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Effectiveness of mixtures of vivianite and organic materials in preventing iron chlorosis in strawberry

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Abstract

Application of Fe salts with different organic matter sources has been demonstrated to be effective in preventing Fe deficiency chlorosis. The main objective of this work was to study the effectiveness of different Fe sources based on mixtures of humic substances or compost with vivianite in preventing this nutritional disorder in strawberry (*Fragaria* × *ananassa* cv Camarosa). To this end, a randomised block experiment involving four replications (3 plants per replication) and one factor (Fe source) was performed in a greenhouse using a calcareous growing medium. Iron sources studied were: (i) control without Fe, (ii) control with an effective Fe source in calcareous media (EDDHA-Fe, 0.1 g kg^{-1}), (iii) vivianite (1 g kg^{-1} medium, which is the recommended rate), (iv) vivianite (1 g kg^{-1}) + humic substances (HS 0.06 g kg^{-1}), (v) vivianite (0.5 g kg^{-1}) + HS (0.06 g kg^{-1}) and (vi) a mixture of composted cork residue and vivianite at a 6:1 mass ratio (CORVIV) applied at a dose of 6 g kg^{-1} medium. All Fe sources were effective in increasing SPAD readings when compared with control without Fe. Treatments based on vivianite provided non-significantly different SPAD readings from that obtained with EDDHA-Fe. However, only CORVIV showed non-significantly different dry matter (DM) production, leaf area index, and total Fe content in the aerial part than EDDHA-Fe. Humic substances and vivianite at 1 g kg^{-1} increased DM yield in plants when compared with vivianite without HS, results with this last treatment being similar to those obtained with vivianite at 0.5 g kg^{-1} with HS. It can be concluded that studied sources of organic matter increased the efficiency of vivianite in preventing Fe chlorosis in strawberry, especially vivianite enriched cork compost which was as effective as EDDHA-Fe.

Additional key words: composted cork; Fe deficiency; *Fragaria* × *ananassa;* humic substances; iron fertilizers.

Introduction

Sustainable production of many horticultural crops must implement systems for reducing the elevate inputs cost for production (Tagliavini *et al*., 2005). Iron deficiency chlorosis is probably the main nutritional disorder affecting crops on calcareous soils which usually constrain their profitability due to the high prices of effective preventive treatments (Fe chelates). Vivianite [$Fe_3(PO_4)_2 \cdot 8H_2O$] is a Fe salt poorly soluble in calcareous media, which can be solubilised by compounds with affinity for Fe, such as citrate and diethylenetriamine pentaacetic acid (DTPA) (De Santiago *et al*., 2008a). Therefore, it can act as a slow-release Fe fertilizer which can be dissolved by the action of carboxylates compounds secreted by roots, resulting in a high Fe bioavailability (Abadía *et al*., 2011). Also, dissolution is enhanced by the phosphate adsorption of plant roots, which decreases the activity of this anion in the soil solution (Eynard *et al*., 1992; Rosado *et al*., 2002). Besides the slow Fe release, the dissolution products in soil are poorly crystalline Fe oxides (Rosado *et al*., 2002; De Santiago *et al*., 2008a) which also contribute to maintain a Fe pool available to plants in soil (De Santiago & Delgado, 2006; De Santiago *et al*., 2008b). All these reasons explain that vivianite mixed with, or injected, into the soils has been used as an effective treatment in preventing Fe chlorosis in different crops

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Abbreviations used: CORVIV (vivianite-enriched cork compost); DM (dry matter); EDDHA (ethylenediaminedihydroxyphenylacetic acid); HS (humic substances); V (vivianite applied at 1 g kg^{-1}); V0.5 (vivianite applied at 0.5 g kg^{-1}).

during several growing seasons (Iglesias *et al*., 2000; Rosado *et al*., 2002; Rombolà *et al*., 2003; Díaz *et al*., 2010). Besides its long-term effectiveness, it can be readily prepared by farmers using low-cost products (ferrous sulfate and bi-or mono-ammonium phosphate).

Treatments based on mixtures of Fe compounds and different organic matter sources can be effective in preventing Fe chlorosis. Humic substances or beet vinasses have been proved to increase the efficiency of vivianite and ferrous sulphate in decreasing the incidence of Fe chlorosis in sensitive plants grown on calcareous media (De Santiago *et al*., 2008a; De Santiago & Delgado, 2010). This enhancement of the effect of inorganic Fe sources increasing Fe availability to plants when they are applied with an organic source has been explained by: (i) the effect of organic matter contributing to metal reduction (Chen *et al*., 2003), (ii) the decrease in the crystallization of Fe oxides resulting from applied Fe fertilizers (Schwertmann *et al*., 2005; De Santiago *et al*., 2008a), and (iii) the formation of complexes with Fe that can be an effective Fe source for plants (Pinton *et al*., 1998, 1999; Bocanegra *et al*., 2006; De Santiago & Delgado, 2007).

The use of different organic byproducts used as organic amendments in soils in treatments to overcome Fe chlorosis can contribute to add value to these organic products. Iron-enriched organic amendments, such as manure, compost or peat, has been demonstrated to be effective in decreasing Fe chlorosis symptoms, $FeSO_4$ being the usual source of Fe (Mathers *et al*., 1980; Sakal *et al*., 1982; Chen *et al*., 1982; Bar-ness & Chen, 1991). This has been explained by the effectiveness of organic compounds present in amendments in keeping Fe in plant-available forms (Bar-ness & Chen, 1991). In particular it is of potential interest the study of the effectiveness of vivianite enriched composts in preventing the problem, since vivianite has been proved to be an effective Fe fertilizer in preventing chlorosis and this effectiveness has been increased by mixing with different organic sources (De Santiago *et al*., 2008a; De Santiago & Delgado, 2010).

The main objective of this work was to study the effectiveness of humic substances and a composted material obtained from the cork industry residue mixed with vivianite in preventing the incidence of Fe deficiency chlorosis with a view of performing Fe chlorosis control strategies involving the application of products cheaper than Fe-chelates. Strawberry (*Fragaria* × *ananassa* cv Camarosa) has been selected for this study because it is one of the most profitable horticultural crops in Mediterranean countries, accounting for net profits around 300 million euros each year only in Spain (CAP, 2007; Medina *et al*., 2007), and strawberry is very sensitive to Fe deficiency chlorosis and can be only cultivated in calcareous soils if Fe chelates are routinely applied in fertigation (Almaliotis *et al*., 2002; Karp *et al*., 2002).

Material and methods

Experimental design

Commercial strawberry plants (*Fragaria* × *ananassa* cv Camarosa) with similar size and development were chosen for the experiment; this cultivar is known to be iron-inefficient (Hancock, 1999). The experiment was performed following a randomized block design involving four replications, with 3 plants per replication (each one in one 5-L pot), and one factor (Fe source). The experiment was developed in a greenhouse in the ETSIA, University of Seville (37° 21' N, 5° 56' W) from 20th February to 19th May 2008, with a mixture of calcareous sand as growing medium (> 99.5% $CaCO_3$; 65% "active" $CaCO_3$; < 4 mm diameter), quartz sand (previously washed with a Na_2CO_3 diluted solution and then with deionised water until neutrality), and perlite in a volume proportion of 10:45:45. Four litres of this substrate were use in each pot. The mass of 4 L of substrate was 2.5 kg proximately.

Irrigation was done daily with 0.25 L of a Hoagland type nutrient solution without Fe having the following composition (all concentrations in mmol L^{-1}): $MgSO_4$ (4), $Ca(NO_3)_2$ (5), KNO_3 (5), KH_2PO_4 (2), H_3BO_3 (0.092), $MnCl_2$ (0.018), $CuSO_4$ (0.0016), $ZnSO_4$ (0.0025), and H_2MoO_4 (0.0023).

Treatments involved were as follows: (1) control without Fe; (2) control with an effective Fe source, EDDHA-Fe at a rate of 0.1 g kg^{-1} from Syngenta Agro SA (Madrid, Spain), with a Fe content in *ortho,ortho* and *ortho,para* isomers of 32 and 28 g kg^{-1} respectively; (3) vivianite at its recommended dose according to previous evidences compiled by De Santiago *et al*. (2008a) (V, 1 g kg^{-1} medium); (4) vivianite at 1 g kg^{-1} (V) + humic substances (HS) (0.06 g kg^{-1}); (5) vivianite at 0.5g kg^{-1} (V0.5) + HS (0.06 g kg^{-1}); and (6) a mixture of composted cork residue and vivianite at a 6:1 mass ratio (CORVIV) applied at a dose of 6 g kg^{-1} medium (thus equivalent to 24 g $plant^{-1}$).

Vivianite solution was prepared from 25 g of (NH_4) $2HPO_4$ and 75 g of $FeSO_4 \cdot 7H_2O$ in one litre of distilled water as described by Rosado *et al*. (2002), raising the pH to 6.5 with KOH. The resulting suspension of vivianite was washed several times with deionised water until the electrical conductivity was lower than 0.5 dS m^{-1}. The content of vivianite in the suspension was approximately 50 g L^{-1}. The vivianite enriched composted cork was prepared by dissolving 75 g of $FeSO_4 \cdot 7H_2O$ in 1-L of water containing 300 g of composted residue of the cork industry (basically small pieces of cork, < 2 mm). After 2 h, 25 g of $(NH_4)_2HPO_4$ was added stirring until homogeneous solubilization and then the pH was raised to 6.5 with KOH. The final compost:vivianite proportion was 6:1 in mass. The applied rate of 6 g kg^{-1} medium thus accounts for a vivianite supply of 0.9 g kg^{-1} that provided to the growing media 0.29 g Fe(II) kg^{-1}. Some characteristics of composted cork used for CORVIV were: real density of 1.48 kg L^{-1}, bulk density of 0.14 kg L^{-1}, DTPA extractable Fe according to Lindsay & Norwell (1978) of 0.59 g kg^{-1}, total Fe concentration of 2.2 g kg^{-1}, and C/N ratio of 16.5. Iron applied with the compost accounted for an application of 3 and 11.2 mg kg^{-1} medium of DTPA extractable Fe and total Fe, respectively. This accounts for a non-effective Fe supply to plants as revealed by the Fe chlorosis inductive capacity of this composted material when used as growing medium (Caballero *et al*., 2007; 2009). The amount of Fe supplied to the growing medium with V, V0.5, and Fe-EDDHA was 0.32 g kg^{-1}, 0.16 g kg^{-1} and 0.003 g kg^{-1} respectively.

Humic substances (HS) for the other Fe-fertilizers based on vivianite were prepared using a commercial liquid mixture of humic and fulvic acids (Solfer humicos®, Valencia, Spain). First HS was extracted according to the Stevenson´s method (1994) and then was dialyzed into 15 kDa cut-off Visking tubing (Sigma, Barcelona, Spain) against deionised water until the electrical conductivity of the solution was < 1 dS m^{-1}. After that, pH was adjusted to 8 using HCl. The dialyzed fraction contains compounds with a greater molecular mass than that used by Pinton *et al*. (1999) but in the range of that used by Bocanegra *et al*. (2006). According to De Santiago *et al*. (2008a) an application rate of 0.06 g kg^{-1} can be considered adequate to improve Fe supply to plants.

Vivianite (alone or mixed with HS) was applied as a suspension which was carefully mixed with the growing media before planting. EDDHA-Fe and CORVIV were applied in solid form; the first product to the surface, and the second mixed with the superficial medium in the pot at a depth of 5 cm.

Plant analysis

Chlorophyll was measured with a Minolta SPAD-502 chlorophyll meter (Minolta Camera Co, Ltd., Osaka, Japan) at the following approximate stages of cultivation: before blooming ($SPAD_{BB}$), full flowering ($SPAD_{FF}$), beginning of fruiting ($SPAD_{BF}$) and at the end of experiment when ripening of strawberry was full ($SPAD_{MS}$). Those stages corresponded to 3, 5, 8 and 12 weeks after transplanting. Leaf area index was calculated as leaf length × width (in the major young leaf completely extended).

At the end of the experiment, iron chelate reductase assay was performed according to Johnson *et al*. (2002) to prove the existence of a reduction based strategy (Chaney *et al*., 1972). In this method, root-associated Fe(III) reduction was determined for whole-root systems of intact plants using the spectrophotometric measurement of Fe(II) chelated to bathophenanthrolinedisulfonic acid (BPDS). Plants were brought into a darkened laboratory and roots were given in nutrient solution (0.25 L) consisting of a 0.4 Hoagland (without Fe) solution (concentration of the nutrient solution described above), 10 mM MES at pH 5.5, 300 µM ferrozine, and 100 µM ferric hydroxyethylethylenediaminetriaceticacid (FeHEDTA) was used as chelate. Reduction experiments were conducted under laboratory conditions of light and temperature with the roots maintained in opaque containers with continual aeration. After 1 h of incubation, ferric chelate reduction was determined by measuring the intensity of the ferrous ferrozine complex spectrophotometrically at 562 nm. Absorbance measurements were corrected for absorbance of the complete reaction mixture incubated without roots. The amount of Fe reduced was calculated using the extinction coefficient (27,900) given by Stookey (1970).

Dry weight of plants (separately shoots and roots after reductase activity determination) was determined after drying plant material at 65°C for 48 h in a forced-air oven. After that, plant matrial was milled to pass though a 1-mm sieve. An aliquot of the milled plant material (0.25 g) was mineralized by ashing at 550°C for 8 h and the obtained ash was dissolved in 1 M HCl. The resulting solution was used to determine phosphorous according to Murphy & Riley (1962) and Ca, Mg, Fe, Cu, Mn, and Zn were measured after diluting the samples by atomic absorption spectrometry.

Growing media analysis

After crop, DTPA extractable Fe was determined according to Lindsay & Norvell (1978), which has been usually considered as a Fe availability index (Sims, 2000); another Fe availability index, the extraction with unbuffered 0.5 M hydroxylamine chloride for 17 h (Fe_{ha}) at a medium to extractant ratio of 1:20 was determined according to De Santiago *et al*. (2008b). Also, a sequential Fe fractionation was performed according to De Santiago and Delgado (2006) involving the use of four extractants consecutively, namely: (i) 0.27 M Na citrate + 0.11 M $NaHCO_3$ (CB-Fe); (ii) 0.25 M Na citrate at pH 6 and then 0.2 M Na citrate at pH 6 (C-Fe); (iii) 0.2 M Na citrate + 0.05 M ascorbate at pH 6 (CA-Fe), and (iv) 0.27 M Na citrate + 0.11 M $NaHCO_3$ + 2% Na dithionite (CBD-Fe). Citrate bicarbonate and the sum of citrate extractions (the combination of the two consecutive extractions, C-Fe) seems to be effective dissolving Fe by complexation by citrate, and also Fe bound to HS. Most of the Fe associated with poorly crystalline Fe oxides is extracted by CA. Crystalline Fe oxides not dissolved in these steps can be dissolved by using a stronger reductant (Na-dithionite) in the last step. All Fe extractions were carried out in triplicate using polypropylene flasks.

Statistical analysis

A one-way analysis of variance on the effects of Fe source on chlorophyll meter readings, dry matter (DM) production, number of leaves, length × width of leaves and micronutrients and phosphorous concentration and total content in aerial part was done using Statgraphics Plus 5.1 (StatPoint, 2000). Mean comparison was performed according to the Tukey test at a probability level of 0.05.

Results and discussion

Effect of Fe-fertilizers on SPAD meter readings and strawberry development

Intervenial chlorosis of leaves and necrosis formation was observed in the control treatment in agreement with previously described symptoms of Fe deficiency chlorosis in strawberry (Erdal *et al*., 2004; Kafkas *et al*., 2007). The source of Fe applied had a significant effect on SPAD readings, DM accumulated by the crop in shoots and roots, leaf area index, and number of leaves (Table 1). The shoot and root DM in plants treated with Fe sources was always greater than that of plants without Fe addition (Table 1). Overall, EDDHA-Fe provided the best results improving DM in plants when compared with control without Fe. However, results with CORVIV treatment were non-significantly different from those obtained with EDDHA-Fe. The other vivianite-based treatments provided lower DM in root and shoots, and total DM than Fe chelate (Table 1). Non-significant differences in DM production was observed in shoots of plants among others vivianite-based treatments different from CORVIV. However, total DM was greater in V + HS than in V or V0.5 + HS, differences between these both last treatments being non-significant (Table 1). Fe-chelate and CORVIV were the most effective treatments in improving the

Table 1. Effect of different Fe sources on growth parameters of strawberry plants (cv. Camarosa) grown in a calcareous medium

Source of iron	DM roots ($g\ plant^{-1}$)	DM shoots ($g\ plant^{-1}$)	Total DM ($g\ plant^{-1}$)	Root:Shoot	Length*Width (cm^2)	Leaves (No.)	$SPAD_{end}$
Control	1.69^d	2.50^c	4.19^d	0.67^b	21.4^c	8^b	6^b
EDDHA-Fe	12.12^a	9.92^a	22.04^a	1.34^a	42.9^a	16^a	31^a
Vivianite (V)	4.03^c	5.44^b	9.47^c	0.76^b	38.5^{ab}	18^a	33^a
V0.5 + HS	5.24^{bc}	5.33^b	10.57^c	0.91^{ab}	36.1^{ab}	15^a	32^a
V + HS	6.64^b	6.69^b	13.33^b	0.95^{ab}	34.3^b	15^a	35^a
CORVIV	9.06^{ab}	8.64^{ab}	17.70^{ab}	1.02^{ab}	46.2^a	15^a	34^a

DM, dry matter; $SPAD_{end}$, value of chlorophyll at the end of the experiment; EDDHA, ethylenediaminedihydroxyphenylacetic acid; V, vivianite applied at 1 g kg^{-1}; V0.5, vivianite applied at 0.5 g kg^{-1}; HS; humic substances; CORVIV, vivianite enriched cork compost. Means followed by the same letter in a column were not significantly different according to the Tukey test at a probability level of 0.05.

leaf area index (Table 1) when compared with control without Fe.

Decrease in root development as been described as one of the main symptoms of Fe chlorosis (De Santiago *et al*., 2008a). All the Fe sources provided significantly higher root DM than control without Fe (Table 1). However, only EDDHA-Fe increased the root to shoot ratio in comparison to the control without Fe, mixtures of organic sources and vivianite providing non-significantly different results to that obtained with the chelate (Table 1).

At the first stage observed (BB; Fig. 1) all sources of Fe had a similar behaviour. At the fully flowering stage, CORVIV and EDDHA-Fe promoted higher SPAD readings than the others treatments. Humic substances increased the efficiency of V in increasing SPAD readings: at FF and BF, V + HS provided better results than V without HS; also, non-significant differences between V and V 0.5 + HS were observed. It is known that organic matter applied as compost may improve strawberry growth, which can be explained at least in part by the improvement of nutrient uptake (Yavari *et al*., 2009). Also, the application of organic matter in the form of HS or sugar vinasses has been shown to be effective in increasing the efficiency of Fe salts in preventing the incidence of Fe deficiency chlorosis in lupin plants (De Santiago *et al*., 2008a; De Santiago & Delgado, 2010).

At the beginning of fruiting, all Fe sources increased SPAD readings when compared with control without Fe. At this stage, Fe chelate was the most effective Fe source increasing SPAD readings. At the end of the experiment (maturation stage), control without Fe showed lower SPAD readings that all the Fe sources studied and non-significant differences between vivianite-based treatments and Fe chelate were observed at the end of the experiment (growing stage MS, Fig. 1; Table 1). These results are consistent with previous findings revealing the efficiency of vivianite in increasing chlorophyll content of sensitive plants grown on calcareous media (Rombolà *et al*., 2003; De Santiago *et al*., 2008a).

Nutrient status in strawberry plants

Induction of FC-R activity in root tips is considered a key response to iron chlorosis in Strategy I plants (Gogorcena *et al*., 2004). All the treatments increased FC-R activity when compared with control without Fe. However, this activity was higher in plants treated with vivianite-based mixtures than with Fe chelate (Fig. 2). Increased FC-R activity in treatments supplying Fe with induced decreased chlorosis symptoms contradicts observations by Schmidt & Steinbach (2000). This difference could be due to the age of the roots of studied plants, or also might reveal that enhanced FC-R activity is not a general response to Fe chlorosis as Pestana *et al*. (2011) argued. Besides this, the use of chelating agents (BPDS) in the method to estimate FC-

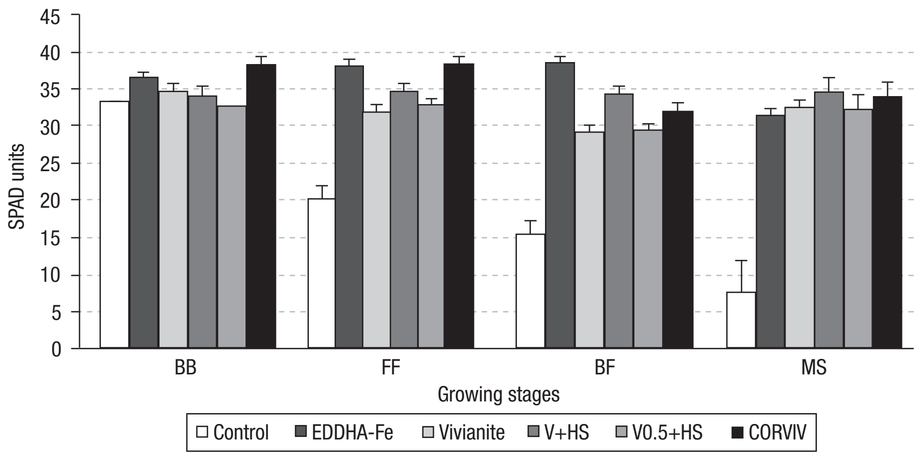

Figure 1. Evolution of SPAD meter readings in the youngest completely expanded leaf of *Fragaria* × *ananassa* cv. Camarosa depending of applied Fe sources: control without Fe; EDDHA-Fe at 0.25 g plant^{-1}; vivianite, applied at a rate of 1 g kg^{-1} (V); vivianite at 1 g kg^{-1} + HS at a rate of 0.06 g kg^{-1} (V + HS); vivianite at 0.5 g kg^{-1} + HS at a rate of 0.06 g kg^{-1} (V0.5 + HS), and composted cork enriched with vivianite (CORVIV). Measurements were done at different growing stages: BB, before blooming (at 3 weeks after transplanting); FF, full flowering; BF, beginning of fruiting (at 5 and 8 weeks after transplanting), and MS, maturation stage at the end of the experiment (at 12 weeks after transplanting). Error bars indicate one standard error above and below the mean.

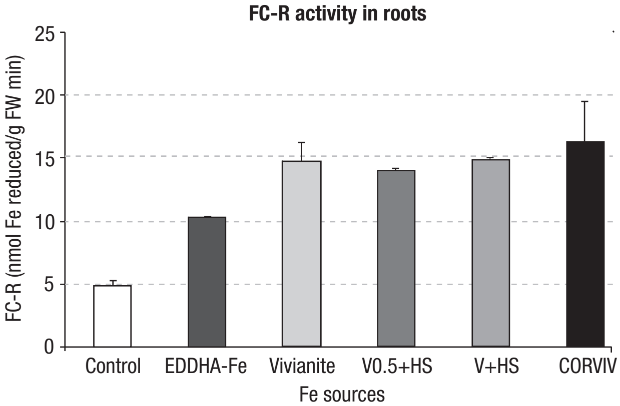

Figure 2. Total FC-R activity (measured in complete roots) at the end of the experiment in strawberry plants cultivated with different sources of Fe; control without Fe; EDDHA-Fe at 0.25 g plant^{-1} ; vivianite, applied at a rate of 1 g kg^{-1} (V); vivianite at 1 g kg^{-1} + HS at a rate of 0.06 g kg^{-1} (V + HS); vivianite at 0.5 g kg^{-1} + HS at a rate of 0.06 g kg^{-1} (V0.5 + HS), and composted cork enriched with vivianite (CORVIV). Error bars indicate one standard error above and below the mean.

R activity with a strong affinity for Fe(II) forms can displace the reaction's equilibrium (Pierre *et al*., 2006); BPDS can complex Fe(II) applied as vivianite, not reduced by the action of FC-R). This can accounts for FC-R activities measurements much higher than those occurring *in vivo* (Lucena & Chaney, 2006; Orera *et al*., 2010).

As stated for other plants in many previous works (*e.g.* De Santiago & Delgado, 2006, 2007), different degrees of incidence of Fe chlorosis did not result in different Fe concentration in aerial parts of strawberry plants (Table 2). However, all the Fe sources increased the total content of Fe in aerial parts when compared with the control without Fe, EDDHA-Fe providing a Fe accumulation in plants higher than vivianite-based treatments except CORVIV (Table 2). Thus, it can be concluded that Fe sources, and particularly EDDHA-Fe and CORVIV, were effective in increasing the amount of available Fe in the growing medium and the Fe uptake by plants.

Besides Fe chlorosis, calcareous media can induce others deficiencies of micronutrients (Mn, Zn and Cu). In some cases, some Fe sources applied to overcome Fe chlorosis can result in a decreased accumulation of other micronutrients in plants (Caballero *et al*., 2009). In this case, concentrations of Mn and Cu were in a sufficiency range without clear trends between treatments; lower total Mn and Cu accumulation in control can be ascribed to the lower DM accumulation (Table 2). Concentration of Mn tended to be lower at increased efficiency of Fe sources in decreasing the incidence of Fe chlorosis as measured by the increment in DM and total Fe in shoots (Table 1). This can be ascribed to an antagonistic effect of Fe supply on Mn nutrition of plants (Chatterjee *et al*., 2006; Caballero *et al*., 2009), and agrees with previous evidences that Fe supplied as Fe-chelate decreased the Mn concentration in plant shoots to a greater extent than that for inorganic Fe sources under conditions of restricted Fe availability in the growing media (Heitholt *et al*., 2003; Caballero *et al*., 2009). Low Zn concentrations in all the treatments can be ascribed to a diminished absorption induced by the growing medium.

Phosphorus concentrations in aerial parts of plants were in a sufficiency range according to Tagliavini *et al*. (2005) in all the treatments. In spite of the P supply done with the nutrient solution, the concentration and total content in aerial parts of this nutrient in plants treated with vivianite-based products was higher than

Table 2. Effect of different Fe sources on the concentration and total content of nutrients in aerial parts of strawberry plants (cv Camarosa) grown in a calcareous medium

Source of iron	Total content in aerial part					Concentration in aerial part				
	Fe (µg plant^{-1})	Mn (µg plant^{-1})	Zn (µg plant^{-1})	Cu (µg plant^{-1})	P (mg plant^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})	P (g kg^{-1})
Control	701^{c}	235^{b}	28^{c}	32^{b}	3.7^{c}	277^{a}	94^{ab}	11^{a}	17^{a}	1.45^{ab}
EDDHA-Fe	2,660^{a}	547^{a}	62^{ab}	82^{a}	6.7^{b}	266^{a}	53^{d}	9^{a}	14^{a}	0.74^{c}
Vivianite (V)	1,485^{b}	547^{a}	47^{abc}	53^{a}	8.8^{a}	274^{a}	103^{a}	9^{a}	10^{a}	1.79^{a}
V0.5 + HS	1,211^{b}	414^{a}	43^{bc}	14^{c}	8.0^{a}	228^{a}	80^{abc}	8^{a}	11^{a}	1.50^{ab}
V + HS	1,479^{b}	498^{a}	57^{a}b	62^{a}	8.3^{a}	219^{a}	72^{bcd}	9^{a}	18^{a}	1.25^{b}
CORVIV	2,410^{ab}	537^{a}	73^{a}	47^{a}	10.6^{a}	274^{a}	61^{cd}	8^{a}	11^{a}	1.23^{b}

EDDHA, ethylenediaminedihydroxyphenylacetic acid; V, vivianite applied at 1 g kg^{-1}; V0.5, vivianite applied at 0.5 g kg^{-1}; HS, humic substances; CORVIV, vivianite enriched cork compost. Means followed by the same letter in a column were not significantly different according to the Tukey test at a probability level of 0.05.

those treated with EDDHA-Fe. This reveals that vivianite can contribute to P nutrition of plants (Bavaresco *et al*., 2010), acting as a slow-release P fertilizer due to its low solubility in calcareous media. In this media, this slow-release of P from vivianite can result in a high efficiency of applied P because low P concentrations in soil solution tended to promote the precipitation of insoluble Ca phosphate to a lesser extend than high P concentrations promoted by soluble fertilizers (Delgado *et al*., 2002).

Effect of Fe sources on Fe forms in the growing medium

Among the studied vivianite-based mixtures, only CORVIV provided similar results to that obtained with EDDHA-Fe in preventing the incidence of Fe chlorosis in strawberry plants. This is not explained by an increased value of Fe availability indexes (hydroxylamine or DTPA extractable) when compared with other vivianite-based treatments (Table 3). However, the significant differences effect observed in the fractionation of Fe can contribute to explain the efficiency of CORVIV. When vivianite is mixed with composted cork, the proportion of applied Fe recovered by citrate bicarbonate (CB) was the highest (Table 3). This is the fraction which likely includes the most readily available Fe forms to plants (De Santiago *et al*., 2008a), and according to de Santiago & Delgado (2010), it is the best estimator of the efficiency of Fe fertilizers in preventing the incidence of Fe chlorosis in calcareous media. Also, with CORVIV, the proportion of applied Fe recovered by the action of the strong reductant (CBD) was the lowest (Table 3). These evidences indicate that this Fe source had promoted the lowest precipitation rate of Fe oxide which does not contribute to Fe supply to plants (De Santiago *et al*., 2008a) and that a highest portion of applied Fe remained extractable by the complexant effect of citrate, which mimics one of the Fe acquisition mechanisms in strategy I plants. Thus, the increased portion of applied Fe recovered by CB and the decreased recovered by CBD in the growing medium treated with CORVIV when compared with other vivianite-based treatments (Table 3) support the evidence of a greater efficiency of Fe applied in this form to overcome Fe chlorosis in strawberry plants described above (Tables 1 and 2). Also, these results are consistent with previous works arguing that complexation effects (De Santiago & Delgado, 2006, 2010; De Santiago *et al*., 2008a) and a decreasing rate in the crystallization of oxides led to an increased micronutrient availability to plants (De Santiago *et al*., 2008a; Pedrot *et al*., 2011). According to De Santiago *et al*. (2008a), the decreased Fe extraction by CB is not a reason for a diminished efficiency of vivianite when mixed with HS.

As final conclusions, vivianite mixed with composted cork was the most effective Fe source in increasing DM yield, leaf area index, and Fe accumulation in strawberry plants grown in a calcareous medium and was not significantly different from EDDHA-Fe treatment. Humic substances also increased the efficiency of vivianite in preventing the problem, and results obtained with vivianite applied at half of the recommended dose (0.5 g kg^{-1}) with HS were similar to that obtained with vivianite without HS at the recommended dose.

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Table 3. Effect of different vivianite-based Fe sources on sequential and simple (index of availability) chemical extractions of Fe from the calcareous growing medium after cultivation of strawberry plants (cv Camarosa)

Source of iron	Sequential extractions (% of Fe applied)						Simple extractions (mg kg^{-1})	
	CB	C1	C2	C1+ C2	CA	CBD	Fe_{ha}	Fe_{DTPA}
Vivianite (V)	35^{b}	11^{b}	17^{a}	27^{a}	7^{b}	30^{bc}	23^{a}	7.7^{a}
V0.5 + HS	10^{c}	15^{a}	13^{b}	28^{a}	11^{a}	46^{a}	14^{b}	4.2^{b}
V + HS	17^{bc}	12^{b}	17^{a}	29^{a}	8^{ab}	37^{b}	17^{ab}	7^{a}
CORVIV	48^{a}	8^{c}	11^{b}	19^{b}	9^{ab}	24^{c}	23^{a}	9.2^{a}

CB, citrate-bicarbonate; C1 and C2, successive extractions with citrate; CA, citrate-ascorbate; CBD, citrate-bicarbonate-dithionite; Fe_{ha}, hydroxylamine-extractable Fe; Fe_{DTPA}, diethylenetriaminepentaacetic acid extractable Fe. Means followed by the same letter in a column were not significantly different according to the Tukey test at a probability level of 0.05.

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