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EFFECT OF SOIL INOCULATION WITH MYCORRHIZA AND APPLICATION WITH SULPHUR ON VEGETATIVE GROWTH AND NUTRITIONAL STATUS OF YOUNG PERSIMMON TREES GROWN IN CALCAREOUS SOIL

BY

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ABSTRACT

The experiment was conducted during 2007 and 2008 seasons to study the effect of mycorrhizal inoculation (M), elemental sulphur application(S) and their interaction (M x S) on vegetative growth and nutritional status of three years-old young "Costata" persimmon trees budded on Diospyrous lotus rootstock and grown on calcareous soil at Abo El-Matamer District, El-Beheira Governorate. The obtained results indicated that, mycorrhizal inoculation stimulated vegetative growth and increased leaf chlorophyll content, total carbohydrates in shoot and leaves and C/N ratio values in leaves as well as leaf N, P, Fe, Mn and Zn contents. Meanwhile, C/N ratio in shoots and leaf K,Ca, and Mg-contents were insignificantly affected in the two seasons of study. Moreover, elemental sulphur addition at 0.5 or 1.0 kg/tree improved vegetative growth and significantly increased leaf chlorophyll content, total carbohydrates and C/N ratio in shoots and leaves as well as leaf N, P, Ca, Mg, Fe, Mn and Zn-contents. On the other hand leaf K-content was slightly reduced in both seasons.

In the two experimental seasons, the application of mycorrhizal inoculation under sulphur application with 0.5 kg/tree (M_1 x S_1) or mycorrhizal inocalation under sulphur application with 1.0 kg/tree (M_1 x S_2) combination treatments recorded the highest values of shoot and leaf growth parameters, total carbohydrates as well as leaf chlorophyll and mineral contents without significant differences between them while, the least values was obtained from to the control treatment, non inoculation with mycorrhiza without sulphur (M_0 x S_0).

Thus, this study recommends "Costata" persimmon growers on calcareous soil to add 0.5 kg/tree elemental sulphur with VA-Mycorrhizal inoculation in (M_1 x S_1) combination treatment for stimulating vegetative growth and improving tree nutritional status.

INTRODUCTION

Japanese persimmon (*Diospyrous kaki* L.) is a deciduous tree, belongs to family Ebenaceae. It was introduced to Egypt in 1911 by the Ministry of Agriculture and successfully grown under the Egyptian environmental conditions. That was due to its low chilling requirements (George *et al.,* 1994). Persimmon trees grow well in a wide range of soil, while they grow and produce best on deep fertile medium textured and will drained soil (Laruc *et al.,* 1982 and Gabr, 2006). However, it's acreage in Egyptian newly reclaimed soils is stimulated .

Different types of new reclaimed soils particularly calcareous soils have alkaline reaction. Application of some mineral fertilizers may be ineffective because of the expected problem of its availability expected. Where phosphorus in those soils are fixed in insoluble forms unavailable to plants. Phosphorus deficiency may be limit plant growth than do nitrogen deficiency (Awad, 1999). In addition, persimmon trees grown on calcareous soil developed in many instances chlorotic symptoms. This lime induce chlorosis is due to iron (Fe) deficiency whereas, micronutrients e.g. Fe, Mn and Zn are in unavailable forms under condition of high soil pH and $CaCO_3$ content (Clark and Kajiura, 1986).

Inoculation with VA-Mycorrhiza positively influenced several aspects of plant physiology especially enhanced absorption of phosphorus and other relatively immobile micronutrients cations, particularly zinc and copper (Lambert *et al.*, 1979, Vidal *et al.*, 1992 and Marin *et al.*, 2003), increased water uptake (Graham *et al.*, 1987) improved resistance to root diseases (Rosendahl and Rosendahl, 1990) and produced more growth promoting compounds viz auxins, cytokinins and gibberellins (Slankis, 1973), thus affected different aspects on plant growth and development.

Where the soil pH is high, correction of Fe Mn and Zn deficiency can be attempted by adjusting pH with acidifying compounds such as elemental sulphur (Clark and Smith, 1986). Whereas, sulphur is oxidized by soil micro-organisms to sulphuric acid in amount enough to lower soil pH and improve availability of most soil nutrients and uptake by plants is expected (Kassem *et al.*, 1995).

Therefore, the objective of this investtigation was to study the possible effects of mycorrhizal inoculation, sulphur application and their interaction on vegetative growth and nutritional status of young "Costata" persimmon trees grown under calcareous soil conditions.

MATERIALS AND METHODS

This work was carried out during 2007 and 2008 seasons on three year old "Costata" persimmon trees budded on Diospyrous lotus rootstock, planted at 5 x 5 meters and grown in private orchard located at Abo El-Matamer District, Beheira Governorate, Egypt. The orchard soil was classified as calcareous (14.5% $CaCO_3$) and slightly alkaline (pH = 8.2). The depth of water table was about 140-160 cm. Some chemical and physical properties of the experimental soil are presented in Table (1).

Table (1): **Some chemical and physical properties of the experimental soil (0-60 cm, depth).**

Soil variable	Values	Soil variable	Values
		Soluble ions meq/L	
		Cations	
pH	8.2	Na^+	0.34
EC mmhos/cm	0.54	K^+	0.13
OM %	0.99	Ca^{++}	1.18
$CaCO_3$ %	14.5	Mg^{++}	1.36
Porosity %	64.7	**Anions**	
Bulk dneisty gm/cm^3	0.98	Cl^-	0.95
Total P (ppm)	11.8	HCO^-_3	1.24
Available P (ppm)	2.1	$CO^=_3$	0.00
		$SO^=_4$	0.82
Partical size distriubiton %		**DTPA extractabel micronutrietns (ppm)**	
Sand	51.3	Fe	18.5
Silt	15.2	Mn	11.7
Clay	33.5	Zn	12.6
Textural grad	Sandy clay loam		

OM: organic matter

Thirty six uniform trees were selected and subjected to the normal horticultural practices NPK fertilization program applied was 900 gm ammonium nitrate (33.5% N), 500 gm calcium super phosphate (16% P_2O_5) and 400 gm potassium sulphate (48% K_2O) per tree.

Elemental agriculture sulphur at three levels 0.0, 0.5 and 1.0 kg/tree (expressed as S_0, S_1 and S_2, respectively) were added during winter agricultural management at the beginning of January in both seasons and mixed with farm manure enriched layer with in 20 cm, depth under the tree canopy. Two months later, the experimental trees were divided into two groups. The first were inoculated with vesicular arbuscular mycorrhiza spores (M_1), while, the second non inoculated (M_0). The inoculation of VA-Mycorrhiza fungi was done using 20 ml spores suspension (50 spore/ml) for each tree according to the methods descrybed by Gerdemann and Nicolson (1963). The spores were mostly *Glomus* sp. and *Gigospora* sp.

The two inoculation VA mycorrhiza treatments (M_0 and M_1) and the three levels of sulphur application (S_0, S_1 and S_2) were arranged in six (2 x 3) combination treatments in a complete randomized block design as factorial experiment, each treatment replicated three times with two trees plot (3 replicates x 2 trees). Besides, guard rows were left to separate the plots.

Measurements and determinations:

a. Vegetative growth parameters:

Four branches in different directions on each tree were labeled. All current shoots developed on these branches in spring were counted and used for measuring vegetative growth parameters i.e. shoot length and diameter (cm) and number of leaves per shoot. Four shoots (one shoot per branch were sampled and all leaves were measured by using Li-core 3100 Aerometer to get area per leaf (cm^2). Shoot and leaf samples were oven dried at 70°C and weighted to get shoot and leaf dry weight (gm) and then leaf specific weight (L.S.W) was calculated as (mg/cm^2) according to Hunt (1989), also seasonal increment in Trunk cross section area (TCSA) (cm^2) was calculated.

b. Chemical determinations:

Thirty mature mid-shoot leaves in mid-August of both seasons were sampled to determined leaf mineral content. Nitrogen was estimated by microkjeldahl gunning method (A.O.A.C., 1990). Phosphorus was determined with a colourimetric method as described by Foster and Cornelia (1967). Potassium was determined by a flame photometer model E.E.L. (Jackson, 1967). Calcium, magnesium, iron, zinc and manganese were determined by Perking-Elmer Atomic absorpation spectro photometer model 2380 AL, according to Jackson and Ulish (1959) and Yoshida *et al.* (1972).

Chlorophyll a and b were extracted from fresh leaves with N.N. dimethyl formamide and determined spectrophotometrically, then total value were calculated according to the method of Moran (1982).

Total carbohydrate content was determined in leaf and shoot samples which were previously dried as percent on dry weight basis according to Dubois *et al.* (1956). Then C/N ratio was calculated.

The present data was statistically analyzed according to Snedecor and Cochran (1990) and the least significant differences (L.S.D.) were used to compare between the means representing the effect of mycorrhizal inoculation treatments, sulphur levels and their interaction.

RESULTS AND DISCUSSION

Effect of mycorrhizal inoculation (M), elemental sulphur application (S) and their interaction (M x S) on:

1. Vegetative growth parameters:

Obtained data in Tables (2 and 3) and Fig. (1) clear vegetative growth parameters of "Costata" persimmon trees as affected by VA-Mycorrhizal inoculation (M), elemental sulphur application (S) and their interaction (M x S) in 2007 and 2008 seasons. The data revealed that VAM inoculated trees produced higher number of new shoots per branch and leaves per shoot, taller and thicker shoots (cm), larger area per leaf and TCSA-increase

(Cm^2) as well as heavier shoot Dwt (gm/cm), leaf Dwt gm and leaf specific weight (LSW) mg/cm^2 than those of non-inoculated ones. The differences were significant in both seasons. This improvement in vegetative growth of mycorrhizal inoculated trees could be attributed to produce a greater root having relatively higher total potential absorbing surface over than of the uninfected system which enhance nutrient absorption particularly phosphorus and zinc (Nawar *et al.*, 1988). In addition, Marks and Kozlowski (1973) reported that mycorrhiza fungi provides the host plant with growth hormones including auxins, cytokinins gibbrellins and vitamins which stimulate plant growth. These results are in harmony with those obtained by Reddy *et al.* (1996), Yamashita *et al.* (1998), Yang *et al.* (2002), Gaber and Nour El-Dein (2005) and Marin *et al.* (2003) on different fruit species.

As for the effect of sulphur application it is clear that, sulphur addition with 0.5 and 1.0 kg/tree (S_1 & S_2) significantly increased all studied vegetative growth parameters of young "Costata" Persimmon trees as compared to the control (S_0). Differences between each of them and the control were significant in both seasons. The highest values belonged to high level (S_2) while, the least values were recorded with the control (S_0). This hold was true in both seasons. The obtained increase in vegetative growth parameters as a result of sulphur application might be due to the role of S in reducing soil pH after oxidization by soil micro-organisms to sulphuric acid and improving the availability of most soil nutrients (Koriem, 1994).

Table (2): Effect of soil inoculation with mycorrhiza, sulphur application and their interaction on shoot parameters of young "Costata" Persimmon trees in 2007 and 2008 seasons.

Treatments		No. of shoots/1-year old branch		Av. shoot length (cm)		Av. shoot diameter (cm)		Shoot D.Wt. (gm./cm)	
VA-Mycorrhiza (M) levels	Sulphur (S) levels	2007	2008	2007	2008	2007	2008	2007	2008
Non-inoculation (M_0)	S_0*	3.1	2.9	14.1	15.8	0.42	0.44	0.156	0.177
	S_1	3.7	3.3	18.7	18.5	0.47	0.48	0.189	0.201
	S_2	3.9	3.8	19.4	19.9	0.49	0.50	0.204	0.216
Average (M_0)		3.6	3.3	17.4	18.1	0.46	0.47	0.183	0.198
VAM-inoculation (M_1)	S_0	3.8	3.5	19.2	20.5	0.48	0.51	0.195	0.210
	S_1	4.3	4.3	23.3	24.2	0.56	0.58	0.217	0.229
	S_2	4.6	4.5	24.0	24.7	0.57	0.60	0.221	0.236
Average (M_1)		4.2	4.1	22.2	23.1	0.54	0.56	0.211	0.225
Average (S)	S_0	3.5	3.2	16.7	18.2	0.45	0.48	0.176	0.194
	S_1	4.0	3.8	21.0	21.4	0.52	0.53	0.203	0.215
	S_2	4.3	4.2	21.7	22.3	0.53	0.55	0.213	0.226
LSD 5%	M	0.23	0.27	0.54	0.50	0.015	0.021	0.0750	0.0680
	S	0.28	0.33	0.66	0.61	0.018	0.026	0.0919	0.0833
	M x S	0.39	0.46	0.94	0.86	0.026	0.036	0.1299	0.1178

M_0, and M_1 = Non-inoculation and VAM -inoculation, respectively.
S_0, S_1 and S_2 = 0.0, 0.5 and 1.0 kg sulphur/tree, respectively.
* This treatment served as control.

In addition, Kowalenko (1979) indicated that sulphur addition reduced nitrate losses and stimulated the reduction of NO_3 to NH_4, thereby, enhanced N availability and uptake. The above mentioned results are in accordance with those obtained by Gabr (2006) on Persimmon. Similar results on other tree fruit species were also reported by Kassem *et al.* (1995), El-Morshedy (1997), Zeerban *et al.* (2000), Dawood (2001) and

Zayan *et al.* (2006). However, the most important data were disclosed by the interaction (M x S) which was significant in both seasons. The highest number of shoots/branch and leaves/shoot beside, the highest values of shoot length and diameter (cm), leaf area cm^2, shoot and leaf dry weights (gm), LSW (mg/ cm^2) as well as TCSA-increase (Cm^2) were obtained with (M_1 x S_1) and (M_1 x S_2) combination treatments without significant differences between them in both seasons and differences between each of them and the control was significant. Whereas, the supplementary addition of sulphur to mycorrhizal inoculated trees induced stimulative effect.

Conclusively, VA-Mycorrhiza inoculation under soil application with 0.5 kg elemental sulphur in (M_1 x S_1) considered the suitable combination treatment for improving vegetative growth of young "Costata" persimmmon trees grown in calcareous soil.

2. Leaf chlorophyll content:

It is clear from data in Table (4) that, chlorophyll a, chlorophyll b and total chlorophyll contents (μg/cm^2) were higher in leaves of mycorrhizal inoculated "Costata" persimmon trees than those of non-inoculated ones. The differences were significant in both seasons except for chlorophyll b in the first season. These results are in line with the findings of Ezz and Nawar (1994) who found that, inoculation of sour orange seedling with mycorrhizal fungi increased leaf chlorophyll content.

Table (3): **Effect of soil inoculation with mycorrhiza, sulphur application and their interaction on leaf parameters of young "Costata" Persimmon trees in 2007 and 2008 seasons.**

Treatments		No. of leaves/shoot		Leaf area (cm^2)		Leaf D.Wt. (gm/leaf)		L:S.W. ** (mg/cm^2)	
VA-Mycorrhiza (M) levels	Sulphur (S) levels	2007	2008	2007	2008	2007	2008	2007	2008
Non-inoculation (M_0)	S_0*	6.5	6.9	44.33	48.60	0.598	0.671	13.5	13.8
	S_1	7.8	8.3	59.79	58.52	0.861	0.854	14.4	14.6
	S_2	8.0	8.5	63.82	64.76	0.913	0.939	14.3	14.5
Average (M_0)		7.4	7.9	55.98	57.29	0.791	0.821	14.1	14.3
VAM-inoculation (M_1)	S_0	7.7	8.4	51.13	53.14	0.752	0.755	14.7	14.2
	S_1	8.7	9.2	67.24	66.50	1.035	1.011	15.4	15.2
	S_2	8.9	9.1	72.75	71.42	1.113	1.107	15.3	15.5
Average (M_1)		8.4	8.9	63.71	63.69	0.967	0.958	15.1	15.0
Average (S)	S_0	7.2	7.7	47.73	50.87	0.675	0.713	14.1	14.0
	S_1	8.3	8.8	63.52	62.51	0.948	0.978	14.9	14.9
	S_2	8.5	8.8	68.29	68.09	1.013	1.023	14.8	15.0
LSD 5%	M	0.22	0.19	2.542	2.971	0.0407	0.0446	0.20	0.19
	S	0.27	0.23	3.114	3.639	0.0499	0.0546	0.25	0.23
	M x S	0.38	0.33	4.405	5.146	0.0705	0.0772	0.35	0.33

M_0, and M_1 = Non-inoculation and VAM -inoculation, respectively.
S_0, S_1 and S_2 = 0.0, 0.5 and 1.0 kg sulphur/tree, respectively.
* This treatment served as control.
** LSW = Leaf specific weight.

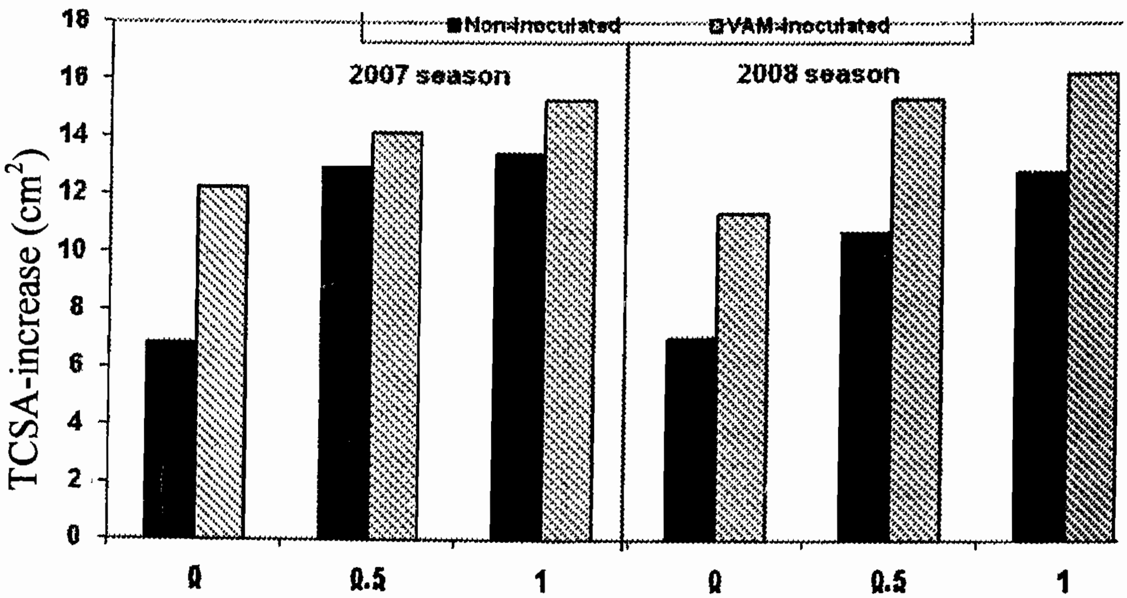

Fig. (1): **Effect of soil inoculation with mycorrhiza and sulphur application on TCSA-increase cm² of young "Costata" persimmon trees in 2007 and 2008 seasons. + TCSA (Trunk Cross Section Area)**

Table (4): **Effect of soil inoculation with mycorrhiza, sulphur application and their interaction on leaf chlorophyll content of young "Costata" Persimmon trees in 2007 and 2008 seasons.**

Treatments		Chlorophyll contents (µg/cm²)					
		Chl. a		Chl. b		Total chl.	
VA-Mycorrhiza (M) levels	Sulphur (S) levels	2007	2008	2007	2008	2007	2008
Non-inoculation (M_0)	S_0*	60.21	60.09	25.56	27.60	85.77	87.78
	S_1	65.35	64.50	26.77	27.57	92.12	92.11
	S_2	67.80	67.32	28.02	27.94	95.82	95.25
Average (M_0)		64.45	63.96	26.78	27.70	91.24	91.71
VAM-inoculation (M_1)	S_0	66.72	67.28	27.05	26.50	93.77	93.88
	S_1	72.14	71.90	26.82	29.24	98.97	100.84
	S_2	72.46	72.65	29.30	30.55	101.76	103.48
Average (M_1)		70.44	70.61	27.72	28.76	98.17	99.40
Average (S)	S_0	63.47	63.69	26.31	27.05	89.77	90.83
	S_1	68.75	68.20	26.80	28.41	95.55	96.48
	S_2	70.13	69.99	28.66	29.25	98.79	99.37
LSD 5%	M	0.414	0.398	N.S	1.333	2.342	1.928
	S	0.507	0.487	1.945	1.633	2.869	2.360
	M x S	0.717	0.689	1.399	2.309	4.058	3.339

M_0, and M_1 = Non-inoculation and VAM -inoculation, respectively.
S_0, S_1 and S_2 = 0.0, 0.5 and 1.0 kg sulphur/tree, respectively.
* This treatment served as control.

Moreover, Nawar *et al.* (1988) concluded that total leaf chlorophyll and chlorophyll a contents were significantly higher in inoculated sour orange seedling than that of uninoculated ones while, chlorophyll b were not affected. Furthermore, Marks and

Kozlowski (1973) indicated that, the increase in chlorophyll content of mycorrhizal plant could be attributed to the ability of mycorrhiza fungi for providing cytokinin which enhance chloroplast development. In addition, sulphur application with 0.5 or 1.0 kg/tree significantly increased chl. a, chl. b and total chl. contents in leaves of "Costata" Persimon trees as compared to untreated one in both seasons. The positive effect of sulphur might be attributed to the role of this amendment in improving soil physical and chemical properties and increased the availability of macro and micronutrients especially nitrogen, iron and magnesium. Such elements were chlorophyll constituents or have a close association in chlorophyll synthesis (Hall and Rao, 1996). These results are in harmony with those reported with El-Morshedy (1997), Zeerban *et al.* (2000), Gabr (2006) and Zayan *et al.* (2006). They indicated that, addition of sulphur markedly increased leaf a, b and total chlorophyll contents. As for the interaction, the data revealed that, the interaction (M x S) was significant in the two seasons of study and the highest values of chl. a, chl. b and total chl. contents recorded with (M_1 x S_1) and (M_1 S_2) interactions. On the other hand, the control treatment (M_0 x S_0) recorded the least values.

3. Total carbohydrates and C/N ratio:

Data being presented in Table (5) indicates that, micorrhizal inoculation of "Costata" persimmon trees significantly increased total carbohydrate concentration in leave and shoots as well as C/N ratio values only in leaves in comparison with non infected control. Meanwhile, C/N ratio in shoot was not affected in both seasons. This increment in total carbohydrate may be due to increasing the rate of photosynthesis, (Eissenstat *et al.,* 1993). Also, Sherstha *et al.* (1995) mentioned that, photosynthesis and transpiration rates of AM inoculated trees were greater than those of non-AM ones and had leaf area 3 times larger than others. The obtained data are confirmed with those reported by Nawar *et al.* (1988) on sour orange seedling and Gabr and Nur El-Dein (2005) on apple trees they concluded that, total sugars, starch and total carbohydrates were significantly increased in leaves and shoots of infected trees when compared to the uninfected ones. However, El-Shamma *et al.* (2001) indicated that, the calculated C/N ratio in shoots of young olive trees were not clearly affected by VAM.

Data in Table (5) clear that, total carbohydrates and C/N ratio values in both leaves and shoots of "Costata" persimmon trees were significantly increased by adding elemental sulphur with 0.5 or 1.0 kg/trees, without significant differences between them. Data of the second season take the same trend. This findings in completely seedless" grapevines and El-Shamma *et al.* (2001) on young olive trees. The interaction (M x S) was significant in both seasons and maximum values of total carbohydrates and C/N ratio were recorded with mycorrhizal inoculation trees under sulphur application with 0.5 or 1.0 kg/tree in (M_1 x S_1) or (M_1 x S_2) combination treatments without significant differences between them in both seasons while, the least values were belonged to the control. Thus, (M_1 x S_1) economically considered the best combination treatment under the conduction of this study.

4. Leaf mineral content:

a. Leaf macronutrient:

According to data presented in Table (6), it is clear that, N and P concentrations in leaves of inoculated "Costata" persimmon trees were significantly increased in comparison with non-inoculated control. However K, Ca and Mg did not clear any significant differences in both seasons. The obtained data are confirmed with those reported by Awad (1999) on "Flame" grape who indicated that, leaf P-content was higher in infected plants. On the other side, leaf K, Ca and Mg-contents were slightly influenced due to mycorrhizal inoculation. Similar results were also obtained by Abd El-Maksoud *et al.* (1988) on sour orange, Maksoud *et al.* (1994) on tamarinds, El-Morsy and El-Ansary (1997) on ruby seedless grape, El-Shamma *et al.* (2001) on young olive trees, Marin *et al.* (2003) on persimmon and Gaber and Nur El-Dein (2005) on ANA apple.

Table (5): Effect of soil inoculation with mycorrhiza, sulphur application and their interaction on total carbohydrate and C/N ratio of young "Costata" Persimmon trees in 2007 and 2008 seasons.

Treatments		Total carbohydrate %				C/N ratio			
		Leaves		Shoots		Leaves		Shoots	
VA-Mycorrhiza (M) levels	Sulphur (S) levels	2007	2008	2007	2008	2007	2008	2007	2008
Non-inoculation (M_0)	S_0*	5.26	5.73	12.07	13.18	2.83	2.97	10.68	10.99
	S_1	6.92	6.84	15.15	15.51	3.25	3.32	11.39	11.65
	S_2	7.16	7.41	16.10	16.64	3.27	3.43	11.50	11.36
Average (M_0)		6.45	6.66	14.44	15.11	3.12	3.24	11.19	11.33
VAM-inoculation (M_1)	S_0	6.28	6.37	15.12	15.31	2.77	2.73	11.34	10.93
	S_1	7.86	7.91	17.26	16.76	3.42	3.35	11.81	11.44
	S_2	8.05	8.12	17.36	17.88	3.31	3.38	11.87	11.68
Average (M_1)		7.40	7.47	16.58	16.65	3.17	3.15	11.67	11.35
Average (S)	S_0	5.77	6.05	13.60	14.25	2.80	2.85	11.01	10.96
	S_1	7.39	7.38	16.21	16.14	3.34	3.34	11.60	11.55
	S_2	7.61	7.77	16.73	17.26	3.29	3.41	11.69	11.52
LSD 5%	M	0.225	0.247	1.033	0.890	0.179	0.226	N.S	N.S
	S	0.279	0.300	1.265	1.090	0.219	0.277	0.808	0.680
	M x S	0.395	0.424	1.790	1.541	0.310	0.392	1.143	0.961

Table (6): Effect of soil inoculation with mycorrhiza, sulphur application and their interaction on leaf macronutrients (%) of young "Costata" Persimmon trees in 2007 and 2008 seasons.

Treatments		Macronutrients (%) on D.W.t									
		N		P		K		Ca		Mg	
VA-Mycorrhiza (M) levels	Sulphur (S) levels	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
Non-inoculation (M_0)	S_0*	1.87	1.93	0.145	0.148	2.19	2.12	2.23	2.16	0.31	0.32
	S_1	2.13	2.07	0.166	0.170	2.15	2.08	2.31	2.25	0.44	0.46
	S_2	2.20	2.17	0.173	0.176	2.04	2.00	2.52	2.41	0.43	0.49
Average (M_0)		2.07	2.06	0.161	0.165	2.13	2.07	2.35	2.27	0.39	0.42
VAM-inoculation (M_1)	S_0	2.27	2.33	0.179	0.182	2.27	2.35	2.18	2.04	0.33	0.30
	S_1	2.30	2.37	0.192	0.189	2.23	2.31	2.36	2.30	0.48	0.44
	S_2	2.43	2.40	0.195	0.192	2.15	2.23	2.58	2.56	0.50	0.47
Average (M_1)		2.33	2.37	0.189	0.188	2.22	2.30	2.37	2.30	0.44	0.40
Average (S)	S_0	2.07	2.13	0.162	0.165	2.23	2.24	2.21	2.10	0.32	0.31
	S_1	2.22	2.22	0.179	0.180	2.19	2.20	2.34	2.28	0.46	0.45
	S_2	2.32	2.29	0.184	0.184	2.10	2.12	2.55	2.49	0.47	0.48
LSD 5%	M	0.132	0.124	0.006	0.005	N.S	N.S	N.S	N.S	N.S	N.S
	S	0.161	0.152	0.007	0.006	N.S	N.S	0.111	0.120	0.069	0.075
	M x S	0.228	0.215	0.011	0.009	N.S	N.S	0.158	0.164	0.097	0.106

M_0, and M_1 = Non-inoculation and VAM -inoculation, respectively.
S_0, S_1 and S_2 = 0.0, 0.5 and 1.0 kg sulphur/tree, respectively.
* This treatment served as control.

Data also showed that leaf N, P, Ca and Mg-contents were significantly increased by adding sulphur with 0.5 or 1.0 kg/tree with compared to the control (S_0). At the same time, K determinations recorded a slight decrease without significant differences between the two sulphur rates and the control. These findings are in line with those of Gabr (2006) who mentioned that, addition of sulphur amendment caused significantly increased in leaf N, P, Ca and Mg concentrations but slightly reduced leaf K-content. Also, Koriem (1994) reported a marked increase in leaf N and P concentration, meanwhile, decrease in leaf K-content. Similar results were also reported by Zayan *et al.* (1989) and El-Shamma *et al.* (2001). The increment in P could be attributed to solubility and availability of this nutrient as result of reducing pH. While, the reduction in leaf K-content due to dilution effect (Kareim, 1994). However, the most important data were disclosed by the interaction and the highest leaf N, P, Ca and Mg concentrations belonged to (M_1 x S_1) or (M_1 x S_2) treatment without significant differences between them, the least value recorded with untreated trees (M_0 x S_0) in both seasons.

b. **Leaf micronutrients:**

Data presented in Table (7) show, VAM inoculation and sulphur applications as well as their interaction resulted in increase leaf Fe, Mn and Zn content in both seasons. The most effective combination treatments were (M_1 x S_1) and/or (M_1 x S_2) which recorded the highest concentrations while, the minimum values came from the control (M_0 x S_0). Other treatments gave the intermediate values. These results are in agreement with those reported by El-Shamma *et al.* (2001) who indicated that micorrhizal inoculation significantly increased leaf micronutrients may be due to its activity by producing CO_2. Moreover, Singh and Kappor (1999) pointed out that, plant hormones being released by mycorrhiza increase plant root growth, cause in turn increasing plant root surface which improves nutrients absorption. However, the observed benefits of sulphur application might be attributed to increase of nutrients availability as a result to reduction in soil pH. These results confirmed with those reported by many previous investigators such as Abd El-Maksoud *et al.* (1988), Awad (1999), El-Shamma *et al.* (2001) and Gaber and Nur El-Dein 2005 on mycorrhiza inoculation and Shehata *et al.* (1992), Azzazy *et al.* (1994), Abdel-Nasser and El-Shazly (2000), Zeerban *et al.* (2000) and Gabr (2006) on sulphur.

Table (7): **Effect of soil inoculation with mycorrhiza, sulphur application and their interaction on leaf micronutrients (ppm) of young "Costata" Persimmon trees in 2007 and 2008 seasons.**

Treatments		Micronutrients (ppm) on D.Wt.					
		Fe		Mn		Zn	
VA-Mycorrhiza (M) levels	Sulphur (S) levels	2007	2008	2007	2008	2007	2008
Non-inoculation (M_0)	S_0*	98.5	114.3	58.6	60.1	17.7	16.8
	S_1	123.2	125.3	70.2	72.4	19.6	19.2
	S_2	125.5	127.5	76.8	81.5	19.8	19.5
Average (M_0)		115.7	122.4	68.5	71.3	19.0	18.5
VAM-inoculation (M_1)	S_0	120.6	128.4	67.8	69.3	20.1	19.6
	S_1	132.1	137.6	79.3	84.7	22.4	21.8
	S_2	130.6	140.3	86.5	88.2	23.0	22.7
Average (M_1)		129.4	135.4	77.9	80.7	21.9	21.4
Average (S)	S_0	109.6	121.4	63.2	64.7	18.9	18.2
	S_1	127.7	131.5	74.8	78.6	21.0	20.5
	S_2	130.6	133.9	81.7	84.9	21.4	21.1
LSD 5% M		3.20	2.37	1.15	1.78	0.21	0.25
S		6.98	2.90	1.41	2.19	0.26	0.30
M x S		5.54	4.11	1.99	3.09	0.37	0.43

M_0, and M_1 = Non-inoculation and VAM -inoculation, respectively.

S_0, S_1 and S_2 = 0.0, 0.5 and 1.0 kg sulphur/tree, respectively.

* This treatment served as control.

Therefore, this study recommends "Costata" persimmon growers on calcareous soil to add sulphur at 0.5 kg/tree with VAM inoculation in (M_1 x S_1) combination treatment which is considered the best economic treatment in this study. This treatment not only stimulated vegetative growth but also improved tree nutritional status.

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تأثير تلقيح التربه بالميكروهيزا والمعاملة بالكبريت على النمو الخضرى والحالة الغذائية لأشجار الكاكى الحديثة النامية فى الأرض الجيرية

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أجريت تجربة حقلية خلال موسمى ٢٠٠٧ ، ٢٠٠٨م بهدف دراسة تأثير التلقيح بالميكروهيزا والمعاملة بالكبريت والتفاعل بينهما على النمو الخضرى والحالة الغذائية لأشجار الكاكى الحديثة (عمر ٣ سنوات) المطعومة على أصل الكاكى Diospyrous lotus والنامية فى الأرض الجيرية بمركز أبوالمطامير ــ محافظة البحيرة.

أظهرت النتائج أن التلقيح بالميكروهيزا شجع النمو الخضرى وزاد محتوى الأوراق من الكلوروفيل ومحتوى النموات والأوراق من الكربوهيدرات الكلية ونسبة (C/N) فى الأوراق بالإضافة لمحتوى الأوراق من النيتروجين والفوسفور والحديد والمنجنيز والزنك بينما لم تتأثر معنويا نسبة (C/N) فى النموات ومحتوى الأوراق من البوتاسيوم والكالسيوم والماغنيسيوم فى كل من سنتى الدراسة.

كما أوضحت النتائج أن إضافة الكبريت المعدنى للتربة بمعدل ٠,٥ ، ١,٠كجم/شجرة قد حسن من النمو الخضرى وزاد محتوى الأوراق من الكلوروفيل ومحتوى النموات والأوراق من الكربوهيدرات الكلية ونسبة (C/N) علاوة على محتوى الأوراق من النيتروجين والفوسفور والكالسيوم والماغنسيوم والحديد والمنجنيز والزنك ومن جهة أخرى فإن محتوى الأوراق من البوتاسيوم قد انخفض بدرجة طفيفة فى كلا الموسمين.

بينت نتائج كلا الموسمين أن أى من المعاملتين المركبتين التلقيح بالميكروهايزا مع أضافه ٠,٥ كجم كبريت / شجره (م١ × ك١) أو التلقيح بالميكروهايزا مع أضافه ١,٠ كجم كبريت / شجره (م١ × ك٢) و قد سجلت أعلى قيم لمقاييس النمو والورقة والكربوهيدرات الكلية بالإضافة إلى محتوى الأوراق من الكلوروفيل والعناصر المعدنية بدون فروق معنوية بينهما ، بينما أقل القيم تتبع معاملة المقارنة بدون تلقيح بالميكروهيزا مع عدم أضافه الكبريت (م٠ × ك٠).

لذلك توصى هذه الدراسة مزارعى الكاكى فى الأراضى الجيرية بإضافة ٠,٥كجم/شجرة كبريت معدنى مع تلقيح التربة بالميكروهيزا فى المعاملة المركبة (م١ × ك١) لتشجيع النمو الخضرى وتحسين الحالة الغذائية للأشجار.