

Using Amino Acids, Silicon, Selenium, Humic Acid and Em for Counteracting the Inferior Effects of Salinity on Fruiting of Picual Olive Tress

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Abstract: During 2014, 2015 and 2016 seasons, Picual olive trees growing under salinity stress were subjected to application of two amino acids namely arginine and glutamic acid, silicon (Si) and selenium (Se) each at 25 ppm and humic acid + effective microorganisms (EM) each at 50 ml/tree/year in single and combined forms. The merit was alleviated the adverse effects of salinity on yield as well as both physical and chemical characteristics of the fruits. Salinity was 4.69 dsm-1 and 3.13 dsm-1 for soil and irrigation water, respectively. All amino acid, Si, Se and humic acid + EM treatments were responsible for promoting initial fruit setting %, fruit retention %, yield, fruit weight, fruit pulp % and fruit oil % relative to the control. Using humic acid+ EM+ Si and / or Se had striking effect on all the investigated parameters than using amino acids plus Si and/ or Se. Silicon effects were greater than Se impacts in this respect. Combined applications of Si and Se was considerably preferable than using each alone in this connection. For improving yield and fruit quality of Picual olive trees growing under salinity stress, it is suggested to add humic acid+ EM via soil each at 50 ml/tree/year besides spraying Si and Se each at 25 ppm three times.

[Abd El-Hameed, M.M Wassel ; Faissal F. ahmed ; Mohamed H. E El-Sheikh and Waleed M. E. F. Mohamed. **Using Amino Acids, Silicon, Selenium, Humic Acid and Em for Counteracting the Inferior Effects of Salinity on Fruiting of Picual Olive Tress.** *N Y Sci J* 2017;10(11):106-113]. ISSN 1554-0200 (print); ISSN 2375-723X (online). <http://www.sciencepub.net/newyork>. 13. doi:[10.7537/marsnys101117.13](https://doi.org/10.7537/marsnys101117.13).

Keywords: Picual olives, arginine, glutamic acid, silicon, selenium, humic acid, effective microorganisms, yield, fruit quality.

1. Introduction

Salt stress imposes a major environmental threat to agriculture and its adverse impacts are getting more serious problem in regions where saline water is used for irrigation. Salinity is one of the major environmental factor limiting plant growth and productivity **Nikolskii-Gavrilovet *et al.*, 2015 and Grattan *et al.*, 2015**).

Recently, many attempts were accompanied for counteracting the inferior effects of salinity stress on yield and fruit quality by using non- traditional methods. Out of these methods were the application of amino acids, silicon, selenium and organic and biofertilization.

Using amino acids is responsible for enhancing the biosynthesis of proteins, DNA, RNA, enzymes, antioxidants, vitamins, cell division, sugars and natural hormones namely IAA and ethylene. There are very effective in inhibiting the formation of reactive oxygen speeds (ROS) that caused great damage on the permeability of cell walls and the dead of plants. (**Mengelet *et al.*, 2001**).

Application of silicon was found by **Sauvasset *et al.*, (2002)** and **Meloet *et al.*, (2003)** and **Ma (2004)** as well as selenium as reported by **Zhang and Gladyshev (2009)** and **Pilon-Smits *et al.* (2009)** to enhance the tolerance of fruit crops to biotic and abiotic stresses,

the biosynthesis of most organic foods, uptake of water and nutrients and the formation of natural hormones. Their impact as antioxidants in reducing reactive oxygen speeds (ROS) surely reflected in protecting plant cells from death.

Humic substances have many important roles in plant nutrition and soli fertility. Plants grown in soils which contain adequate humic substances are less subject to stress and are healthier status **Ferraraet *al.*, (2001)**.

Effective microorganisms (EM) consists of different beneficial microorganisms. It is responsible for plant development and soli fertility as it improves biological activity and availability of nutrients. The occurrence of this microorganisms led to maximize the uptake of nutrients and the release of vitamins B, plant hormones and antibiotics **Kannaiyan, (2002)**.

Higher salinity has an obvious inhibition on yield and fruit quality in different olive cvs. (**Loreto *et al.*, 2003; Chartzoulakis, 2005; Melgaret *et al.*, 2009 and Gad 2013**).

The results of **El-Badway and Abd El-aal (2013)**, **Ahmed *et al.*, (2014a & 2014 b)**, **Hassan (2014)** and **Hassan- Huda (2014)** emphasized the beneficial effects of amino acids on stimulating yield and fruit quality of the fruit crops grown under salinity stress.

Previous studies showed that using silicon (Gad El-Kareem, 2012; Ahmed *et al.*, (2014a & 2014 b); Al-Wasfy, 2013; Abdelaal and Oraby, Mona, 2013; El-khwaga and Mansour, 2014 and Mohamed, 2015) and selenium (Gad El-Kareem, *et al.*, 2014; Ibrahiem and Al-Wasfy, 2014 and Masoud, 2017) had an announced promotion on yield and fruit quality in different crop fruits.

Organic and biofertilization using humic acid (Moffed, 2009; Youssef- Amalet *al.*, 2011; Khaled and Fawy, 2011 and Haggag- Lailaet *al.*, 2013) and effective microorganisms (Kannaiyan, 2002; Gamal, 2006 and Hassan-Huda, 2014) were favourable in enhancing yield and fruit quality in various crop trees.

The purpose of this study was elucidating the effect of amino acids, silicon, selenium, humic acid and effective microorganisms on alleviating the adverse effects of salinity in the soil and water irrigation on yield and fruit quality of Picual olive trees grown under West Samalout, Minia region.

2. Materials and Methods

This study was conducted during 2014, 2015 and 2016 seasons on Picual olive trees. The trees of olive were about 12- years old, propagated by leafy cutting and growing in a private orchard located at village (4) west Samalout district, Minia Governorate.

The Picual olive cv. were planted at 6x6 meter apart in sand soil under drip irrigation system with the same amount of water and subjected to the regular recommended horticultural practices and free from pathogens and physiological disorders. Soil was washed end of year to ensure soil salinity was stable. Salinity of soil was 3000 ppm and salinity of water was 2000 ppm.

Soil analysis was done according to Piper (1950), Black (1965) and Evenhuis and Dewaard (1980).

Table (1): Analysis of the tested soil

Content	Value
Sand %	91.0
Silt %	2.5
Clay	6.5
Texture grade	Sandy
pH (1: 2.5 extract)	7.51
EC (1: 2.5 extract) dsm^{-1}	0.6
Calcium carbonate %	2.5
Total N%	0.08
Available P (Olsen, ppm)	2.1
Available K (ammonium acetate, ppm)	95.0
Available micronutrient (ppm)	-
Zn	1.0
Fe	0.7
Mn	0.8
Cu	0.2

1- Experimental work:

This experiment included seventeen treatments consisted from picual olive cv.

- 1) Spraying water (control).
- 2) Spraying L-Arginineamino acid at concentration 25 ppm.
- 3) Spraying Glutamic amino acid at concentration 25 ppm.
- 4) Spraying L -Arginineamino acid at 25ppm + Glutamic amino acid at 25ppm.
- 5) Addition of Humic at rate 50 ml + addition of E.M at rate 50 ml.
- 6) Spraying L-Arginineamino acid at 25ppm + spraying selenium at 25 ppm.
- 7) Spraying L -Arginineamino acid at 25ppm + spraying silicon at 25 ppm.
- 8) Spraying L -Arginineamino acid at 25ppm + spraying selenium at 25 ppm+ spraying silicon at 25 ppm.
- 9) Spraying Glutamic amino acid at 25 ppm + spraying selenium at 25 ppm.
- 10) Spraying Glutamic amino acid at 25 ppm + spraying silicon at 25 ppm.
- 11) Spraying Glutamic amino acid at 25 ppm + spraying selenium at 25 ppm+ spraying silicon at 25 ppm.
- 12) Spraying L-Arginineamino acid at 25ppm+ Glutamic amino acid at 25ppm + spraying selenium at 25 ppm.
- 13) Spraying L-Arginineamino acid at 25ppm+ Glutamic amino acid at 25ppm+ spraying silicon at 25 ppm.
- 14) Spraying L-Arginineamino acid at 25ppm+ Glutamic amino acid at 25ppm + spraying selenium at 25 ppm+ spraying silicon at 25 ppm.
- 15) Addition of Humic at rate 50 ml + addition of E.M at rate 50 ml + spraying selenium at 25 ppm.
- 16) Addition of Humic at rate 50 ml + addition of E.M at rate 50 ml + spraying silicon at 25 ppm.
- 17) Addition of Humic at rate 50 ml + addition of E.M at rate 50 ml + spraying selenium at 25 ppm+ spraying silicon at 25 ppm.

Each treatment was replicated three times, one tree per each.

Humic acid and E.M were added one time at growth start (1st week of Mar.) one time. Spraying of selenium, silicon and amino acids was carried out three times at growth start (1st week of Mar.), just after fruit setting (mid. of Apr.) and at one month later (mid./ of May). Triton B as a wetting agent was added to all selenium, silicon and amino acid solutions at 25 ppm and spraying was done till runoff (10 L / tree). Selenium and silicon were dissolved in ethyl alcohol. Silicon and selenium were applied in potassium silicate and pure selenium forms, respectively. Amino

acids, silicon and selenium, humic acid and EM were used at the recommended concentrations (according to **Gad El- Kareem, 2012; El- Sayed- Esraa, 2007 and Gamal, 2006**).

2- Experimental design:

This study was statistically analyzed using randomized complete block design (RCBD), where the experiment included seventeen treatments from single and combined applications of amino acids, silicon, selenium and humic acid+ EM. Each treatment was replicated three times one tree per each.

3- Different measurements:

3.1. Percentages of initial fruit setting and fruit retention:

Twenty shoots (one - years - old) on each tree were labeled for counting the initial number of flowers at full bloom. Number of fruitlets and fruits were recorded at monthly intervals up to harvest. Numbers of fruits were recorded on each of the selected shoots according to **Ferguson et al., (1994)** as follows:

Fruit set (%)= No. of developing fruitlets/ Total initial No. of flowers at full bloom x 100

Fruit retention % was calculated by dividing number of fruits just before harvesting date by total number of setted fruits and multiplying the product x 100.

3.2- yield per tree (Kg)

Fruits of each experimented tree was harvested at ripe stage (olive with superficial pigmentation on more than 50% of the exo-carp) and the average yield was calculated.

3.3.- Fruits quality

A sample of 50 fruits from each tree was randomly chosen to determine fruit weight (g) and percentage of fruit pulp.

Oil content (%) was determined by extraction the oil from the dried flesh samples using the Soxhlet fat extraction apparatus and using petroleum ether (60-80°C) boiling point as a solvent for about 16 continuous hours and the percentage of oil on dry weight was calculated (**A.O.A.C, 2000**).

4.- Statistical analysis

Each treatment had three replicates with one tree per a replicate. The trees of control treatment were sprayed with tap water. The results in this study were exposed to proper statistical analysis of variance for a randomized complete block design (RCBD). New L.S.D. test at 5% was used for making all various treatment comparison between means (**Snedecor and Cochran, 1980 and Mead et al., 1993**).

3. Results

1. Percentages of initial fruit setting and fruit retention

Data in Tables (2 & 3) clearly show that supplying the trees grown under salinity condition s

with the two amino acids namely arginine and glutamic acid, silicon (Si) and selenium (Se) each at 25 ppmas well as humic acid plus effective microorganisms (EM) each at 50 ml/tree/year significantly improved the percentages of initial fruit setting and fruit retention over the control treatment (trees grown under salinity conditions alone). Spraying arginine at 25 ppm had no significant promotion compared to the control treatment. Using glutamic acid was preferable than using arginine in this respect. Using both amino acids together was superior than using each alone in this connection. Application of Si with amino acids/ or with humic acid + EM significantly was favourable than using Se with the other materials. Using Si and/or Se with humic acid + EM significantly was superior than using Si and/or Se with amino acids in improving such two parameters. The highest values were recorded on the trees that received humic acid + EM + Si + Se than the other treatments. The best results were recorded on the trees that received humic acid + EM + Si+ Se. Under such promised treatment initial fruit setting reached 47.23 & 47.12 & 46.47 % while the percentages of fruit retention were 15.20 & 15.44 & 15.09 during 2014, 2015 and 2016 seasons, respectively. The untreated trees growing under salinity stress without treatment produced initial fruit setting reached 20.40 & 20.74 & 20.69 % and fruit retention % reached 5.00 & 5.13 & 5.08 % during the three seasons, respectively. These results were true during the three seasons.

2. Yield/tree

Data in Table (4) obviously reveal that varying amino acid, Si, Se and humic acid + EM treatments had significant differences on the yield relative to the control treatment. Subjecting the trees growing under salinity stress conditions with amino acids, Si, Se and humic acid + EM in combinations treatment significantly improved the yield above the check treatment. A slight promotion on the yield was observed due to supplying the trees with arginine and/ or glutamic acid compared with the control. Using Si was preferable than using Se in improving the yield. Using Si and /or Se along with amino acids or with humic acid+ EM was significantly preferable than using amino acids or humic acid + EM alone in this respect. Treating the trees with humic acid + EM+ Si+ Se gave the highest values of the yield (112.20 & 114.44 & 111.61 kg) during the three seasons, respectively. The yield of the untreated trees reached 79.90 & 81.64 & 81.02 kg during 2014, 2015 and 2016 seasons, respectively. The percentage of increment on the yield due to using humic acid + EM+ Se+ Si above the check treatment reached 40.43 & 40.81 & 37.76 % during 2014, 2015 and 2016 seasons. This results were nearly the same during the three seasons.

3. Fruit weight and fruit pulp%

It is clear from the data in Tables (5 & 6) that subjecting the trees to combined applications of amino acids, Si, Se and humic + EM significantly was very effective in enhancing fruit weight and pulp % relative to the control treatment. Using Si and Se in the investigated treatments significantly was superior than using any one alone in this respect. Using humic acid+ EM + Si and /or Se significantly surpassed the application of amino acid plus Si and/or Se. The best results were obtained due to treating the trees with humic acid + EM+ Si+ Se. The untreated trees produced the lowest values. Similar results were announced during the three seasons.

4. Percentage of fruit oil

As shown in Table (7) oil % was varied according to the present treatments. It was varied significantly when amino acids as well as humic acid+ EM treatments were applied with Si and/or Se. Using arginine and /or glutamic acid and humic acid+ EM had a slight promotion compared with the check treatment. Using Si and/or plus amino acids or humic acid+ EM significantly was very effective in enhancing oil % than using amino acids and humic acid+ EM without Si and Se. The maximum values of fruit oil % (14.65 & 14.61 & 14.41 %) were recorded on the fruit from the trees treated with humic acid+ EM + Si + Se during the three seasons, respectively. Percentage of fruit oil of the fruits picked from the untreated trees reached 12.06 & 12.26 & 12.20% during the three seasons, respectively. Similar results were announced during the three seasons.

4. Discussion

The deleterious effects of salinity on plant growth are associated with low osmotic potential of the soil solution (water stress), nutritional imbalance, specific effects (salt stress) or a combination of these factors (Grattan *et al.*, 2015).

Using amino acids is responsible for enhancing the biosynthesis of proteins, DNA, RNA, enzymes, antioxidants, vitamins, cell division, sugars and natural hormones namely IAA and ethylene. There are very effective in inhibiting the formation of reactive oxygen speeds (ROS) that caused great damage on the permeability of cell walls and the dead of plants. (Mengelet *et al.*, 2001).

Application of silicon was found by Sauvasset *et al.*, (2002) and Meloet *et al.*, (2003) and Ma (2004) as well as selenium as reported by Zhang and Gladyshev (2009) and Pilon-Smits *et al.* (2009) to enhance the tolerance of fruit crops to biotic and abiotic stresses, the biosynthesis of most organic foods, uptake of water and nutrients and the formation of natural hormones. Their impact as antioxidants in reducing

reactive oxygen speeds (ROS) surely reflected in protecting plant cells from death.

Humic substances have many important roles in plant nutrition and soil fertility. Plants grown in soils which contain adequate humic substances are less subject to stress and are healthier status Ferraraet *al.*, (2001).

Effective microorganisms (EM) consists of different beneficial microorganisms. It is responsible for plant development and soil fertility as it improves biological activity and availability of nutrients. The occurrence of this microorganisms led to maximize the uptake of nutrients and the release of vitamins B, plant hormones and antibiotics Kannaiyan, (2002).

These results regarding the adverse effects of soil and water salinity on yield and fruit quality are in agreement with those obtained by Loreto *et al.*, (2003); Chartzoulakis, (2005); Melgaret *et al.*, (2009) and Gad (2013). of El-Badway and Abd El-aal (2013), Ahmed *et al.*, (2014a & 2014 b), Hassan (2014) and Hassan- Huda (2014) emphasized the beneficial effects of amino acids on improving yield and fruit quality of the fruit crops grown under salinity stress.

Previous studies showed that using silicon (Gad El-Kareem, 2012; Ahmed *et al.*, (2014a & 2014 b); Al-Wasfy, 2013; Abdelaal and Oraby, Mona, 2013; El-khwaga and Mansour, 2014 and Mohamed, 2015) and selenium (Gad El-Kareem, *et al.*, 2014; Ibrahim and Al-Wasfy, 2014 and Masoud, 2017) had an announced promotion on yield and fruit quality in different crop fruits.

Organic and biofertilization using humic acid (Moffed, 2009; Youssef- Amalet *et al.*, 2011; Khaled and Fawy, 2011 and Haggag- Lailaet *et al.*, 2013) and effective microorganisms (Kannaiyan, 2002; Gamal, 2006 and Hassan-Huda, 2014) were favourable in enhancing yield and fruit quality in various crop trees.

Table (2): Effect of spraying of silicon, selenium, L-arginine acid, glutamic acid and addition of Humic and E.M on the percentage of initial fruit setting of Picual olive cv. during 2014, 2015 and 2016 seasons

Treatments	2014	2015	2016
Control	20.40	20.74	20.63
Arginine	21.67	22.05	21.89
Glutamic	23.15	24.73	24.01
Arginine.+ Glutamic	24.07	24.95	24.56
Humic acid.+EM	24.92	27.49	26.32
Arginine +Se	27.96	28.03	27.53
Arginine +Si	28.90	30.60	29.89
Arginine +Se+ Si	31.11	31.71	31.35
Glutamic +Se	31.87	32.73	32.20
Glutamic +Si	34.45	35.62	34.86
Glutamic +Se+Si	36.02	36.83	36.19
Arginine + Glutamic +Se	38.00	40.19	39.46
Arginine +Glutamic.+Si	38.70	41.18	40.36
Arginine +Glutamic +Se +Si	41.48	43.54	42.80
Humic +EM+ Se	42.43	44.20	43.62
Humic +EM+ Si	44.80	46.21	45.67

Humic +EM +Se+ Si	47.23	47.12	46.47
LSD 0.05	1.90	2.06	2.07

EM: Effective microorganisms Se: Selenium Si: Silicon

Table (3): Effect of spraying of silicon, selenium, l-arginine acid, glutamic acid and addition of Humic and E.M on the percentage of fruit retention of Picual olive cv. during 2014, 2015 and 2016 seasons.

Treatments	2014	2015	2016
Control	5.00	5.13	5.08
Arginine	4.82	5.34	5.08
Glutamic	6.14	6.49	6.33
Arginine.+ Glutamic	6.51	6.83	6.69
Humic acid.+EM	7.08	7.42	7.28
Arginine +Se	7.83	7.87	7.72
Arginine +Si	8.27	9.06	8.70
Arginine +Se+ Si	9.06	9.83	9.42
Glutamic +Se	9.77	10.19	9.95
Glutamic +Si	10.64	11.24	10.88
Glutamic +Se+Si	11.04	11.78	11.34
Arginine + Glutamic +Se	11.60	12.55	12.18
Arginine +Glutamic.+Si	11.83	12.67	12.38
Arginine +Glutamic +Se +Si	12.87	13.34	13.20
Humic +EM+ Se	13.71	14.20	14.05
Humic +EM+ Si	14.30	14.90	14.65
Humic +EM +Se+ Si	15.20	15.44	15.09
LSD 0.05	1.21	1.11	0.90

EM: Effective microorganisms Se: Selenium Si: Silicon

Table (4): Effect of spraying of silicon, selenium, l-arginine acid, glutamic acid and addition of Humic and E.M on yield per tree (kg) of Picual olive cv. during 2014, 2015 and 2016 seasons.

Treatments	2014	2015	2016
Control	79.90	81.64	81.02
Arginine	82.17	83.65	83.04
Glutamic	83.55	87.15	85.62
Arginine.+ Glutamic	83.57	85.14	84.51
Humic acid.+EM	87.61	89.30	88.76
Arginine +Se	91.50	89.48	88.98
Arginine +Si	91.49	93.62	93.01
Arginine +Se+ Si	94.63	95.52	94.90
Glutamic +Se	94.72	95.16	94.62
Glutamic +Si	100.12	99.33	99.23
Glutamic +Se+Si	101.34	101.97	100.99
Arginine + Glutamic +Se	102.50	107.71	106.11
Arginine +Glutamic.+Si	100.78	106.33	104.63
Arginine +Glutamic +Se +Si	104.45	108.96	107.43
Humic +EM+ Se	105.57	111.70	109.41
Humic +EM+ Si	108.80	115.26	112.47
Humic +EM +Se+ Si	112.20	114.44	111.61
LSD 0.05	5.00	5.30	6.10

EM: Effective microorganisms Se: Selenium Si: Silicon

Table (5): Effect of spraying of silicon, selenium, l-arginine acid, glutamic acid and addition of Humic and E.M on the fruit weight (g) of Picual olive cv. during 2014, 2015 and 2016 seasons.

Treatments	2014	2015	2016
Control	7.11	7.25	7.20
Arginine	7.33	7.35	7.35
Glutamic	7.47	7.50	7.51
Arginine.+ Glutamic	7.49	7.52	7.53
Humic acid.+EM	7.51	7.63	7.60
Arginine +Se	7.87	7.63	7.62
Arginine +Si	7.81	7.87	7.88
Arginine +Se+ Si	7.95	7.98	7.95
Glutamic +Se	7.86	7.90	7.86
Glutamic +Si	8.11	8.13	8.08
Glutamic +Se+Si	8.09	8.04	8.01
Arginine + Glutamic +Se	8.11	8.42	8.34
Arginine +Glutamic.+Si	7.90	8.20	8.13
Arginine +Glutamic +Se +Si	8.10	8.53	8.37
Humic +EM+ Se	8.14	8.61	8.43
Humic +EM+ Si	8.26	8.77	8.55
Humic +EM +Se+ Si	8.53	8.80	8.61
LSD 0.05	0.43	0.34	0.48

EM: Effective microorganisms Se: Selenium Si: Silicon

Table (6): Effect of spraying of silicon, selenium, l-arginine acid, glutamic acid and addition of Humic and E.M on the fruit pulp percentage of Picual olive cv. during 2014, 2015 and 2016 seasons

Treatments	2014	2015	2016
Control	85.03	86.62	86.10
Arginine	85.95	87.44	86.82
Glutamic	86.00	88.06	87.75
Arginine.+ Glutamic	86.00	88.10	870.80
Humic acid.+EM	86.31	87.42	87.16
Arginine +Se	88.20	85.76	85.53
Arginine +Si	86.61	87.92	87.70
Arginine +Se+ Si	87.54	87.82	87.53
Glutamic +Se	85.90	85.48	85.40
Glutamic +Si	88.26	88.37	87.88
Glutamic +Se+Si	87.43	87.53	86.90
Arginine + Glutamic +Se	87.20	90.71	89.81
Arginine +Glutamic.+Si	84.64	88.80	87.62
Arginine +Glutamic +Se +Si	86.44	90.30	88.97
Humic +EM+ Se	86.23	91.90	89.71
Humic +EM+ Si	87.50	92.80	90.51
Humic +EM +Se+ Si	89.37	93.98	90.58
Control	1.47	2.41	1.86

EM: Effective microorganisms Se: Selenium Si: Silicon

Table (7): Effect of spraying of silicon, selenium, l-arginine acid, glutamic acid and addition of Humic and E.M on the fruit oil percentage of Picual olive cv. during 2014, 2015 and 2016 seasons

Treatments	2014	2015	2016
Control	12.06	12.26	12.20
Arginine	12.25	12.40	12.35
Glutamic	12.49	12.66	12.62
Arginine.+ Glutamic	12.34	12.45	12.42
Humic acid.+EM	12.61	12.72	12.71
Arginine +Se	13.10	12.69	12.68
Arginine +Si	13.09	13.27	13.24
Arginine +Se+ Si	13.33	13.34	13.31
Glutamic +Se	13.23	13.15	13.15
Glutamic +Si	13.70	14.47	13.61
Glutamic +Se+Si	13.69	13.58	13.54
Arginine + Glutamic +Se	13.79	14.17	14.12
Arginine +Glutamic.+Si	13.47	13.85	13.80
Arginine +Glutamic +Se +Si	13.86	14.23	14.14
Humic +EM+ Se	13.90	14.25	14.17
Humic +EM+ Si	14.25	14.56	14.45
Humic +EM +Se+ Si	14.65	14.61	14.41
LSD 0.05	0.70	0.90	0.80

Conclusion

For improving yield and fruit quality of Picual olive trees growing under salinity stress, it is suggested to add humic acid+ EM via soil each at 50 ml/tree/year besides spraying Si and Se each at 25 ppm three times.

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11/13/2017