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Importance of Arbuscular mycorrhizal fungi inoculation and iron chelates addition on "Rich Lady "peach (*Prunus persica* L. Batsch) variety: enhancement of mineral status and peaches quality

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Abstract - Peach trees are highly susceptible to lime induced chlorosis which can strongly affect fruit quality and yield resulting on an unbalance of nutrients uptake. The present study was assessed in order to evaluate the impact of arbuscular mycorrhizal fungi (AMF) inoculation and the Fe-ethylenediamine-di (*O*-hydroxy-phenylacetic-acid) (Fe-EDDHA) on the 'Rich Lady' peach variety behavior, under iron deficiency conditions. At 30 days after full bloom (DAFB), a mixture of six commercial strains of AMF *Glomus* sp. (T1), a synthetic Fe-EDDHA (T2) and a combination of both treatments (T3) were supplied and compared to a non inoculated and non fertilized peach trees (Tc) . The shoots length, the peaches size and the leaves mineral contents were evaluated at 60 and 120 DAFB. The fruit quality was performed at ripening.Results showed greatest effects of the overall applied treatments, compared to the control, in improving the shoots growth, the mineral uptake and equilibrium and the fruit quality. At 60 DAFB, T1, T2 and T3 treatments, significantly improved the foliar total nitrogen and magnesium levels (29%, 34%, 52% and 10%, 16%, 20% respectively) (at $P<0.05\%$). They also significantly decreased the foliar potassium and calcium leaves contents (-22%, -18%, -17% and -21%,-21%, -29% respectively) (at $P<0.05\%$). At 120 DAFB, the T1, T2 and T3 treatments significantly increased the leaves phosphorus levels and leaded to the equilibrium of the iron and magnesium leaves levels. Nevertheless, the T3 treatment was the most effective in decreasing the potassium leaves contents as response to iron chlorosis correction. In addition, it significantly enhanced the peaches size, weight (32.5%) and firmness (15%) and the juice weight (40%), volume (28.9%), pH (4.8%), soluble solids contents (13.3%). Else, it significantly decreased the titratable acidity of peaches by 14.2%. The approach offers challenge of use of AMF for iron chlorosis correction in sustainable agriculture.

Keywords: *Prunus persica* (L. Batsch), AMF, Fe, Fruit quality

1. Introduction

The arbuscular mycorrhizal fungi (AMF) are used as biofertilizers on many economically cultivated fruit trees. They are with a great interest in the management of plant nutrition in the sustainable agriculture and their role in improving Fe uptake through the soil solution was proved (Chadwik et al. 2015 ; Gong et al. 2009; Li et al. 2015). In fact, it was demonstrated that AMF showed a greater iron uptake when bioassayed for hydroxamatesiderophores: an iron-binding agents secreted by bacteria and fungi, under iron deficient conditions (Haselwandter 2008; Rains et al. 2015). In addition, the ability of AFM in forming symbiosis with many strategic fruit stone crops, such as peach (*Prunuspersica* L. Batsch) trees: a well spread culture through the world and the Mediterranean area, has been largely highlighted (Dinghra et al. 2014; Pérez-Jiménez et al. 2014). The iron (Fe) chlorosis caused by Fe deficiency, is the major problem that affect the nutritional steadiness, the fruits quality and the yield occurring on calcareous soils with high HCO_3^- and pH levels (Álvarez-Fernández et al. 2011). So, the Fe-ethylenediamine-di (*O*-hydroxy-phenylacetic-acid) (Fe-EDDHA) are the most effective Fe-synthetic-fertilizers which are peach cropping improvers (Álvarez-Fernàndez et al. 2007; Gharbi et Sanâa 2014; Karagiannidis et al. 2008). Nevertheless, they are costly expensive and environment polluters because of their persistence in the soil caused by their low biodegradability (Álvarez-Fernández et al. 2005; Rahman et al. 2009). The main objective of this research is highlighting the effectiveness of both AMF and iron chelates alone or in combination on the vegetative growth, the mineral uptake and the peaches quality under lime induced chlorosis.

2. Materials and methods

2.1. The study area

The experiment was conducted in a peach (*Prunuspersica* L. Batsch) orchard in Morneg delegation belonging to the governorate of Ben Arous in the North-East of Tunisia. The plot coordinates were taken with a device brand Juno st Trimble GPS (Global positioning system) and its cartography was made with the Arc-GIS (*Geographic System Information*) software version 10.1 (developed by the American society Esri: *Environmental Systems Research Institute*) (Figure 1). The geographical extent of the site covers an approximate of 10000 m^2. In the last four seasons, a monthly minimum average temperature of 7.8 °C and a Maximum average of 35 °C were respectively recorded in February and August. The average of total annual precipitation recorded 493 mm (National institute of meteorology of Tunisia 2015). The soil of the study area is heavy and clayey with 85.8 % of clay, 12.54% of silt, 12.54 % of sand. The pH in water =8.14, the EC =175.3 milli-Simens/cm, the active lime = 13.33% and organic matter = 1.23%. All the mentioned parameters are iron chlorosis promoters in peach trees.

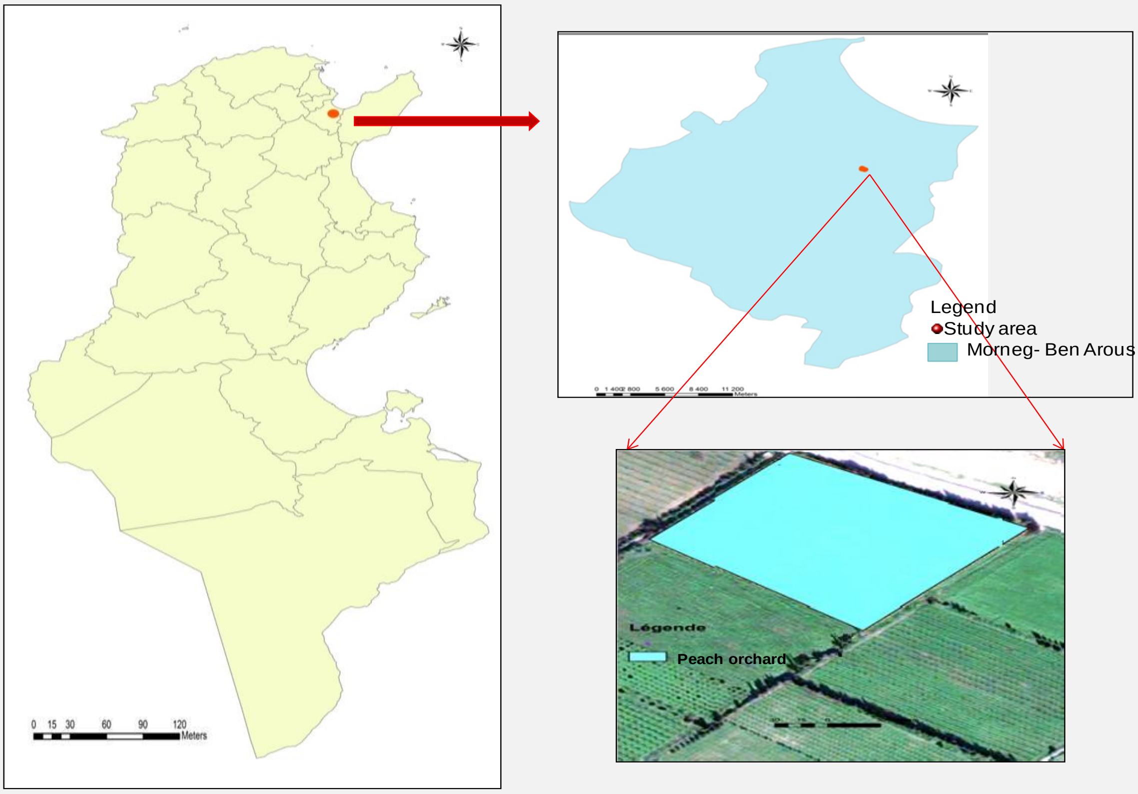

Figure 1: Location of the study area (C) within Ben Arous government (B) in Tunisia (A).

2.2. Plant material and applied treatments

The trial was conducted on a ten years old peach (*Prunuspersica* L. Batsch) orchard variety "Rich Lady", grafted on hybrid GF677 (*Prunuspersica*x*Prunusamygdalus*). Peach trees were planted respectively at the distance of 5m and 3 m between rows and trees (660 trees per hectare). The plot was drop irrigated. Only a supplement of a cow organic matter has been added as organic fertilizers for the last ten years. At 30 days after full bloom (DAFB) peach trees were inoculated with AMF alone or in combination with a commercial iron chelate : Fe-EDDHA. Homogenous plants were selected and received four treatments: T1: +AMF-(Fe-EDDHA): inoculated and not iron chelates supplied; T2: - AMF +(Fe-EDDHA): non inoculated but iron supplied and T3: +AMF+ (Fe-EDDHA):inoculated and iron chelates supplied. A non-inoculated and non iron fertilized peach trees: Tc: -AMF-(Fe-EDDHA) were used as a control. The used inoculums is a commercial product "Symbivit" provided by Inoculum*plus* Company that containing propagules of six species of AMF *Glomus* sp.. One hundred grams of AMF inoculums were applied alone or simultaneously with 30 grams of Fe-EDDHA distributed on either four sides of the peach tree, at a depth of 30 cm of the rhizospheric zone. Doses of inoculums were applied respectively as recommended by the manufacture and those of the Fe-EDDHA were applied at half the recommended dose (50% of 60 grams) and Gharbi-Hajji and Sanâa (2014).

2.3. Shoots and fruits development

At 60 and 120 DAFB, corresponding to 30 and 90 days after the AMF inoculation (DAI), the shoots length and the diameter of peaches were monitored. The shoots length was measured with a measuring tape, and the fruit diameter was measured with a caliper.

2.4. Foliar diagnosis and mineral nutrition

Tow foliar diagnoses have been done at 60 and 120 DAFB (corresponding to 30 and 90 DAI). Leaves were taken from the median zone of shoots having 30 to 35 cm of length (Basar 2006). Leaves were washed and dried at 70°C before they were hashed and stored. Samples were analyzed for macronutrients [total nitrogen (N_{tot}), phosphorus (P), potassium (K) and Calcium (Ca)] and micronutrients [iron (Fe) and magnesium (Mg)] contents evaluation according to Klara (1998). The N_{tot} leaves contents were determined by Kjeldhal method consisting on digesting 0.1g of dry matter with 10 ml of H_2SO_4 at 400°C before distillation and titration with a 0.1N HCl solution. P, K and Ca and Mg and Fe leaves contents were determined using a spectrophotometer, a flame photometer and an atomic absorption spectrophotometer respectively.

2.5. Fruit development and quality

For all considered treatment, 15 peaches were sampled (5 for each repetition) and they were directly analyzed for weight (g), flesh firmness (kg/cm^2), weight (g) and volume (ml) of the juice, soluble solids content (SSC) (°Brix), pH and titratable acidity (TA) (g/l). The flesh firmness was determined using a 8 mm diameter penetrometer with a metallic point, the pH was determined using a pH meter. SSC and TA were determined in the pressed peaches juice using a digital hand refractometer (PR-1, Atago, Tokyo, Japan) and by titration of 10 ml of peach juice with 0.1N mol/l NaOH respectively.

2.6. Data analysis

The data for plantlets were statistically subjected to analysis of variance (ANOVA) to assess the response of the peach trees (*Prunuspersica*L.Batsch) to AMF inoculation and iron chelates supplying. Analyses were performed using the statistical software Statistica 5.0 (StatSoft France 1998). Means differences were tested by the Fisher's test to check the hypothesis of means equality ($P<0.05$). When F test was significant, means were separated by the Newman-Keuls post-test. Graphical outputs were made using the Excel software.

3. Results and discussion

3.1. Effects of AMF and iron chelate on shoots development

From 60 DAFB to 120 DAFB, the shoots length was respectively increased from 57.2 to 62 cm , from 56.5 to 68.56 cm, from 57.14 to 69.61 cm and from 59.67 to 73.22 cm with the treatments: –AMF-(Fe-EDDHA); –AMF+(Fe-EDDHA); +AMF-(Fe-EDDHA) and +AMF+(Fe-EDDHA) (Figure2).
At 120 DAFB, the AMF and the EDDHA-Fe, used each alone, have respectively increased the shoot length by 10.6% and 12.3%. But, no significant effects of the both added treatments on shoots length were shown. However, the associated AMF and Fe-EDDHA significantly enhanced the shoot length by 18% ($p<0.05$). These result confirms those showing the imperative effect of AMF and iron chelate in increasing the vegetative growth and the mineral nutrition of plants (Karaginnidis et al. 2008; Rutto et *al*. 2002; Traquair and Berch 1988).

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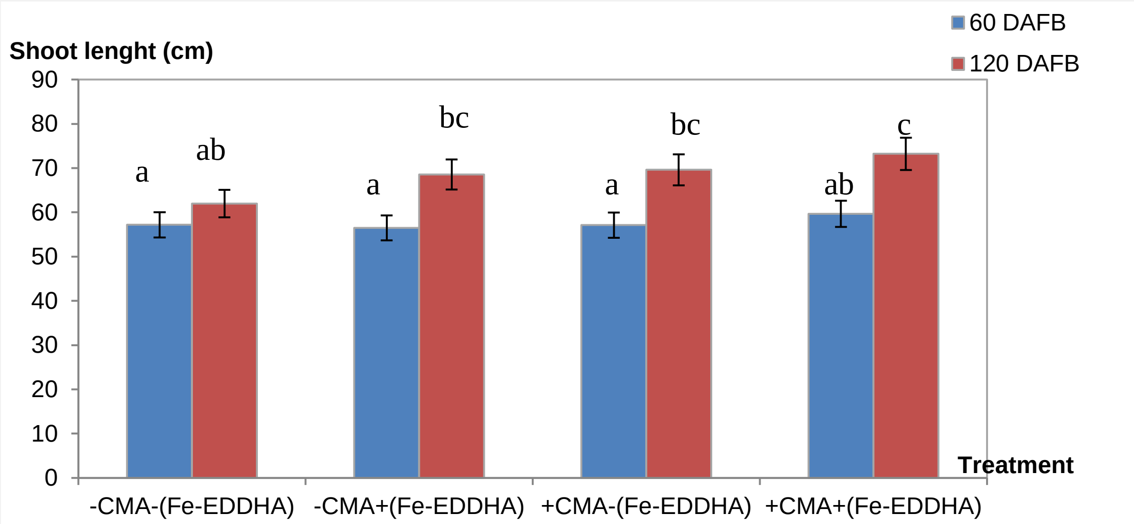

Figure 2: Shoot length evolution of 'Rich Lady' peaches in response to AMF inoculation and iron chelate supply.
-AMF-(Fe-EDDHA) : without AMF inoculation and without iron chelate supply), -AMF+(Fe-EDDHA): without AMF inoculation and with iron chelate supply, +AMF-(Fe-EDDHA): with AMF inoculation and without iron chelate supply, +AMF+(Fe-EDDHA)
Values followed by the same letters are not significantly different at $p<0.05$

3.2. Effects of AMF and iron chelate on peach mineral nutrition

In this study, two foliar diagnoses were done at 60 DAFB and 120 DAFB and leaves mineral contents were determined (Table 1). Results showed differences in levels variation during the two period of diagnosis. This could be attributed to the mobility of the nutritive elements through plant tissues. At 60 DAFB the AMF inoculation, the Fe-EDDHA addition and the combined AMF and Fe-EDDHA showed a respectively significant increase on the N_{tot} (29%, 34% and 52%) and Mg (10%, 16% and 20%) leaves levels ($p<0.05$). However, the same treatments respectively decreased the excessive K (-22%, -18% and -17%) and Ca (-21%, -21% and -29%) leaves levels. No significant effects were shown between treatments for the P and the Fe leaves levels.At 120 DAFB, the AMF and the Fe-EDDHA single or combined application engendered a significant increase on the P leaves contents and a significant decrease on the excess of Fe and the Mg observed in stressed peach trees(non inoculated and non fertilized). Only the combination showed a significant decrease on K leaves contents. However, no significant effects were shown for N_{tot} and Ca contents in leaves. The results were according with studies which highlighted the implication of AMF symbiosis in the uptake of different nutrients, in general, and that of the Fe in particular, under stressed conditions (Baum et al. 2015; Schreiner 2007). In fact, it has been demonstrated that hyphae of AMF *Glomusmossea* can mobilize and/or take up Fe from phytosiderophore-iron complex and are able to translocate it to the host plant (Caris et al. 1998). In the other hand, they were in concordance with researches that demonstrated the effectiveness of the Fe-EDDHA fertilizers in nutrition of plants by providing the appropriate element uptake and mobilization (Hernàndes-fuentes et al. 2004). In addition, the effectiveness of the combination of AMF and Fe-EDDHA supply is attributed to their enhancing effects on the Fe solubilization and the iron deficiency correction. So, a significant increase on N_{tot} and P in leaves tissues and a significant decrease on K, Fe and Mg were registered. The results are contradictory with those obtained by Perur et al. (1961) that demonstrated no significant differences in N_{tot} from green and chlorotic leaves of Corn. However, they are with a great synergy with those obtained respectively by Lindner et Harley (1944) that exposing a positive correlation between lime induce chlorosis and K in leaves and a negative correlation between lime induce chlorosis and Ca an Mg in leaves. However, atthe end of the experiment, the reduced Fe leaves contents in the treated peach trees may be attributed to the iron paradox phenomena making leaves analysis not always useful for assessing the Fe nutritional status (Morales et al. 1998) .

Table 1 : Effects of AMF inoculation and iron chelate on leaves mineral contents of peach *Prunuspersica* (L. Batsch) variety 'Rich Lady'

		Ntot (%)	P (%)	K (%)	Ca (%)	Fe (ppm)	Mg (ppm)
60 DAFB	-AMF-(EDDHA-Fe)	2,14b	0,54ab	3,11c	3,28d	53,44a	0,23a
	- AMF +(EDDHA-Fe)	2,87c	0,54ab	2,53a	2,60c	62,18a	0,27b
	+ AMF -(EDDHA-Fe)	2,76c	0,54ab	2,42a	2,58bc	58,53a	0,28b
	+ AMF +(EDDHA-Fe)	3,24d	0,55abc	2,59ab	2,33b	64,05a	0,32c
120 DAFB	- AMF -(EDDHA-Fe)	1,57a	0,53a	3,42c	1,73a	140,53c	0,43e
	- AMF +(EDDHA-Fe)	1,66a	0,56cd	3,08c	1,57a	103,60b	0,30bc
	+ AMF -(EDDHA-Fe)	1,79a	0,55bcd	3,04bc	1,63a	99,80b	0,32c
	+ AMF +(EDDHA-Fe)	1,87ab	0,57d	2,55a	1,73a	113,60b	0,39d

Values in the same column and followed by the same letters are not significantly different at $p<0.05$

3.3. Effects of AMF and iron chelate on fruit quality

- Effects on the fruit size

The assessment of peach *Prunuspersica* (L.Batsch) variety 'Rich Lady' showed that tree diameter ranged from 2.61 cm to 6.08 cm (respectively at 60 and 120 DAFB) (Figure3). At 60 DAFB, the AMF inoculation and/or Fe-EDDHA addition showed no significant effects on diameter value if compared to this of the control (non-inoculated and non iron fertilized) peach trees($p<0.05$). However, at 120 DAFB, the added AMF and Fe-EDDHA engendered a significant increase ($p<0.05$) on peaches diameter (7.63% and 7.98% respectively). In addition, the +AMF+Fe-EDDHA was the most effective combination (+14.8%) on fruit diameter enhancement. These results are with a strong accordance with those obtained by Loupassaki et al. (1997) and Zaiter et al. (1993) respectively showing that the Fe-EDDHA increased significantly kiwi and strawberry fruit size and tree yield. In the other hand, Lehman and Rillig (2015) meta-analysis, showed that there is a significant positive impact of the AMF inoculation on crop plant Fe nutrition, only detectable for immediate: during the experiment (lasting 56 to112 days).

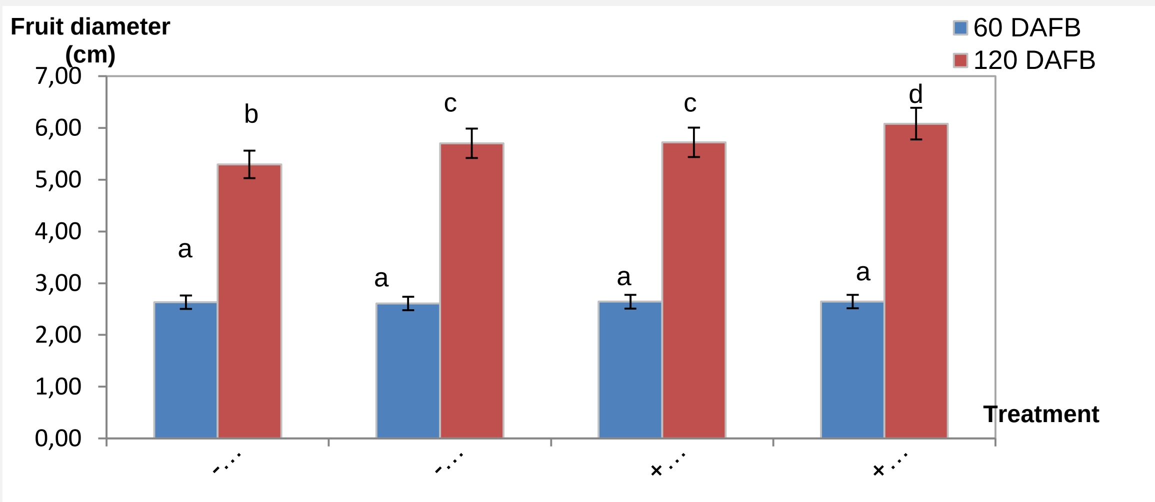

Figure 3: Fruit diameter evolution of 'Rich Lady' peaches in response to AMF inoculation and iron chelate supplying.
-AMF-(Fe-EDDHA) : without AMF inoculation and without iron chelate supply), -AMF+(Fe-EDDHA): without AMF inoculation and with iron chelate supply, +AMF-(Fe-EDDHA): with AMF inoculation and without iron chelate supply, +AMF+(Fe-EDDHA)
Values followed by the same letters are not significantly different at $p<0.05$

- **Effects on the fruit quality**

The AMF and the Fe-EDDHA treatments, used each alone or in combination, greatly influenced the fruit qualitative parameters (average weight of five fruits, firmness, weight and volume of juice, soluble solids content, pH and titratable acidity) (Table 2). The picked peaches from the non inoculated and non fertilized peach trees, showed a closed fresh weight and firmness values to those obtained by Gullo et al. (2014) and a closed SSC and TA values to those showed by Ortiz et al. (2009) for respectively ' Rich May' and the 'Rich Lady' peach varieties.

The overall studied fruits sensorial characteristics were enhanced by the AMF and Fe-EDDHA treatments. Even so, the combination of AMF with Fe-EDDHA was the most effective for all studied parameters. It significantly improved the average weight of five fruits (32.5%), the firmness (15%), the weight and the volume of juice (40% and 28.9% respectively), the SSC (13.3%), the pH (4.8%). However, it significantly decreased the TA (-14.2%). This agrees with numerous research results showing the importance of the Fe-EDDHA addition and the AMF inoculation in improving the peaches quality (Alvarez-Fernàndez et al. 2007; Baslam et al. 2013; Castellanos-Morales et al. 2010; Ulrichs et al. 2008).

Table 2 : Effects of AMF inoculation and iron chelates addition on quality of mature 'Rich Lady' peaches

Treatments	**Average weight of 5 fruits (g)**	**Firmness (Kg/ cm^2)**	**weight of juice (g)**	**Volume of juice (ml)**	**Soluble Solids content (SSC) (°brix)**	**pH**	**Titrabl eacidity (g/l)**
-AMF-(EDDHA-Fe)	517,00^a ± 32,97	7,11^a ± 0,42	330,99^a ± 13,50	346,67^a± 40.41	11,11^a± 0,23	3,37^a± 0,01	12,7^b± 0, 70
-AMF+(EDDHA-Fe)	623,33^{bc} ± 40,08	7,89^{ab}± 0,70	370,09^a ± 61,85	366,67^a± 50.33	11,71^{ab}± 0,57	3,40^a± 0,02	11,8^{ab}± 1,30
+AMF-(EDDHA-Fe)	555,33^{ab}± 77,50	7,71^{ab}± 0,10	400,47^{ab}± 16,64	413,33^{ab}± 32,15	12,07^{ab}± 0,78	3,39^a± 0,09	11,0^a± 0,70
+AMF+(EDDHA-Fe)	685,00^c± 56,32	8,17^b± 0,14	463,11^b± 54,19	446,67^b± 37.86	12,59^b± 0,37	3,53^b± 0,09	10,9^a± 0,60

Values in the same column and followed by the same letters are not significantly different *at* $p<0.05$.

4. Conclusion

The present study revealed that both AMF and Fe-EDDHA addition played major roles in improving the vegetative growth of shoots, the mineral uptake and the fruit quality of peach trees 'Rich Lady' variety under lime induced chlorosis : in a calcareous soil with high pH levels. Moreover, the combination between both AMF and Fe-EDDHA was the most effective for increasing the Ntot, K, Ca, Fe and Mg contents in leaves tissus and their uptake regulation and improving the fruit quality by increasing the size, the average weight, the pressed juice volume and weight, the SSC, the pH and the TA. The reduction of iron chelates doses at 50% can contribute the theenvironment safety.The approach offers challenge of use of AMF for iron chlorosis correction in others lime induced sensitive crop trees, in orchard. Therefore, most interest must be given to soil properties for symbiosis establishment.

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