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Impact of Foliar Application of Zinc on Micro and Macro Elements Distribution in *Phyllanthus amarus*

Nguyen Cao Nguyen, Krasimir I. Ivanov, Penka S. Zapryanova

Abstract**—**The present study was carried out to investigate the interaction of foliar applied zinc with other elements in *Phyllanthus amarus* plants. The plant samples for our experiment were collected from Lam Dong province, Vietnam. Seven suspension solutions of nanosized zinc hydroxide nitrate ($Zn_5(OH)_8(NO_3)_2{\cdot}2H_2O$) with different Zn concentration were used. Fertilization and irrigation were the same for all variants. The Zn content and the content of selected micro (Cu, Fe, Mn) and macro (Ca, Mg, P and K) nutrients in plant roots, and stems and leaves were determined. It was concluded that the zinc content of plant roots varies narrowly, with no significant impact of ZnHN fertilization. The same trend can be seen in the content of Cu, Mn, and macronutrients. The zinc content of plant stems and leaves varies within wide limits, with the significant impact of ZnHN fertilization. The trends in the content of Cu, Mn, and macronutrients are kept the same as in the root, whereas the iron trends to increase its content at increasing the zinc content.

Keywords**—**Zinc fertilizers, micro and macro elements, *Phyllanthus amarus.*

I. Introduction

THE healthy development of plants requires the absorption of sufficient amounts of macro- and microelements, and deficiencies in these nutrients have a significant impact on the quantity and quality of plant production. Zinc (Zn) is one of the eight microelements (along with manganese, copper, boron, iron, chlorine, molybdenum and nickel) that are essential for normal and healthy plants and plant reproduction. The mean Zn concentration in plant tissues is in the range of 15 - 80 $mg.kg^{-1}$ [1]. Its physiological role in plants is associated with its participation in many enzyme systems (carbonic anhydrase, superoxide dismutase, dehydrogenases), the activation of many other enzyme systems, protein synthesis, the integration of cell membranes, etc. [2]. A Zn deficiency in plants causes a number of structural and functional disorders such as increased membrane permeability, a high concentration of active oxygen forms, a reduced photosynthetic rate, and growth restriction, etc.

Phyllanthus amarus (*P. amarus*) belongs to the family *Euphorbiaceae* and is a small herb well known for its medical properties. It has been used widely in various traditional medicines to treat swelling, sores, jaundice, inflammatory diseases, kidney disorders, diabetes and viral hepatitis as bitter, astringent, stomachic, diuretic, febrifuge and antiseptic. It grows in the tropical and subtropical climate over well-drained sandy-loam soils. The majority of soils in these regions are deficient in available zinc [3]. The need for zinc fertilization triggered the necessity for studying the interaction effects of Zn with other plant nutrients in soils and plants, which is the object of our investigation. In our experiment, zinc hydroxide nitrate (ZnHN) suspension with the content of 7.9 % zinc was used as a foliar fertilizer.

Nguyen Nguyen is with the Department of Chemistry, Agricultural University, 4000 Plovdiv, Bulgaria (e-mail: caonguyen0016@gmail.com).
Krasimir Ivanov is with the Department of Chemistry, Agricultural University, 4000 Plovdiv, Bulgaria (corresponding author, phone: +359-887-745-734; fax: +359-32-633-157; e-mail: kivanov1@ abv.bg).
Penka Zapryanova is with the Department of Ecology, Agricultural University, 4000 Plovdiv, Bulgaria (e-mail: p_alexieva@abv.bg).

II. Materials and Methods

The zinc hydroxide nitrate was prepared by pouring KOH solution into $Zn(NO_3)_2.6H_2O$ solution under vigorous stirring. The initial molar ratio OH/Zn was 1.6 and the time for precipitation was 30 minutes in all cases. The resulting white suspension contained 15.0 % $Zn_5(OH)_8(NO_3)_2.2H_2O$ (7.9% Zn).

The field experiment was conducted in Lam Dong province, Vietnam. Before planting, surface soil samples (0 - 20 cm depth) from each harvesting plot were collected, air-dried, mixed and analysed for the selected physicochemical properties. The results are presented in Table I.

TABLE I
ZINC CONCENTRATION IN *PHYLLANTHUS AMARUS* ORGANS, MG KG^{-1} D.WT

Soil test parameter	Test level	Test rating
Soil pH (1:5)	5.87± 0,02	Acidic
Organic matter (%)	1.57	Low
(Nutrients mg kg^{-1})		
Total Nitrogen	894	Medium
Potassium (K_2O)	79.9 ± 2.4	Very Low
Phosphorous (P_2O_5)	1169 ± 24	Medium
Calcium	540.2 ± 12.6	Very Low
Magnesium	422.5 ± 17.4	Very Low
Copper	7.86 ± 0.38	Low
Manganese	160.26 ± 3.24	Low
Iron	12346 ± 270	Medium
Zinc	28.00 ± 1.02	Low

The soil in this research area was with an acidic pH (5.87) and low content of organic matter. It is characterized by a low content of Ca, Cu, Mn, K and Zn and medium content of N, P and Fe.

Since the soil is low in organic matter and nutrients in our experiment, we used a mixture of four parts of soil, one part of coco fiber and 0.5 parts of organic fertilizer.

Eight suspension solutions with different Zn concentrations were used. The scheme of the experiment is presented in Table II. The foliar fertilizer was applied triple during the growth period – first, fourth and seventh week after germination. The size of the plots was 4.4 m^2 for all variants and the working solution for each spraying – 0.2 l/plot.

TABLE II
SCHEME OF THE EXPERIMENT

Variant	$Zn_5(OH)_8(NO_3)_2$ suspension, g/l	$Zn_5(OH)_8(NO_3)_2$ suspension, g/plot	Zn, g/plot
Control	-	-	-
2	6.6	1.32	0.10
3	10.7	2.14	0.17
4	14.8	2.96	0.23
5	18.9	3.78	0.30
6	23.0	4.60	0.36
7	27.0	5.40	0.43
8	32.8	6.56	0.52

After harvesting, random samples were collected and air dried at 85 °C for 24 hours. All samples were carefully separated into its roots, stems and leaves, after which all parts were milled, mixed and analysed for Zn, Cu, Mn, Fe, P, K, Ca, and Mg. All samples were digested by a mixture of HNO_3 and H_2O_2 in a Microwave Digestion System MARS 6 - CEM Corporation.

The results were analysed statistically using IBM SPSS statistic software.

III. RESULTS

A. Impact of Foliar Fertilization on the Zinc Distribution in the Phyllanthus Amarus Organs

The results in Table III present the impact of the foliar fertilization on the zinc distribution in the *Phyllanthus amarus* organs.

TABLE III
ZINC CONCENTRATION IN *PHYLLANTHUS AMARUS* ORGANS, MG KG^{-1} D.WT

Variant	Roots	Stem	Leaves
Control	75.22 ± 2.02	54.22 ± 2.12	90.24 ± 1.56
2	73.30 ± 1.82	93.12 ± 2.64	171.12 ± 2.02
3	75.42 ± 1.90	104.42± 1.82	191.32 ± 2.58
4	76.12 ± 1.80	110.63 ± 2.22	247.16 ± 2.92
5	67.24 ± 1.72	115.00 ± 2.42	333.04 ± 3.84
6	81.31 ± 1.96	119.02 ± 1.02	333.46 ± 5.36
7	77.02 ± 1.68	158.42 ± 3.12	338.23 ± 5.48
8	79.25 ± 1.94	211.04 ± 3.22	314.12 ± 4.98

The content of zinc in the roots varied within a narrow range of 75.22 for the control sample to 81.31 mg kg^{-1} for variant 6, with the mean value for all variants 75.38 ± 4.21 mg kg^{-1}. The zinc content in the stems ranges widely from 54.22 in the control sample to 211.04 mg kg^{-1} in variant 8, and the mean value for all variants was 120.50 ± 46.58 mg kg^{-1}. The range in the zinc content in the leaves was also significant. It ranged from 90.24 in the control sample to 338.12 mg kg^{-1} in variant 7 with the mean value for all variants was 252.10 ± 93.32 mg kg^{-1}. The presented results show that the concentration of the zinc fertilizer used significantly influences the zinc content in the stems and leaves of the plants, but not in the roots.

B. Impact of Foliar Fertilization on Micro and Macro Elements Distribution in the Phyllanthus Amarus Organs

Figs. 1-3 present the impact of Zn fertilization on micronutrients (Cu, Mn and Fe) content in roots of P. amarus.

The largest part of the total Zn in the soils is associated with Fe and Mn oxides [4]. In the form of divalent ion, Zn interacts with all plant nutrients present in the soil. In many studies antagonistic Cu and Zn interaction has been found, especially in contaminated soils with a high content of both elements [5]. The data presented in Table I show that the copper and zinc content of the soil used in our experiment is low and a noticeable competitive interaction between the two elements cannot be expected. No significant effect of foliar Zn fertilization on Cu content in the roots can be observed (Fig. 1). Its content varied within a narrow range of 9.04 for variant 2 to 11.31 mg kg-1 for variant 3, with the mean value for all variants 10.25 ± 1.04 mg kg-1.

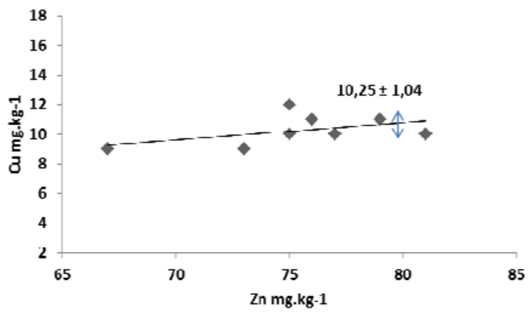

Fig. 1 Impact of Zn fertilization on Cu content in roots of *P. amarus*

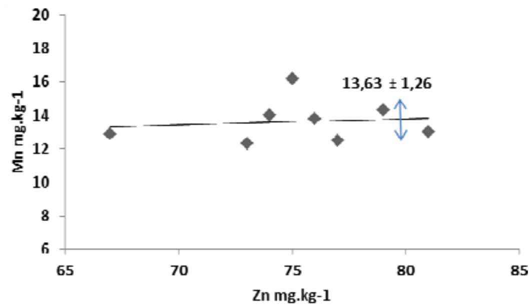

Fig. 2 Impact of Zn fertilization on Mn content in roots of *P. amarus*

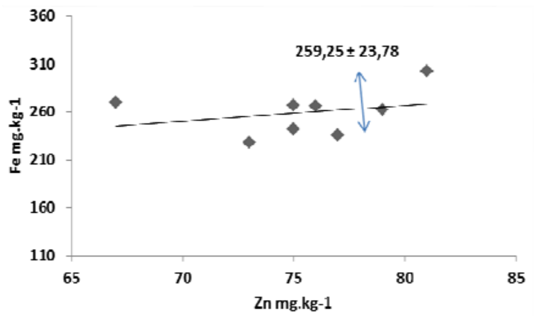

Fig. 3 Impact of Zn fertilization on Fe content in roots of *P. amarus*

White et al. [6] observed that increased levels of Zn in soil solution significantly increased translocation of Mn to tops which indicate the appearance of chlorosis. According to Ambler et al. [7], zinc inhibited Fe translocation in some cases. In our experiment, the content of both elements varies narrowly (13.63 ± 1.26 mg kg^{-1} for Mn and 259.25 ± 23.78 mg kg^{-1} for Fe). Obviously, the limiting factors in determining the content of micronutrient at the roots of *P. amarus* are pH and soil composition.

Figs. 4-7 present the impact of Zn fertilization on macronutrients (P, K, Ca and Mg) content in roots of *P. amarus*.

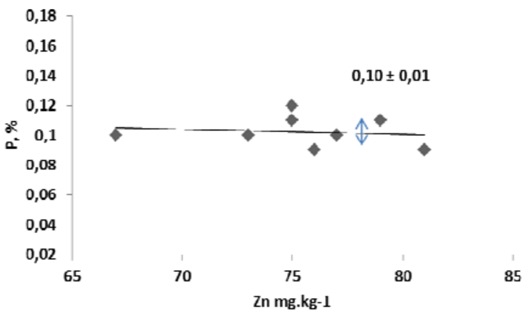

Fig. 4 Impact of Zn fertilization on P content in roots of *P. amarus*

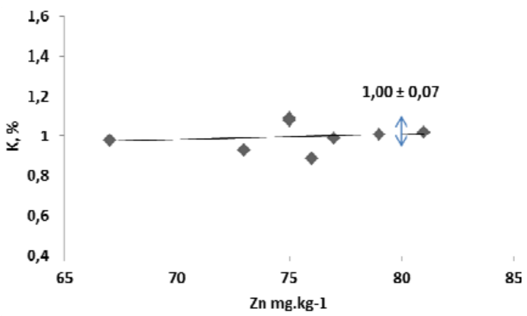

Fig. 5 Impact of Zn fertilization on K content in roots of *P. amarus*

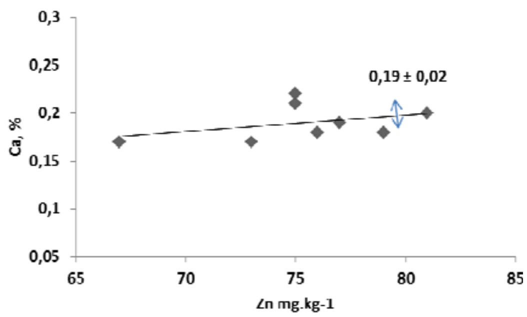

Fig. 6 Impact of Zn fertilization on Ca content in roots of *P. amarus*

Godbold and Huttermann [8] reported that increasing zinc levels in soil solution decreased the shoot to root ratios and translocation of Zn, Mg, K, P and Ca and caused accumulation of these nutrients in the root. Cayton et al. [9] also reported that the absorption and translocation of plant macronutrients like Mg, K, P, and Ca depended on Zn concentration in soil. They notified that zinc is an antagonist to copper at the primary absorption site, in contrast to its action on K, Ca, Mg, P and Fe. Zn interfered at the storage site in the roots and decreased the rate of absorption or translocation of macronutrients to plants or caused mineral imbalances [10]. Accumulation of zinc in the roots or shoots was usually accompanied by accumulation of calcium [11].

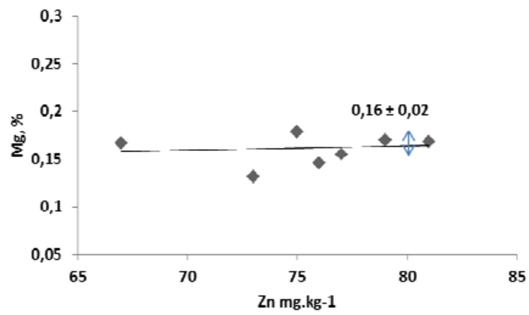

Fig. 7 Impact of Zn fertilization on Mg content in roots of *P. amarus*

The results presented in Figs. 4-7 show that foliar application of zinc in no way affects the content of macronutrients at the roots of *P. amarus*. The content of P, K, Ca, and Mg varies in a narrow range (0.10 ± 0.01% for P, 1.00 ± 0.07% for K, 0.19 ± 0.02% for Ca and 0.16 ± 0.01% for Mg).

The content of micro and macronutrients in the above-ground parts of plants strongly depends on their concentration and the interaction between them in the soil solution. However, unambiguous conclusions cannot be extracted, and the experimental results obtained are contradictory.

Zn and Fe might compete with each other for root absorption sites. According to Soltangheisi et al. [12], manganese and zinc interact with iron and this interaction can result in impacts on the yield of corn plants. They have established that at enhancing the Zn concentration in solution, iron concentration in roots increased linearly up to 1.0 mg L^{-1} level of Zn and its concentration in roots differ at $P \leq 0.05$. Fe concentration reached a maximum at the highest Zn level. These results are in agreement with many other authors [13].

Rosell and Ulrich [14] investigated the impact of Zn supply to sugarbeets by nutrient solution and have established a drastic decrease of Fe in the leaves from 900 to 90 mg kg^{-1}. Adilgolu et al. [15] reported that application of Zn reduced Fe content but increased Mn and Cu concentration in corn plants.

Giordano et al. [16] did not find evidence for the blocking of iron transport to plant tops by zinc.

Figs. 8-10 present the impact of Zn fertilization on micronutrients (Cu, Mn, and Fe) content in stems of *P. amarus*. The results presented in Figs 8 and 9 show that the concentration of the applied zinc fertilizer has no significant influence on the Cu and Mn content in the stems of the plants. The content of both elements varies in a narrow range of 6.38 ± 0.58 mg kg^{-1} for Cu and 27.38 ± 2.00 mg kg^{-1} for Mn.

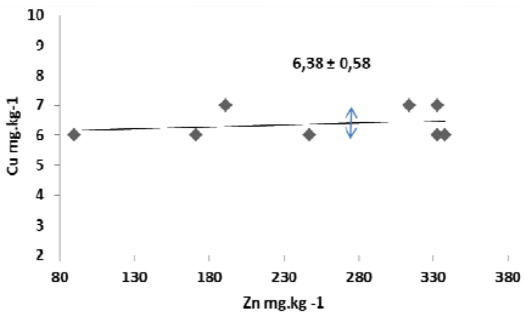

Fig. 8 Effect of Zn fertilization on Cu content in stems of *P. amarus*

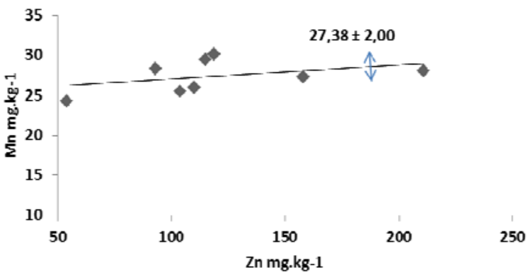

Fig. 9 Effect of Zn fertilization on Mn content in stems of *P. amarus*

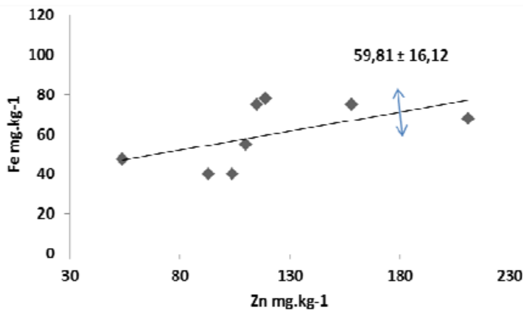

Fig. 10 Effect of Zn fertilization on Fe content in stems of *P. amarus*

A little different is the result of iron. Its content is ranging from 40.0 to 78.2 with the mean value for all variants 59.81 ± 16.12 mg kg^{-1}**.** This difference, however, does not allow for the conclusion of synergy between the two elements. These results show that the interaction of zinc with Cu, Mn and Fe depends not only on the type of plant but also on the mode of application of zinc. Obviously, the interaction of zinc with micronutrients is much more pronounced in the soil solution and the roots of the plants than in their above-ground parts after using zinc-containing leaf fertilizers.

Figs. 11-14 present the impact of Zn fertilization on macronutrients (P, K, Ca and Mg) content in stems of *P. amarus*.

The content of all four elements in the leaves of *P. amarus* varies within a narrow range, which confirms the above conclusion that the interaction of soil-zinc and foliar applied Zn with nutrients differ substantially.

Figs. 15-17 present the impact of Zn fertilization on micronutrients (Cu, Mn, and Fe) content in leaves of P. amarus.

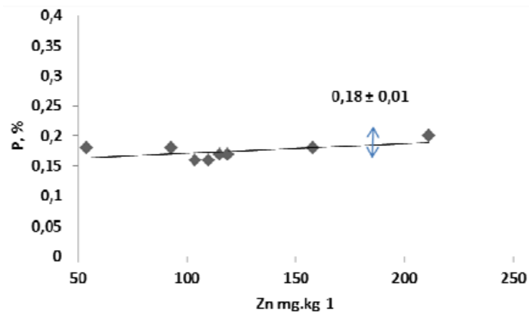

Fig. 11 Impact of Zn fertilization on P content in stems of *P. amarus*

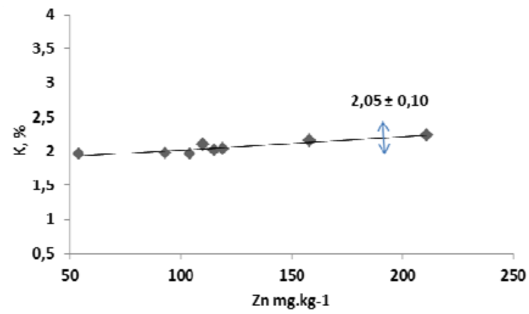

Fig. 12 Impact of Zn fertilization on K content in stems of *P. amarus*

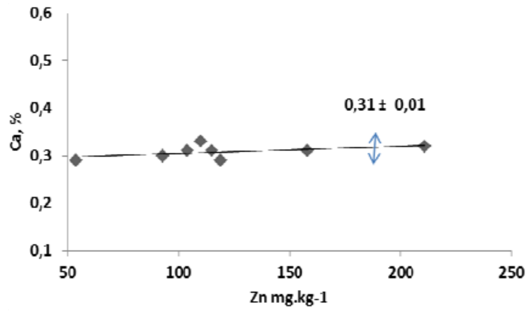

Fig. 13 Impact of Zn fertilization on Ca content in stems of *P. amarus*

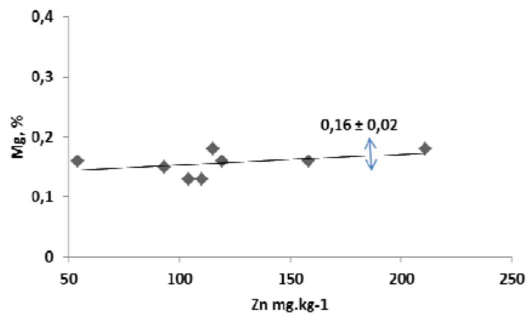

Fig. 14 Impact of Zn fertilization on Mg content in stems of *P. amarus*

Many publications report negative interaction between zinc and copper due to the effect of antagonism and the same membrane transport protein [17], reported that with increasing soil-Zn applications, Mn concentrations in shoot tissue decreased maximum manganese was found in the control plants, where no zinc was applied and high zinc rates lead to a significant reduction in manganese concentration. Application of 16 kg ha^{-1} Zn, with a leaf Mn content of 182 mg kg^{-1}, showed 37% increase relative to the control, with a leaf Mn content of 133 mg kg^{-1}.

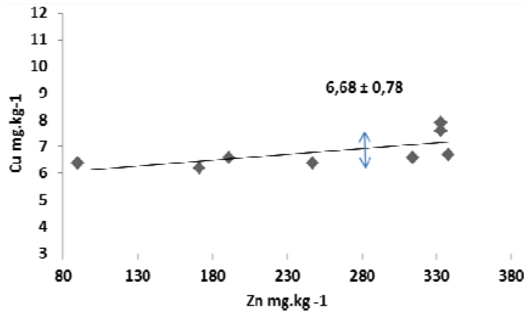

Fig. 15 Impact of Zn fertilization on Cu content in leaves of *P.amarus*

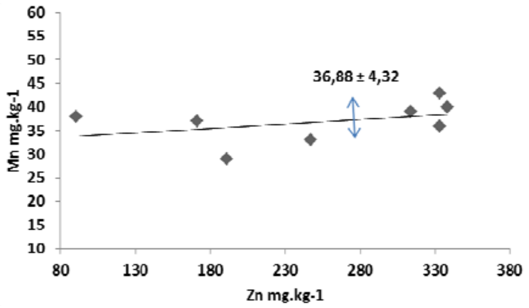

Fig. 16 Impact of Zn fertilization on Mn content in leaves of *P. amarus*

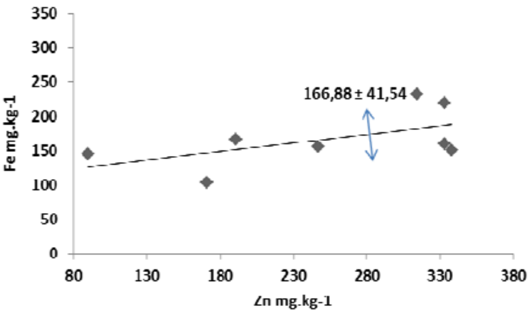

Fig. 17 Impact of Zn fertilization on Fe content in leaves of *P. amarus*

In our study, such an effect is not noticeable. The content of Cu in the leaf of *P. amarus* varies within a relatively narrow range from 4.02 to 7.88 with the mean value for all variants 6,67 ± 0.78 mg kg^{-1}. Manganese ranged from 38.10 to 43.20 with a mean value of 36.88 ± 4.32.

In the leaves, as well as in the stems of *P. amarus*, there is a tendency of increasing the iron content with increasing the zinc content. It varies from 14.20 to 220.16 with the mean value of 166.88 ± 41.54 mg kg^{-1}.

Aref [18] has studied the influence of soil-Zn fertilization on the Fe content in the leaves of maize grown on calcareous soil with high pH and have established no effect on the Fe content in the leaf. According to the same authors, Zn spraying increased the leaf Fe content. The explanation is that, after applying Zn fertilizers, the activity and extractability of Zn added to soils in water-soluble forms continually and decreases, and Zn changes to more stable forms through slow reactions with soil constituents. This result leads to the conclusion that foliar spraying is more efficient than soil-Zn application. The summarized results for micronutrient content in *P. amarus* show that the content of copper increases in order: roots > stems ≅ leaves. The manganese content in the different organs of the plant differs significantly, as in this case, the order is: leaves > stems > roots. The main part of the iron is localized in the roots > leaves > stems.

Figs. 18-21 present the impact of Zn fertilization on macronutrients (P, K, Ca and Mg) content in leaves of *P. amarus*.

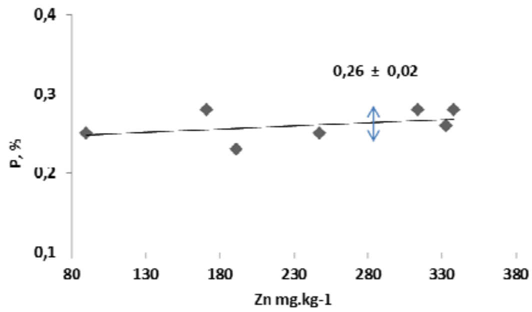

Fig. 18 Effect of Zn fertilization on P content in leaves of *P. amarus*

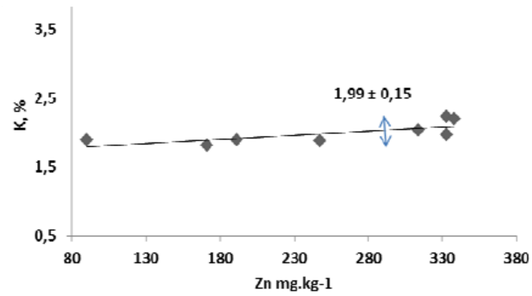

Fig. 19 Effect of Zn fertilization on K content in leaves of *P. amarus*

It is generally accepted that there are antagonistic effects of P application on absorption and uptake of Zn [19]. Formation of Zn phosphate or/and phytate is considered responsible for Zn immobilization on root surfaces and in leaves. Antagonistic effects of Ca and Zn have been reported by many authors. Davis-Carter et al. [20] reported that Ca content in peanut leaves decreased due to Zn fertilization. Erdal et al. [21] showed that the P, K, Ca and Mg concentrations of maize plants decreased with Zn fertilization from the soil.

The results presented in Figs. 18-21 do not show any effect of foliar Zn fertilization on macronutrients in the leaves of *P. amarus* and the content of P, K, Ca and Mg at all variants is comparable with those of control plants. The main part of P and Ca is localized in the leaves, followed by stems and roots.

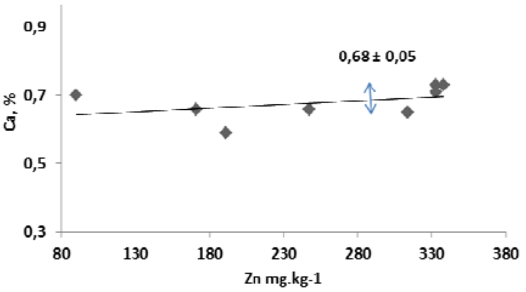

Fig. 20 Effect of Zn fertilization on Ca content in leaves of *P. amarus*

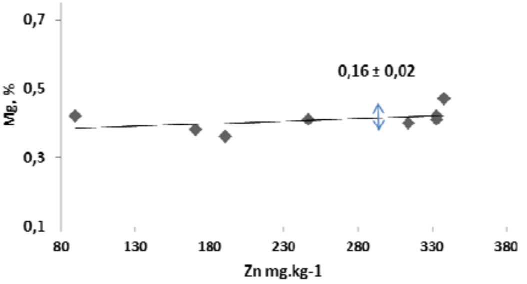

Fig. 21 Effect of Zn fertilization on Mg content in leaves of *P. amarus*

The content of K increases in order: stems ≅ leaves > roots. The magnesium content in the different organs of the plant differs significantly, as in this case, the order is: leaves > stems > roots.

The summary of the results presented in Figs. 1-21 shows that the mineral composition in each of the three parts investigated differs from each other. The minerals in the root were in order of P ≅ K > Ca > Mg for macronutrients and Fe > Zn > Mn> Cu for micronutrients. In the steams, the content for macronutrients was in order of Ca > K > P > Mg and Zn > Fe > Mn > Cu for micronutrients and for the leaves, the order was Ca > Mg > P > K and Zn > Fe > Mn > Cu for micronutrients.

IV. Conclusions

The results of this study give us reason to conclude that the interaction of zinc with other nutrients strongly depends on the way of its application. The water-soluble forms in the soil solution can be converted into more stable forms through reactions with soil constituents, which leads to well-expressed antagonism. Essential for the existence of synergism or antagonism between zinc and other nutrients is and competitive interaction between the elements at the absorption through the root system. Foliar spraying could be much more efficient as it allows plants to be supplied with the required amount of zinc at the desired time without interfering with the absorption of other nutrients.

Acknowledgment

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