	-- c	-- c	-- c	-- c	-- c
T_U	80.90 f	81.60 c	84.27 e	83.32 d	83.67 d	85.79 b	82.27 e	84.35 d
T_S	19.10 e	18.40 f	15.73 a	16.68 c	16.33 b	14.21 d	17.73 f	15.56 a
T_U / T_S	4.24 b	4.43 c	5.36 h	5.0 e	5.12 e	6.04 g	4.64 c	5.39 h

(M. N.) = Micronutrients : Zn + Fe + Mn

T_U = Total unsaturated : F.A. T_S = Total saturated : F.A.

Values in the same column with different letters are significantly different ($P \geq 0.01$).

Table (4): Effect of asorbic acid (As. A.) and some micronutrients (M. N.) on the sterols fractions percent in maize seed oil.

Sterols fractions	With fertilization				Without fertilization			
	Control	As. A.	M. N.	As.A. + M.N.	Control	As. A.	M. N.	As.A. + M.N.
Campsterol %	23.47 g	26.92 c	26.11 d	23.72 f	11.61 h	31.30 b	24.11 e	36.96 a
β-sitosterol	76.53 b	73.08 f	73.86 e	76.28 c	88.31 a	68.70 g	75.89 d	63.04 h

(M. N.) = Micronutrients : Zn + Fe + Mn

Retintion times of campsterol and β-sitosterol were 28.85 and 31.67 respectively .

Values in the same column with different letters are significantly different ($P \geq 0.01$).

Table (5): Effect of asorbic acid (As. A.) and some micronutrients (M. N.) on the relative percentage of the less polar compounds in maize seeds oil.

Fatty acids	With fertilization				Without fertilization			
	Control	As. A.	M. N.	As.A. + M.N.	Control	As. A.	M. N.	As.A. + M.N.
C_{12}	3.59 b	21.87a	3.36 c	3.36 c	-- d	1.61 b	1.41 c	3.60 a
C_{14}	1.17 a	0.39 c	0.53 b	0.53 b	0.99 d	1.46 a	0.33 c	0.69 b
C_{16}	4.91 b	1.97 d	2.97 c	5.42 a	0.77 d	4.44 a	1.47 c	3.28 b
C_{18}	1.41 c	36.82a	1.95 b	0.70 d	30.11 a	1.23 d	4.75 c	5.25 b
Unknown	4.75 a	-- b	-- b	-- b	-- b	4.95 a	-- b	-- b
C_{20}	2.68 a	0.47 d	0.80 c	1.43 b	1.02 c	2.07 a	0.77 d	1.09 b
C_{22}	1.08 c	1.95 b	3.49 a	3.45 a	0.96 c	0.91 c	6.07 a	5.79 b
C_{23}	3.83 a	1.01 c	2.08 b	-- d	0.49 c	4.18 b	19.77a	-- d
C_{24}	22.26a	11.08d	20.75b	14.24c	0.11 d	31.17b	1.45 c	56.86 a
C_{25}	8.00 a	2.15 b	-- c	-- c	0.83 d	9.08 a	2.43 b	0.95 c
C_{26}	3.77 b	0.97 d	3.22 c	4.92 a	0.35 c	4.73 a	2.52 b	0.22 d
C_{28}	5.94 a	4.96 c	5.86 b	0.49 d	0.39 d	3.55 b	15.03a	1.56 c
Unknown	3.62 b	-- c	4.40 a	-- c	-- c	11.26a	-- c	1.49 b
Unknown	3.56 c	1.42 d	4.46 b	6.87 a	11.64 a	1.38 d	5.00 b	2.34 c
Unknown	6.02 b	1.87 d	4.34 c	8.33 a	4.07 c	2.66 d	5.38 a	4.94 b
Squalene	8.42 b	0.61 d	6.99 c	13.20a	40.65 a	5.11 c	8.17 b	3.63 d
Tricontane	4.46 b	2.99 c	8.03 a	-- d	0.24 d	2.19 b	5.25 a	1.83 c
Dotricontane	4.03 c	2.04 d	5.63 b	8.77 a	6.23 a	3.39 c	4.07 b	1.53 d
Compestevol	0.98 d	3.70 c	10.02a	5.80 b	0.24 c	0.09 d	7.40 a	2.43 b
β-sitosterol	0.12 d	3.77 c	11.12b	11.50a	1.81 d	4.54 b	8.73 a	2.49 c

M.N. = Micronutrients (Zn + Fe + Mn)
As.A. = Ascorbic acid

The mixture of (M. N.) and (As. A.) caused significant increase in hexacosane, octacosane, squalene, dotricane, campesterol ester and β-sitosterol ester relative percentage with fertilization. Tetracosane represented 56.8% from the total less polar compounds as the above mixture was applied. In all cases some unknown compounds appeared and disappeared when (As. A.) or (M. N.) were applied individually or as a combination of them. Generally it was noticed that the application of (As. A.) or (M. N.) caused significant increase in total protein of maize seeds (germs and endosperm), whereas their effect was varied on the protein fractions relative concentration when they were applied solely or combined, but in most cases albumin and globulin relative concentrations increased significantly. It also, improved the quality of maize seeds- oil where it increased the essential fatty acids, unsaturated fatty acids, campesterol ester and β-sitosterol ester relative percentage.

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دراسة كيميائية حيوية على تأثير الرش الورقى لبعض العناصر المغذية الصغرى (زنك + حديد + منجنيز) وحمض الاسكوربيك على مكونات حبوب الذرة من البروتينات والليبيدات

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الملخص العربى

اجريت تجارب حقلية لتقييم تأثير الرش الورقى لمخلوط أملاح الكبريتات الزنك + الحديد + المنجنيز بنسبة ١ : ٢ : ١ وكذلك حمض الاسكوربيك كل على حده (مخلوط العناصر أو حمض الاسكوربيك) أو مجتمعين (مخلوط العناصر + حمض الاسكوربيك) على محتويات حبوب الذرة من البروتينات والليبيدات وكانت نتائج التحليل الكيميائى كالآتى :

- زادت نسبة البروتين الخام بنسبة صغيرة فى الجنين وبنسبة أكبر فى الاندوسبرم بإضافة مخلوط العناصر أو حمض الاسكوربيك منفردة أو مجتمعه مع التسميد أو بدونه
- كذلك أدى إضافة مخلوط العناصر وحمض الاسكوربيك مجتمعة أو منفردة الى نقص المحتوى النسبى لكل من الجنين والاندوسبرم من الجلوتينين والبرولامين والبروتين الغير ذائب ، بينما زاد محتواها من الألبيومين والجلوبيولين .
- كذلك أدت كل المعاملات الى زيادة نسبة الاحماض الدهنية الضرورية (لينوليك ولينولينك) وكذلك نسبة الأحماض الدهنية الغير مشبعة الى المشبعة فى زيت حبوب الذرة .
- كذلك أدت المعاملات المذكورة أعلاه الى زيادة نسبة استرات الكامبيستيرول والبيتا سيتوستيرول بينما نقصت نسبة الاسكوالين وبعض الهيدروكربونات لأخرى .