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Response of Spanish Grown In Sandy Soil to Mineral Nutrition

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Abstract

Vegetables are important crops for additional supply of human nutritional requirements, with high nutritive contents. In particular, they are high in vitamins, minerals and fiber. Spinach has a high nutritional value and is extremely rich in antioxidants and vitamins, especially when fresh, steamed, or quickly boiled. It is a rich source of vitamins A, C, E, K, B2, and B6, magnesium, manganese, iron, calcium, potassium, folic acid (vitamin B9), copper, protein, phosphorus, zinc, niacin, selenium, and omega-3 fatty acids. Recently, opioid peptides called rubiscolins have also been found in spinach. To benefit from the folic acid in spinach, it is better to steam it than to boil it. Boiling spinach for four minutes can half the level of folic acid. So the the present study was conducted to investigate whether application of ZnSo4 (Zn) and zinc bis glycinate (Zn AA) could regulate the soluble carbohydrate contents, soluble protein contents, proline contents, activities of enzyme activity. Addition of Zn or ZnAA markedly increased the morphology and activity of both CAT, POX was significantly increased. Generally, it could be concluded that Zn or ZnAA have (to more extent) a beneficial regulatory role in plant.

Key words: zinc bis glycinate, spinach , zinc , Antioxidant enzyme activity.

Introduction

Leafy vegetables are a highly variable group of crop plants that can be broadly defined as vegetables grown for their edible leaves. However, the distinction between leafy and non-leafy crops is not always clear, due to cultural and regional differences. Leafy vegetables include spinach.

Spinach (*Spinacia oleracea* L.) is an annual cool season crop, and in temperate areas it can be grown all year around. It is a green, leafy vegetable, eaten boiled, baked and raw as a baby leaf as well. However, spinach plants accumulate unwanted compounds for human health, such a nitrate and oxalate **(Santamaria *et al.*, 1999; Chen *et al.*, 2004)**. Spinach belongs to the chenopodiaceae family. Leaves are smooth, semi-savoy or savoy, depending on cultivar. Therefore, highly desirable to explore possible ways and means to enhance the productivity of this important crop employing cost effective and easy to use techniques. In this respect vitamins are known to improve plant growth and development. Beneficial effects of vitamins on yield quantity and quality have been reported for various crops by **(El- Bassiouny et al., 2005; Gomaa *et al.*, 2005; Mona *et al.*, 2005; El-Tohamy *et al.*, 2008; Fawzy, 2007; Hussain and Khalaf, 2007; Fawzy *et al.*, 2010 and Ghoname *et al.*, 2010)**

Micronutrients such as Zn, has essential roles in plant's life cycle and very essential for normal growth plants (**Mengel *et al.*, 2001; Fageria, 2007, Sofy 2009)**. Zinc (Zn) is an essential nutrient required in some fertilizer programs for crop production. While some soils are capable of supplying adequate amounts for crop production, addition of zinc fertilizers is needed for others **(Sofy 2009).**

Nanotechnology as a new powerful technology has the ability to create a great revolution and transformation in food supply system in a global scope **(Orama *et al* 2002 ; Mitschker *et al* 2004).** This technology is particularly applied in chelate fertilizers such as zinc, iron and magnesium chelate.

(**Lucena *et al.*, 2008**) Nanotechnology is gradually moving from the experimental stage to the stage of operational and practical.

In contrast, high concentrations of protein and amino acids (AA) in cereals can contribute to higher bioavailability of micronutrients in the diet (**Ferndndez, et al, 2008**).

In Zinc or ferrous or magnesium glycinate chemically, the carboxyl group and the α-amino group of glycine both donate electron pairs into the Zinc or ferrous or magnesium cation forming coordinate covalent bonds (**Atkins and Beran, 1992; McMurry and Fay, 1995**). Zinc or ferrous or magnesium serves as the closing member in the formation of the two resulting 5-membered heterocyclic rings.

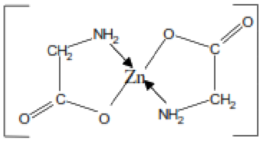


Figure (1) chemical composition of zinc bis glycinate

1- Materials and Methods

These experiments were carried out El-Minia, Cairo, Egypt on the date 6.4.2015. The seeds of spanish cv. Balady. (*Spinacia oleracea* ssp.) were obtained from Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt.

A pot experiment was designed as follows: A homogenous Spanish seeds (were sown in three plots (3 m width and 15 m length for each plot) containing 9 ridges (12 for each plot). The seeds were sown on one side of the ridge, with 20 cm apart between the hills. The developed plants were irrigated whenever required with tap water until the complete germination.

Treatments

- Irrigation water (tap water)

- Zn (75 ppm as zinc sulphat)

- Zn AA (15 ppm as Zinc bisglycinat)

The plants of Spanish were treated twice with the above mentioned treatments (as foliage spraying). The first treatment was made when the age of plants was 8 days, while the second treatment was made when the age of plants was 20 days of sowing. The plant samples were collected for analysis when the plants were 15 (Stage I) and 30 (Stage II) days old.

2.2. Phytochemical Contents

Photosynthetic pigments and carotenoids were estimated using the method of **Vernon and Selly [1966].** Contents of soluble protein of seeds were estimated according to the methods of **Lowery et al. [1951].** Contents of soluble carbohydrate were measured according to the method of **Umbriet et al. [1969]** Determination of proline contents were hand-homogenized in 3% of sulfosalicylic acid and centrifuged at 3000g at 4°C for 10 min. The supernatants were used for proline estimation. **Bates *et al.* (1973).**

2.3. Assay of Enzymes Activities

Protein enzymes were extracted according to the method of **Kherjee and Choudhuri [1983].** Peroxidase (POX) activity was assayed using the method of **Bergmeyer [1974].** Catalase (CAT) activity was assayed according to the method of **Chen et al. [2000**].

Statistical analysis:

We calculate sample size according to Raosoft and All statistical calculations were done using SPSS (statistica package for the social science version 25.00) statistical program. at 0.05 level of probability (**Snedecor and Cochran, 1982**). Quantitative data with parametric distribution were done using Analysis of variance the One-way ANOVA and Post hoc-LSD tests (the least significant difference). The confidence interval was set to 95% and the margin of error accepted was set to 5%. The p-value was considered non significant (NS) at the level of > 0.05, significant at the level of < 0.05, 0.01 and highly significant at the level of < 0.001.

RESULTS AND DISCUSSION:

Plant growth:

Table (1, 2) revealed that, treating spinach with Zn or Zn AA resulted in, significant increases in shoot and root length at two stages of growth when compared to normal plant.

Also, treating spinach with Zn or ZnAA shown more significant increases in number of leaves , fresh and dry weight of shoot , root .at two stages especially treating with ZnAA

This result is agreement with **(Ali, 2012; Bameri et al, 2012; Rehman et al, 2012; Rawashdeh and Sala, 2014**) who reported plant height increased due to micronutrient foliar application.

The use of micronutrients to improve the shoot, root length and F. wt of shoot , root was recommended by other investigators. In this regard, **Ali et al., (2009)** on wheat and

Ali (2012) on wheat, indicated that the sprayed plants with micronutrients (Zn or Fe) showed a marked increase in the shoot/ length, fresh amd dry weight plant.

Table (1): Effect of Zn, Zn AA on shoot length (cm), root length (cm) and number of leaves / plant of spinach. Each value is mean of 10 replicates ± standard error of means.

Treatments	Shoot length (cm)		Root length (cm)		Number of leaves	
	Stage I	Stage II	Stage I	Stage II	Stage I	Stage II
Control	9.90 ±0.20 a	18.72±0.37 a	5.60±0.30 a	10.47±0.29 a	5.13±0.09 a	7.41±0.26 a
Zn	17.67±0.33 b	31.54±0.29 b	8.50±0.29 b	15.00±0.11 b	5.77±0.18 ab	10.17±0.17 b
Zn AA	19.50±0.29 c	35.22±0.19 c	7.20±0.15 c	12.67±0.24 c	6.43±0.30 b	11.87±0.13 c
F ratio	327.706	890.626	31.650	99.114	9.75	132.358
P value	0.000 ^{HS}	0.000 ^{HS}	0.000 ^{HS}	0.000 ^{HS}	0.013 ^{S}	0.000 ^{HS}

Table (2): Effect of Zn, Zn AA on fresh and dry weights of shoots of spinach .Each value is mean of 10 replicates ± standard error of means, NS = Non significant, S = Significant and HS = Highly significant.

Treatments	**F. Wt. of shoot (g)**		**D. Wt. of shoot (g)**		**F. Wt. of root (g)**		**D. Wt. of root (g)**	
	Stage I	**Stage II**	**Stage I**	**Stage II**	**Stage I**	**Stage II**	**Stage I**	**Stage II**
Control	**12.47±0.29 a**	**32.54±0.29 a**	**1.80±0.15 a**	**5.51±0.29 a**	2.10±0.06 a	2.77±0.14 a	0.33±0.33 a	0.93±0.09 a
Zn	**16.57±0.30 b**	**36.39±0.30 b**	**3.47±0.26 b**	**7.47±0.29 b**	3.30±0.15 b	3.50±0.29 b	0.87±0.07 b	1.73±0.12 b
Zn AA	**18.73±0.14 c**	**43.33±0.33 c**	**4.53±0.27 c**	**7.60±0.23 b**	4.57±0.30 c	4.90±0.21 c	0.90±0.11 b	2.00±0.11 b
F ratio	157.178	307.502	34.389	18.678	39.883	23.850	16.059	26.000
P value	**0.000** ^{HS}	**0.000** ^{HS}	**0.001** ^{HS}	**0.003** ^{S}	**0.000** ^{HS}	**0.001** ^{HS}	**0.004** ^{S}	**0.001** ^{HS}

Biochemical assay:

It was found from the obtained results that application of Zn AA was more effective than Zn on photosynthetic pigments contents. This was the case throughout the two stages of growth. Results revealed also that treatment with either Zn or Zn AA markedly increased the carotenoid content. Figs (1-4).

Results in figs. (5 -7) revealed that, the carbohydrate, protein, proline contents in spinach plants were, highly significantly increased with compared to control.

The zinc element in stress condition produce an enhancing role on osmotic adjustment process (due to the increase of soluble carbohydrates). Zinc is a vital and occasional consumption element, that have an natural part in protein, and carbohydrate synthesis, cell metabolic process, protection of cell membrane from reactive oxygen species along with other processes connected with adaptation of plants to worry, to ensure that, under drought stress conditions the role of the element is visible like a cause of osmotic regulation, by using intervention within the synthesis of osmotic compounds for compatibility with stress and keep turgor pressure performed their roles (**Eid et al., 2010).**

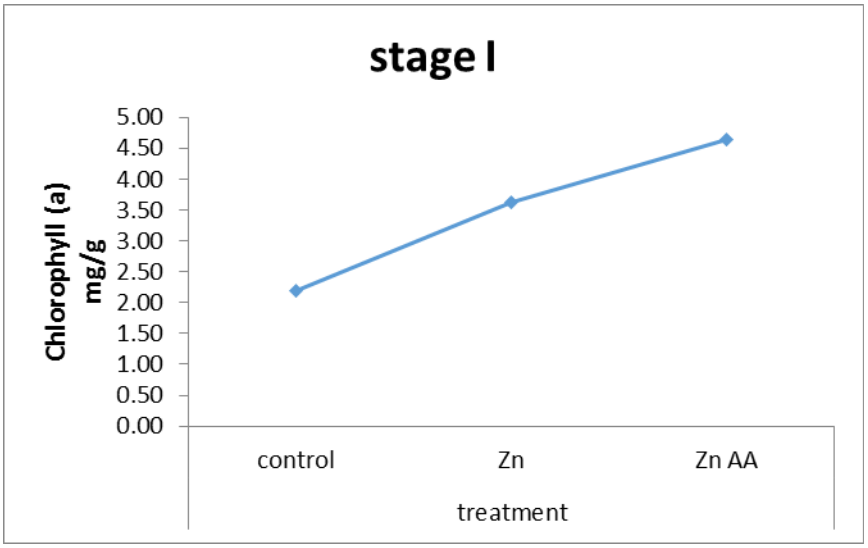
One of the most abundant groups of organic compounds in the plant kingdom is the carbohydrates.

The contents of chlorophyll a; b; total chlorophyll (a+ b) and carotenoids of common bean plants showed, in most cases, consistent and gradual decreases in response to various treatments applied of Zn at all doses. The obtained results agree with those observed by a number of investigators for example, **El-Gready *and* El-Tohamy (2007)** observed that the lowest Zn concentration (50 ppm) caused a decrease in the total chlorophyll content of snap beans L. plants compared to that of untreated plants.

Results of the present work showed that contents of total carbohydrates were, generally, significantly increased in shoots, in response to the treatment with micronutrients (Zn and Zn AA). In this regard, several investigators proved that micronutrients (Zn and Zn AA) plays an important role in carbohydrate metabolism.

The iron and zinc element have an enhancing role on osmotic adjustment process (due to the increase of soluble carbohydrates).

Wanas (2002) reported that, spraying faba bean plants with Zn (50 and 100 ppm) significantly enhanced chlorophylls a & b, carotenoids, total carbohydrates and crude protein contents. **Rashed and Ahmed (1997)** found that, the contents of soluble carbohydrates in faba bean plants were increased in response to the foliar application of Zn (50 ppm).



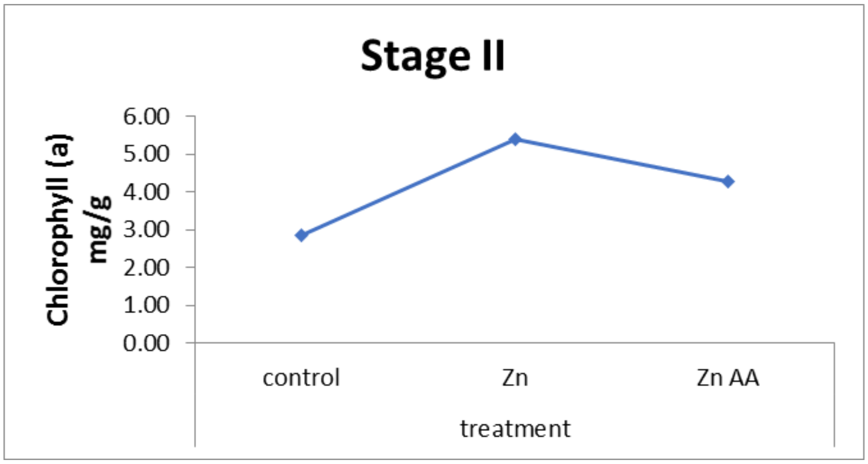
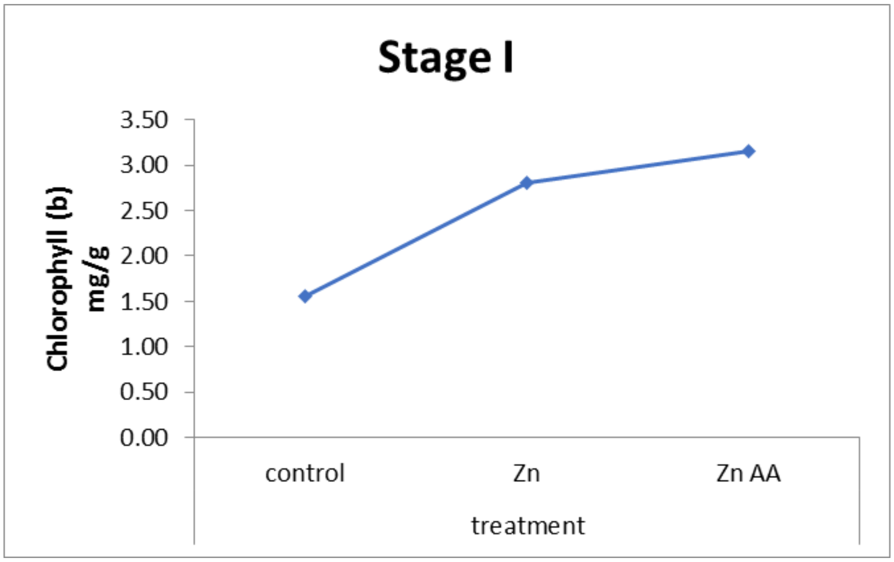


Figure (1): Effect of Zn and Zn AA on the chlorophyll (a) contents (mg/g fresh weight) of spinach.



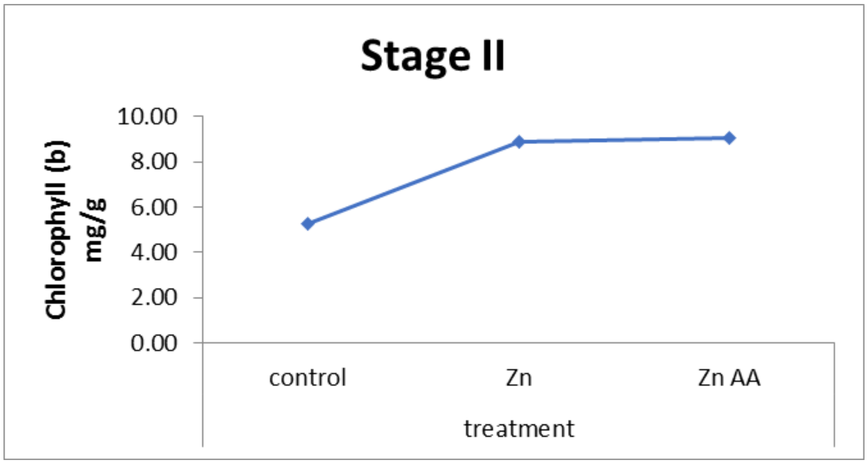
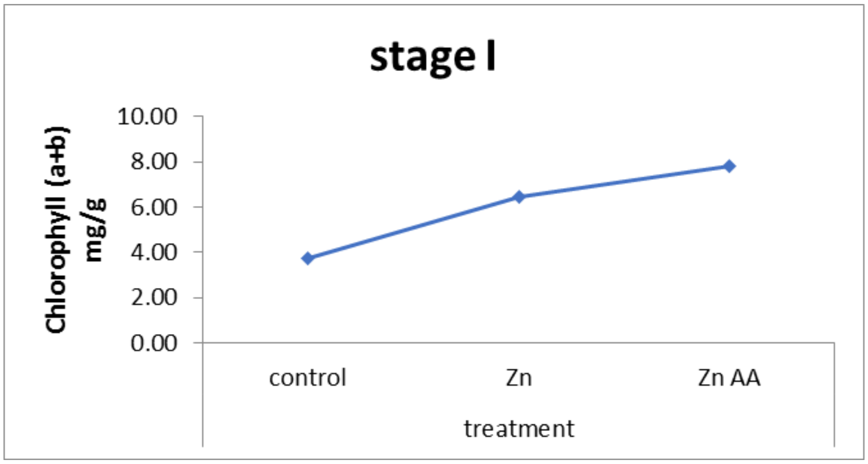


Figure (2): Effect of Zn and Zn AA on the chlorophyll (b) contents (mg/g fresh weight) of spinach.



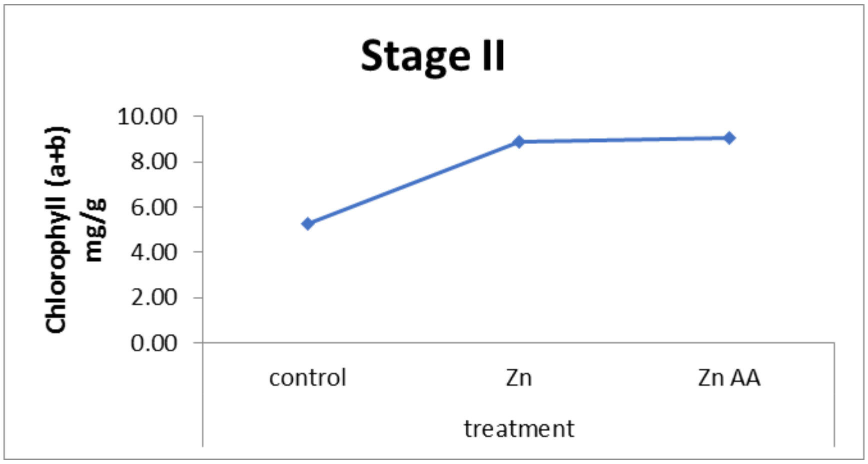
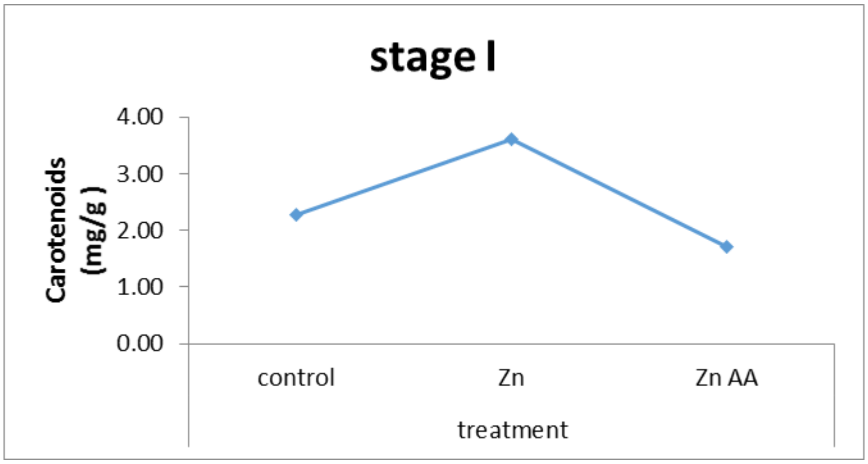


Figure (3): Effect of Zn and Zn AA on the chlorophyll (a+b) contents (mg/g fresh weight) of spinach.



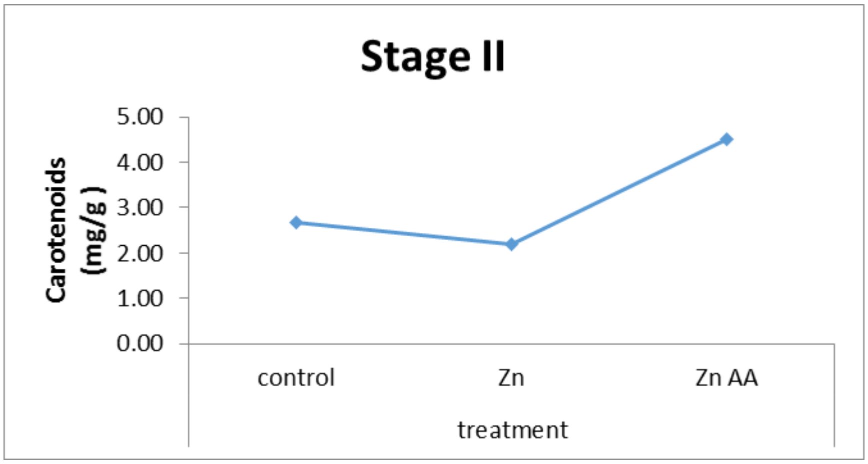
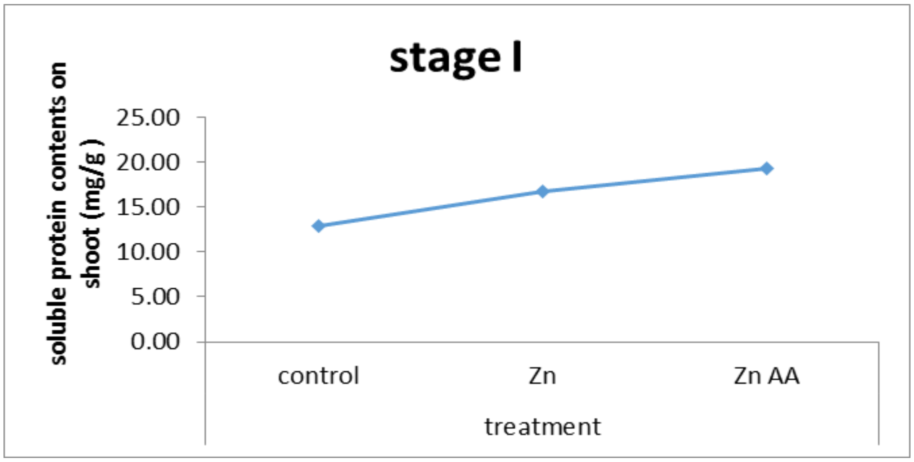


Figure (4): Effect of Zn and Zn AA on the carotenoids contents (mg/g fresh weight) of spinach.



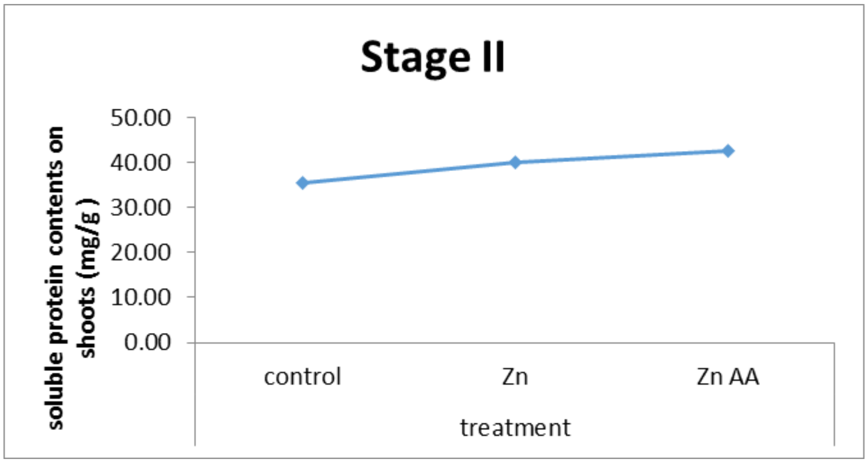
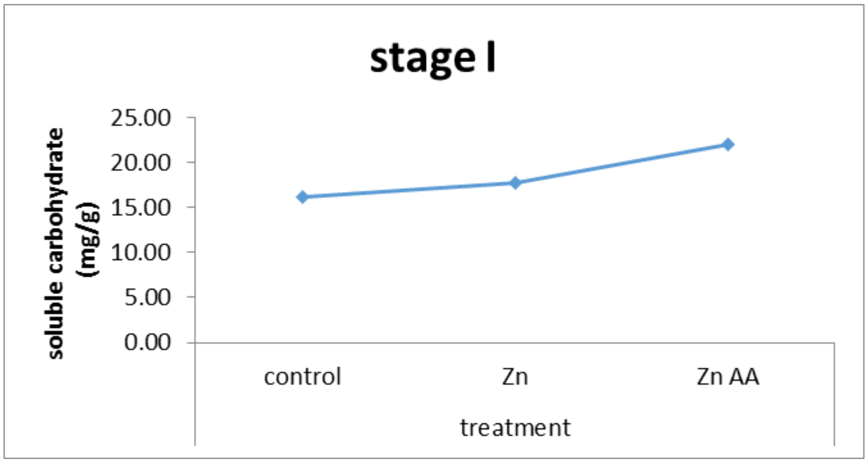


Figure (5): Effect of Zn and Zn AA On the total-soluble protein Contents in shoot (mg/g dry weight) spinach.



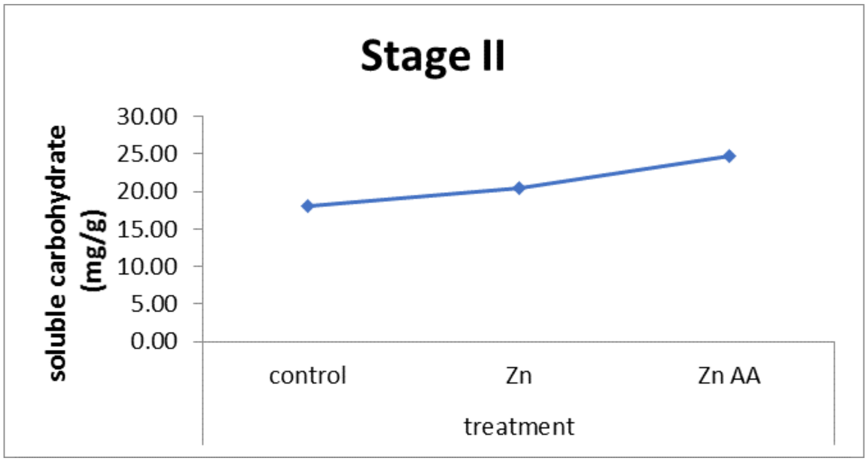
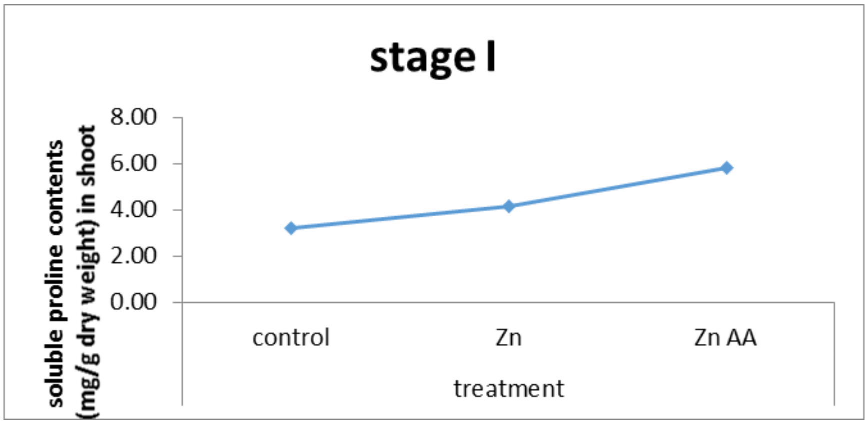


Figure (6): Effect of Zn and Zn AA On the total-soluble carbohydrate Contents in shoot (mg/g dry weight) spinach



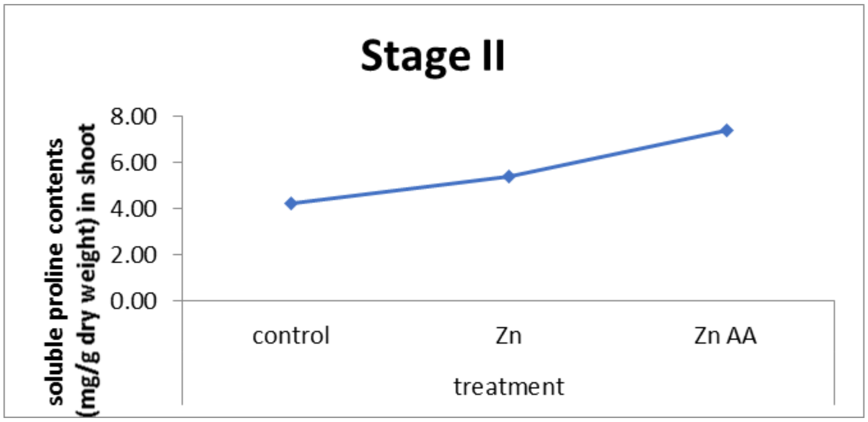


Figure (7): Effect of Zn and Zn AA On the total-soluble proline Contents in shoot (mg/g dry weight) spinach

Antioxidant enzymes

On the contrary, application of Zn, Zn AA caused significant changes changes in peroxidases, catalase activities at the two stages of growth with untreated plant plants grown figs (8&9)

Environmental stresses result in the generation of reactive oxygen species (ROS) in plants. ROS accumulate in cells and lead to the oxidation of proteins, chlorophyll, lipids, nucleic acids, carbohydrates etc. Cells have evolved intricate defence systems including enzymatic (catalase (CAT), ascorbate peroxidase (APX), which can scavenge the indigenously generated ROS. Plant stress tolerance mediated by antioxidants has been shown by many workers. Antioxidant resistance mechanisms may provide a strategy to enhance plant stress tolerance. Various enzymes involved in ROS-scavenging have been manipulated, over-expressed or down-regulated to add to the present knowledge and understanding of the role of antioxidant system.

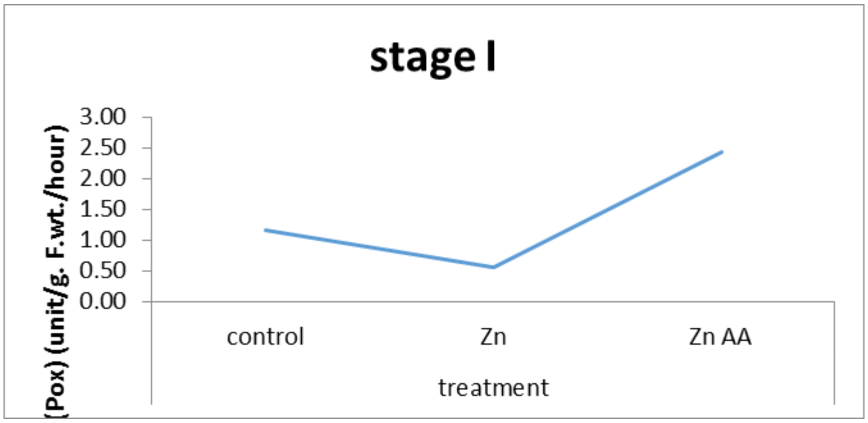
Tobbal (1999) revealed that, activities of catalases were increased. Activities of peroxidases were stimulated in fenugreek plants, but they were, mostly, inhibited in chickpea plants in response to the treatment with Zn (0.1 %).

El-Gebaly et al. (2003) pointed out that, Zn treatment at 0.06% to flax plants slightly affected the activities of peroxidasese and polyphenyleoxidases enzymes.

Sofy (2009) reported that, application of Zn (75 ppm) increased the activities of catalases and peroxidase in faba bean plants.

Nutrient uptake by crop plants grown in soil is greatly influenced by root morphology, soil properties, and climate and plant **species (Chandra, 2005**). Numerous essential plant nutrient elements are known to regulate the plant metabolism (**Alam, 1999**).

Zago and Oteiza (2001) reported also that zn increased anioxidant contents.



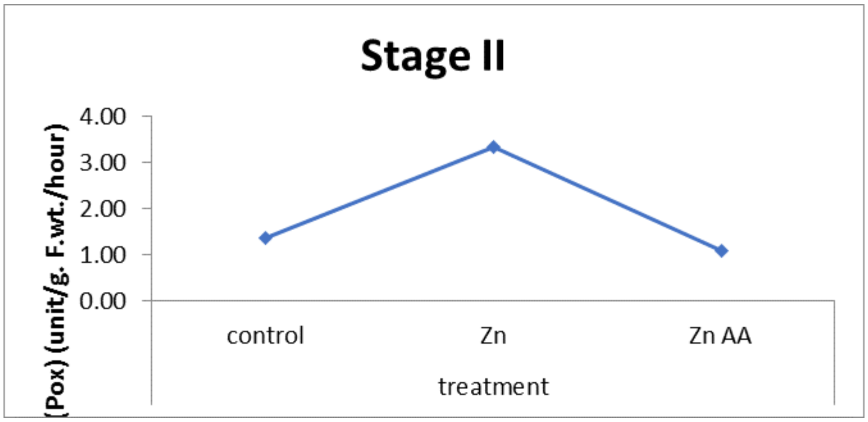
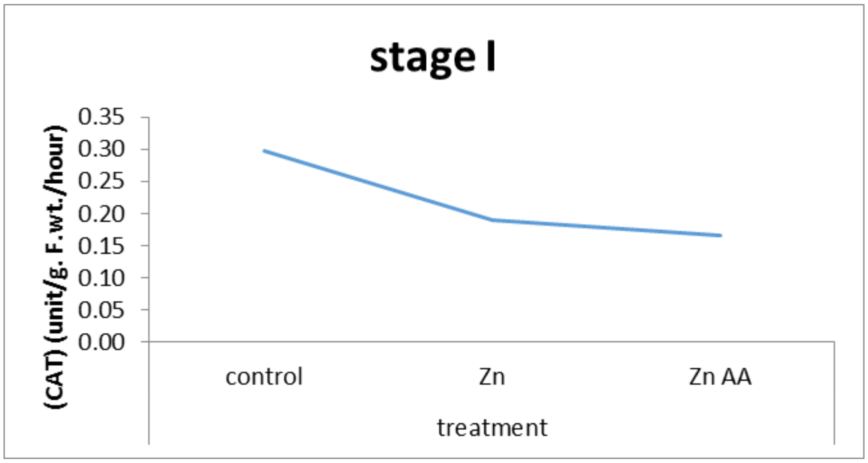


Figure (8): Effect of Zn and Zn AA on the activity of peroxidases (POX) (unit/g. F.wt./hour) of spinach.



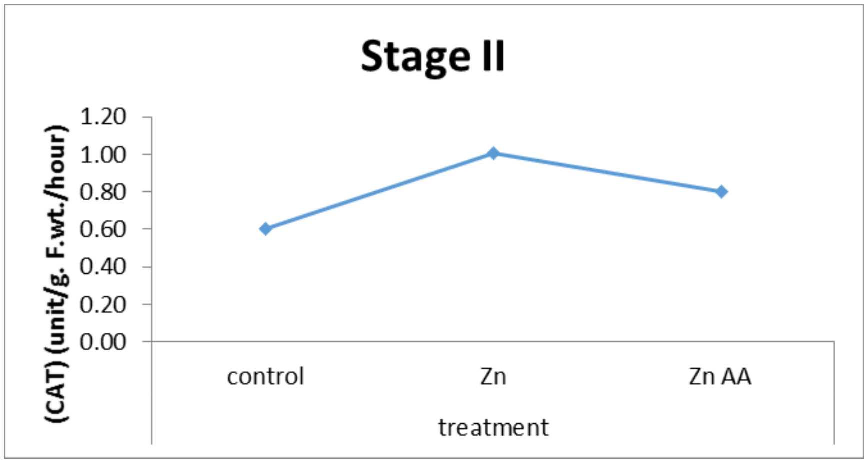


Figure (9): Effect of Zn and Zn AA on the activity of catalase (CAT) (unit/g. F.wt./hour) of spinach.

CONCLUSION

In conclusion, Zn or Zn AA application improved quality of plant and biochemical assay . In general, it is concluded that by using Zinc AA can vegetative better as compared to with or without using this Zn fig (10).

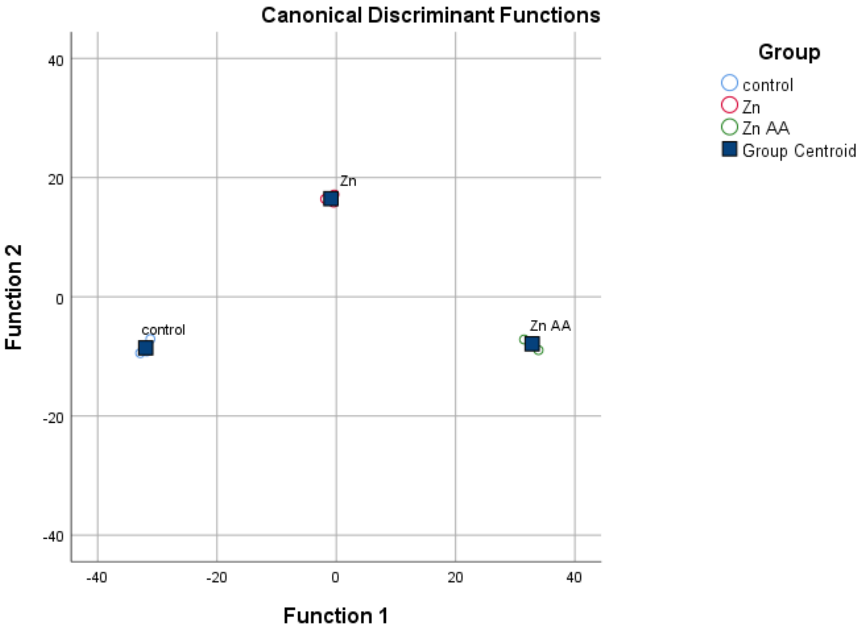


Figure 10. Discriminant of the vegetative of the Spanish in response to Zn and Zn AA

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