Bull. Fac. Agric., Cairo Univ.,51
(2000) : 251-266.

THE CONTRIBUTION OF BIO-CHEMICAL TREATMENTS FOR REMEDY THE PROBLEMS OF ZINC IN CALCAREOUS SOIL

(Received 25 . 7. 1999)

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ABSTRACT

In a compariative study between biochemical compounds (aliphatic and phenolic acids) and/or arbuscular mycorrhizal fungi (AMF) and their effects on Zn and other nutrient acquisition of maize grown in a calcareous soil, a pot experiment was carried out in the greenhouse. In a calcareous soil, corn (*Zea mays* L. Giza 2) was planted, labelled $^{65}ZnSO_4$ was, added at rates of 0, 10, 20 mg Zn kg^{-1} soil. Biochemical compounds (citric and salicylic acid) were added at a rate of 24 mg acid kg^{-1} soil either alone or combined with AMF. Data showed that dry matter yield of corn was significantly increased by organic compounds and/or AMF application. The rates of increase were 78, 55 and 57% for citric, salicylic acids and AMF, respectively comparing with the control treatment. They were 93 and 86% when AMF was combined with citric and salicylic acids, respectively. Zinc concentration was increased by 100% and 125% over the control in the treatments of citric and citric+ AMF, respectively. This is attributed to direct acquisition of Zn by hyphate in maize and also to the ability of organic compounds to chelate divalent ions. Data indicated that under different Zn levels, Fe concentration improved with different biochemical treatments. In conclusion, in calcareous soils where Zn is deficient, the use of biochemical treatments (citric, salicylic acids and/or AMF) can help in improving Zn and Fe forms to be more available for corn plants and reduce the inhibition effect of

Zn on Fe uptake by plants.

Key words: *AMF, calcareous soil, citric acid, iron, salicylic acid, zinc.*

1. INTRODUCTION

Zinc deficiency in crop plants is widespread particularly in calcareous soils (Adriano 1986 and Marschner 1986). Soil amendments and foliar sprays of Zn are common methods to correct Zn deficiency. However, these methods are expensive, time consuming, and may be effective only for one cropping season (Cakmak *et al.*, 1994). Alternatively, the use of organic chelating compounds and biofertilizers to increase the efficiency of Zn acquisition from soil by plants is a realistic approach. Anon (1974) demonstrated that corn is more susceptible to Zn deficiency.

It is well documented that plants can increase the availability of nutrients in the rhizosphere by various response mechanisms (Marschner, 1986). Chelator addition to the soil or release by the roots facilitates the movement of micronutrients to the root surface (Lindsay, 1974). Zhang *et al.*, (1991) added that the release of low-molecular weight root exudates is of great importance for the acquisition of micronutrient cations from rhizosphere.

There is evidence that higher plants followed the same response mechanisms for both Zn and Fe deficiency. Therefore, enhanced Zn uptake under Fe deficiency has been reported in non-graminaceous plant species such as chickpea (Agarwala *et al.*, 1979) and sunflower (Scherer and Hofner 1980; Romheld *et al.*, 1982). This enhancement can be attributed to the mobilization of apoplasmic Zn by Fe deficiency-induced release of H^+ and phenolics (Marschner *et al.*, 1986).

Corn (*Zea mays* L.) is a graminaceous monocot followed the same mechanism (Strategy I) for Fe acquisition (Lytle *et al.*, 1990; Lytle and Jolley 1991) and did not produce measureable phytosiderophores in response to Fe stress (Jolley and Brown 1991). Accordingly, corn may respond to Zn deficiency stress by the same Fe deficiency mechanism strategy I (*i.e.*, root excrete H^+ and phenolics).

Phenolic acids form stable complexes with metal ions, and thus they are important in the transport of micronutrient ions in soils (Stevenson, 1991). He added that the low molecular-weight organic aliphatic acids play a key role in the formation of complexes with divalent and trivalent cations. Citric and oxalic acids are of special importance by virtue of their wide distribution in the pedosphere, since they form highly stable complexes with trace elements. Mench *et al.* (1988) found that organic acids were major components of low-molecular-weight exudates of corn (*Zea mays* L.). In most cultivated soil, the amounts of biochemical compounds (chelating agents) found in the soil solution at any time are low and variable, and represent a balance between synthesis and destruction by microorganism (Stevenson, 1991).

Reports on enhanced acquisition of mineral nutrient by plant inoculated with Arbuscular mycorrhizal fungi (AMF) are numerous, specially for P (Bolan, 1991; Kothari *et al.* 1990; Marschner and Dell, 1994). Information on enhanced acquisition of micronurtient including zinc has been recently reviewed (George *et al.*, 1994; Marschner and Dell, 1994; Kothari *et al.*, 1991). Direct acquisition of P and Zn by hyphate in maize has been reported (Kothari *et al.*, 1991), and many indirect effects of AMF have been attributed to enhanced growth because of improved P status of plants (Cooper, 1984; Marschner and Dell, 1994).

The objective of this study was to determine the effects of biochemical compounds (aliphatic and phenolic acids) and/or AMF on Zn and Fe acquisition and the growth of maize grown on calcareous soils.

2. MATERIALS AND METHODS

A greenhouse experiment was conducted at the Soil & Water Res. Dept., Atomic Energy Authority, Inshas, Egypt. Physical and chemical properties of the calcareous soil used were 81% sand, 13% silt, 6% clay, pH 8.3 (1:2.5 soil : water ratio), 38% $CaCO_3$; 0.2% organic matter ; DTPA extractable Zn and Fe were 2.6 and 1.2 mg kg^{-1} soil, respectively.

A mixture of arbuscular mycorrhizal spores was isolated from a calcareous soil and used as an inoculum. A suspension of AM was added in a hole of 5 cm depth under every seed before planting at a rate of 50 spore ml^{-1}.

Plastic pots were filled with 5 kg air-dry soil. Superphosphate (15.5% P_2O_5) was added at a rate of 120 mg super-P kg^{-1} soil and mixed with the soil. Five seeds of corn (*Zea mays* L. Giza 2) were planted in one group of pots. In the other group, the corn seeds were planted after inoculation with mycorrhizal spores. The pots were arranged in a completely randomized design with three replicates. Ten days latter, seedlings were thinned to two plants per pot. Plants were fertilized with N and K at the rates of 120 and 50 mg kg^{-1} soil as NH_4NO_3 and K_2SO_4, respectively. Iron was added at the rate of 10 mg Fe kg^{-1} soil as $FeSO_4$. Labelled $ZnSO_4$ with ^{65}Zn with specific activity 1 mCi ^{65}Zn/ 1 mg Zn was added at rate of 0, 10 and 20 mg Zn kg^{-1} soil. Biochemical compounds i.e. citric (Cit.) and salicylic (Sali.) acids were added at a rate of 24 mg acid kg^{-1} soil either alone or combined with mycorrhizal inoculum.

After 45 days, the plants were harvested and shoots were dried at 70°C, weighed and digested using a mixture of sulfuric and perchloric acids. Zinc and Fe were determined using atomic absorption spectrophotometer. Radioactivity of ^{65}Zn was determined by gamma counter; the amounts of Zn derived from fertilizer (Zndff) and % Zn fertilizer utilization (U%) were calculated according to Zapata (1990).

The entire root system was suspended in water and then carefully wet sieved to collect the spores. Roots were cut into segments about 1 cm long cleared in 10% KOH and the fungus was stained and the percentage of mycorrhizal infection was determined according to Phillip and Hayman (1970).

3. RESULTS AND DISCUSSION

3.1. AM colonization status

Percentage of root colonization differed with biochemical treatments. It increased with chemical treatments and Zn levels where 20 mg Zn kg^{-1} soil treatment had higher AM colonization percentage in the case of citric and salycilic acid treatments than control ones. At the same time, the biochemical treatments (AM and AM + Citric or

Salicylic) showed high colonization percentage than control or chemical treatments only (Fig. 1). The high levels of AM root colonization related to microbial inoculants by AM fungi may have been due to changes in roomorphology and physiology brought by these treatments (Singh and Singh,1993). Also organic amendements revealed favourable effects on the development of AM fungi. Bady and Manibushanrao (1996) reported that organic materials increased infection percentage in rice plants.

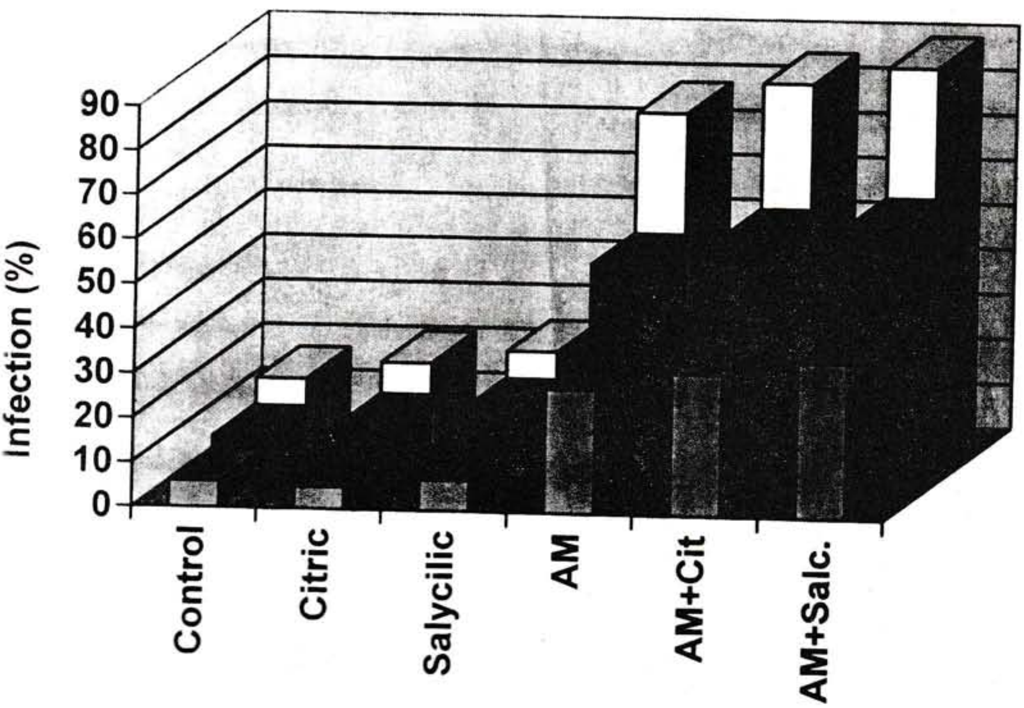
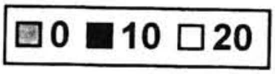


Fig.1 : AM infection as influenced by biochemical treatments and zinc levels



3.2. Plant Growth

Dry matter yield production of corn plants was significantly improved by the application of organic compounds and/or AMF Table (1). The application of organic compounds with AMF caused additional increase in dry matter of corn plants than that applied alone. In this concern, the rates of increase, in general, were 78, 55 and 57% for citric acid, salicylic acid and AMF, respectively. They were 93 and 86% for AMF+ citric acid and AMF+salicylic acid compared with untreated plants, respectively. A major beneficial response of AMF associations with plant roots has enhanced plant growth which often is attributed to improved mineral nutritional status of the host plants (Cooper, 1984; Kothari *et al.*, 1990; Marschner and Dell, 1994; O'keefe and Sylvia, 1991). The important role of organic acids on improving the growth of corn plant, might be accomplished by indirect effects through improved nutrient status in plants by acidifying the rhizosphere and its ability to chelate divalent ions, particularly citric acid and can sequently increased the divalent ions, near the root zone (Hoffland, 1992, Mench and Martin, 1991 and Treeby *et al.,* 1989). Also, many indirect effects of citric acid on enhancing the growth have been attribute to enhancing P uptake by plants (Bar-Yosef, 1991; Fox and Comerford, 1992 and Kafkafi *et al.*, 1988). This clearly explain the additional improving effect of organic compounds when they are combined with AMF on the growth of corn plant.

The application of $ZnSO_4$ significantly increased the dry matter of corn plants compared with unfertilized plants (Table, 1). The mean average of increases was 20 and 34% when Zn was applied at rates of 10 and 20 mg Zn kg^{-1} soil. The role of Zn on improving plant growth by enhancing carbohydrate metabolism and proteins synthesis has been established (Marschner, 1986; Romheld and Marschner, 1991).

The combined applications of bio-chemical treatments with Zn levels caused an additional enhancing effect on the growth of corn plants. In this concern, the highest rates of increases were recorded at treatments of citric acid either alone or combined with AMF. They deduced an increase on the growth by about 96% and 101% over control when Zn was added at a rate of 10 mg Zn kg^{-1} soil. At20 mg Zn kg^{-1} soil , the corresponding values were 76 and 88%. The growth of the inoculated plants with AMF was increased with increasing Zn levels. The percentage rate of increases were 42, 54 and 71% compared to the uninoculated plants at Zn_0, Zn_{10} and Zn_{20}, respectively. The highest dry matter production was obtained when salicylic acid was combined with AMF inoculated plants which were fertilized with 20 mg Zn kg^{-1} soil.

Table (1): Dry matter production of corn shoots (g pot^{-1}) as affected by bio-chemical treatments and Zn levels.

Zn level (mg kg^{-1} soil)	Bio-chemical treatments						
	Control	Citric acid	Salicylic acid	AMF	AMF +(Cit.)	AMF +(Sal.)	Mean
0	3.66	5.82	5.25	5.19	6.92	6.34	5.53
10	4.05	7.95	6.34	6.25	8.15	7.12	6.64
20	4.42	7.79	7.20	7.55	8.33	9.10	7.39
Mean	4.04	7.19	6.26	6.33	7.80	7.52	--
L.S.D. 5%	Treatements			0.98			
	Zn levels			0.69			
	Interaction			NS			

3.3. Zinc status in plant

Zinc concentration in corn shoots was significantly increased by amended calceareous soil with bio-chemical treatments and/or $ZnSO_4$ fertilization (Table 2). In general, Zn concentration was increased by 100% and 125% over control plants in the treatments of citric acid and citric acid+AMF, respectively. The corresponding values for salicylic acid either added alone or with AMF were 75% and 98% compared to untreated plants, respectively. For the AMF treatment, the improvement of plant Zn concentration was 64% over the control plants.

Increased Zn concentration in corn leaves may indirectly improve human health, as the corn leaves are generally used for animals nutrition (Welch, 1993; Moraghan, 1996). In addition, Zn from animal foods is generally considered to be more available to human than plant Zn (Van Campen, 1991).

Zinc levels had a little significant effect on Zn concentration in corn plants, so, the net increments were, in general, 11 and 22% over the control plants when Zn was added at the rate of 10 and 20 mg Zn kg^{-1} soil, respectively.

Table (2): Zinc concentration (ug/g^{-1}D.M) in corn shoots as affected by bio-chemical treatments and Zn levels.

Zn level (mg kg^{-1} soil)	Bio-chemical treatments						
	Control	Citric acid	Salicylic acid	AMF	AMF +(Cit.)	AMF +(Sal.)	Mean
0	70	151	128	130	174	150	133
10	91	158	157	132	186	169	148
20	92	195	157	153	210	180	162
Mean	84	168	147	138	190	166	--
L.S.D. 0.05%	Treatements 15.5						
	Zn levels 10.9						
	Interaction NS						

Organic compounds had a positive effect on improving Zn concentration in corn shoots under different Zn levels (Table, 2); both the amount of Zn derived from fertilizer (Zndff) and the percentage of fertilizer utilization (U%) (Table, 3). In this concern, the improving effect of citric acid exceeded that of salicylic acid. The rates of increases on Zn concentration due to citric acid application were 45, 74, 112% over control at Zn_0, Zn_{10} and Zn_{20}, respectively. For salicylic acid they were 83, 73 and 71% under increasing Zn levels, respectively.

The improving effect of these organic compounds may be explained on the basis of the ability of citrate to chelate divalent ions (Stevenson, 1991; Dinkelater *et al.*, 1989; Mench and Martin, 1991; Treeby *et al.*, 1989) and salicylic acid as a phenolic compound form stable complex with metal ions of micronutient thus, increased its availability in soil (Stevenson, 1991). Also, the enhancing effect of these compounds on the availability of micronutrients, particularly Zn may be by acidifying the rhizosphere and indirectly by improving plant growth.

Arbuscular mycorrhizal fungi have an improving effect on Zn concentration so, the shoot Zn concentration was increased by about 86, 45 and 66% compared to the control at Zn treatment of 0, 10 and 20 mg Zn kg^{-1} soil, respectively. Wellings *et al.*, (1991) reported the improvement of plant Zn nutrition by a mycorrhizal infection. This is due to direct acquisition of Zn by hyphae in maize (Kothari *et al.*,1991; Li *et al.*, 1991) and indirect acquisition have been a ttributed

to improved P status of host plants (Cooper, 1984; Marschner and Dell,
to improved P status of host plants (Cooper, 1984; Marschner and Dell,
1994).

The results in Table (3) obviously indicate the ability of organic compounds and AMF to increasing the amount of Zn derived from fertilizer taken by corn plants. Also, the utilization percentage of applied fertilizer was increased due to the application of bio-chemical treatments, but it was higher in the case of 10 than 20 mg zn kg $^{-1}$ soil treatment.

The virtue of individual treatments on improving Zn status in corn plants, may elucidate the beneficial effect of combined ones, (*i.e.*, AMF + Citric acid, AMF + Salicylic acid) which caused more ameliorating effect on plant Zn content and U%, under calcareous soils (Table 3).

Table (3): Amounts of ^{65}Zn derived from fertilizer (Zndff) and the fertilizer utilization percentage (U%) by corn plants as affected by bio-chemical treatment and Zn levels.

Bio-chemical Treatments	Zn level (mg kg^{-1} soil)			
	10		20	
	Zndff (mg pot^{-1})	U%	Zndff (mg pot^{-1})	U%
Control	44	0.09	73	0.07
Citric acid	364	0.73	394	0.39
Salicylic acid	258	0.52	316	0.32
AMF	257	0.51	323	0.32
AMF+Citric	531	1.06	525	0.53
AMF+Salicylic	325	0.65	442	0.44

3.4. Iron concentration

Besides the role of bio chemical treatments on enhancing Zn uptake, they also improve Fe nutrition of corn plants Table (4). It is well Known that, Fe mainly uptake by plants as Fe^{2+} (Chaney *et al.*, 1972). In spite of Zn inhibits the reduction of Fe^{3+} to Fe^{2+} by plant roots, consequently inducing Fe deficiency (Olsen *et al.*,1982). Application of the organic acids and/or inoculation with AMF had an important role on improving Fe uptake under different Zn levels.

The Fe concentration in shoots was reduced by increasing Zn levels up to 20 mg Zn kg^{-1} soil in untreated plants with biochemical treatments. In this concern, the percentage rate of reductions were 8% and 19% in control plants when Zn was applied at the rates of 10 and 20 mg Zn kg^{-1} soil, respectivel. This clearly demonstrates the benefical role of organic compounds either alone or combined with AMF on improving Fe concentration in corn. The mean average of increases were 50, 49, 42, 43 and 28% for AMF+Citric acid, AMF+Salicylic acid, AMF, citric acid and salicylic acid, respectively.

Table (4): Iron concentration (ug/ g^{-1}D.M) in shoots of corn plants as affected by bio-chemical treatments and Zn levels.

Zn level (mgkg^{-1}soil	Bio-chemical treatments						
	Control	Citric acid	Salicylic acid	AMF	AMF +(Cit.)	AMF +(Sal.)	Mean
0	283	353	304	342	366	343	332
10	261	379	320	383	394	392	355
20	228	372	368	369	398	410	358
Mean	257	368	330	365	386	382	--
L.S.D. 5%			Treatements			36	
			Zn levels		NS		
			Interaction			NS	

Arbuscular mycorrhizal fungi are known to improve plant Fe nutrition in maize grown on alkaline soil (Clark and Zeto, 1996). They reported that improved Fe acquistion by host plant may be due to indirect rather than direct effects. According to our data, this may explain the ability of AMF to improve Fe concentration in corn plants grown on the calcareous soil under different Zn levels. The same observation was obtained with organic compounds either alone or combined with AMF. This may be attributed to the ability of organic compounds to form complexes with divalent and trivalent ions (Stevenson, 1991). Moreover they cause a reduction of Fe^{3+} to Fe^{2+} which improved Fe acquisition by plants (Brown 1982 for citrate and Olsen *et al.*, 1982; Marschner, 1986 for phenolic acids). In addition, Fe translocation in plant has been known by citrate.

Zn levels had no significant differences on Fe concentration at different bio-chemical treatments. Thus, Zn_{10} treatment (10 mg Zn kg^{-1} soil) is considered a suitable rate of $ZnSO_4$ added with bio-chemical treatment particularly AMF+Citric acid in order to improve Fe concentration simultaneously with Zn uptake by corn plants grown on calcareous soil.

CONCLUSIONS

The use of organic acids and AMF has a great improving effect on the growth, Zn and Fe uptake by corn plants grown on calcareous soil. According to our results we suggest that the adverse effect of Zn on Fe uptake due to impair Fe^{3+} reduction to Fe^{2+} can be reduced or alleviated by the application of the bio-chemical treatments. This is important to improve both Zn and Fe nutrition simultaneously of plants grown in calcareous soil.

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دور المعاملات البيوكيميائية في معالجة مشاكل الزنك في الأراضي الجيرية

إسماعيل أبو سريع الغندور - سمير سعد محمد على
قسم بحوث الأراضي والمياه - هيئة الطاقة الذرية

ملخص

أجريت دراسة مقارنة بين المركبات العضوية (الأحماض الاليفاتية والفينولية منفردة أو متحدة مع الأسمدة الحيوية (فطريات الميكوريزا) على امتصاص نبات الذرة لعنصر الزنك والعناصر الأخرى في الأراضي الجيرية. أقيمت تجربة أصص في الصوبة حيث تم زراعة الذرة صنف جيزة 2. أضيفت كبريتات الزنك المشع بمستويات صفر، 10، 20، مجم زنك/كجم تربة كما أضيفت المركبات الكيميائية (حمض الستريك، حمض السليسلك) بمعدل 24 مجم/كجم تربة.
أوضحت النتائج أن كل من المعاملات الكيميائية والحيوية منفردة أو مجتمعة أدت إلى زيادة الوزن الجاف زيادة معنوية حيث كان معدل هذه الزيادة لكل من حمض الستريك والسليسلك وكذلك فطريات الميكوريزا 78، 55، 57% على الترتيب مقارنة بالنباتات غير المعاملة بينما كانت 93 و 86% عن إضافة الأسمدة الحيوية (الميكوريزا) مع حمض الستريك والسليسلك على التوالي.
كذلك بلغت الزيادة في تركيز الزنك في المجموع الخضري 100، 125% بالنسبة لحمض الستريك والستريك+ الميكوريزا مقارنة بالكنترول وتعزى هذه الزيادة إلى قدرة حمض الستريك على خلب الايونات الثنائية وتسهيل امتصاصها بواسطة النبات. وفيما يتعلق بتأثير الزنك على امتصاص الحديد أوضحت النتائج أن امتصاص الحديد لم يتأثر كثيراً بمستويات الزنك المختلفة وذلك بإضافة المعاملات الكيميائية والحيوية.
ونخلص من ذلك أن استخدام المواد الكيميائية العضوية مع الأسمدة الحيوية (الميكوريزا) في الأراضي الجيرية حيث ينتشر نقص الزنك تؤدى إلى تحسين امتصاص الزنك والحديد بواسطة نباتات الذرة وكذلك تقلل من التأثير المثبط للزنك على امتصاص الحديد بواسطة النبات وبالتالي معالجة المشاكل الناتجة عن نقص الزنك والحديد في الأراضي الجيرية.

المجلة العلمية - لكلية الزراعة - جامعة القاهرة المجلد (51) العدد الثاني
إبريل (2000):251-266.