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ORIGINAL PAPER

An Integrated Approach Employing Endotherapy Accompanied with Fertilization and Soil Mulching Recovered Plane Trees from Early Leaf Chlorosis in the Urban Landscape

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Abstract

Chlorosis, a significant issue affecting plane trees in urban green spaces, was the focus of this research. The aim was to investigate the effectiveness of a combined strategy involving soil amendment and feeding in reducing chlorosis and enhancing tree health. A factorial study was conducted using a randomized complete block design with four replications on plane trees in an urban setting. The study included two trunk injection (endotherapy) levels (non-injection and injection) and four fertilization techniques (control, dig-hole fertilizer, mulch, and dig-hole fertilizer + mulch), both individually and combined. The study revealed that the injection treatment played a crucial role in facilitating the absorption of essential nutrients such as iron and phosphorus, leading to increased chlorophyll levels and overall improved physical health of the plane trees. Furthermore, the combination of injection and dig-hole fertilizer and also injection and mulch resulted in enhanced iron and zinc concentrations in the leaves. During the summer, the combined application of injection, dig-hole fertilizer, and mulch significantly enhanced tree health, as evidenced by a remarkable 40% increase in total chlorophyll concentration compared to the control group. This holistic approach not only boosted chlorophyll levels but also improved the physical health of the trees over the months from June to September, with enhancements of 55%, 30%, 55%, and 69% respectively, when compared to the control treatment. Overall, the findings emphasize the importance of improving soil conditions for efficient nutrient uptake by trees in urban green spaces. Tree health significantly improved by enriching the soil with additional elements through dig-hole fertilizer and trunk injection methods.

Keywords Iron deficiency · Dig-hole fertilizer · Tree decline · Urban forestry · Trunk injection

1 Introduction

The plane tree (*Platanus orientalis* L.) is a popular street tree in Iran and some Mediterranean countries, where some ancient trees may be found (Khorsandi et al. 2016). They are recognized for their lengthy lifespan and widespread distribution within regions with moderate temperatures (Aalipour et al. 2019). Over the past few years, chlorosis has been a major physiological condition affecting most plane trees in Iran (Aalipour and Nikbakht 2021).

Chlorosis is a significant physiological issue that impacts plants globally. It is particularly prevalent in Mediterranean areas where trees are planted in calcareous or alkaline soils (Sanz et al. 1992; Lucena and Hernandez-Apaolaza 2017). Chlorosis can be attributed to nutritional problems and deficiencies in essential elements, including nitrogen, zinc, and particularly iron (Aalipour et al. 2019). This phenomenon arose due to the elevated pH and bicarbonate levels in the soil, resulting in a decrease in the absorption of iron by plants. This was achieved by decreasing the presence of iron levels in the ground, leading to iron precipitation in the apoplast of the root cells (Zribi and Gharsalli 2002). Although there is a significant amount of iron present in the soil, it is

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not beneficial to plants because it exists in forms that are not easily accessible to them (Bindra et al. 2019). The iron content in leaf chloroplasts is estimated to be around 60–80% (Kroh and Pilon 2020). Hence, a deficiency of iron inhibits the production of chlorophyll in leaves, while a higher concentration of carotenoids results in the emergence of yellow or pale green colors in leaves (Santos et al. 2016), so diminishing the overall well-being of trees in urban green areas.

The availability of nutrients in soils is one of the key components of plant nutrition. The use of fertilizers like ferrous sulfate ($FeSO_4$) is typically ineffective in calcareous soils with elevated bicarbonate levels, as they tend to get stuck in the soil and are no longer beneficial for the plant (Zokaee Khosroshahi et al. 2023). Iron deficiency symptoms are worsened by high soil density, inadequate drainage, and cold, damp weather. When carbon dioxide and bicarbonate levels rise in the soil, any iron fertilizer that is applied will quickly stabilize and become ineffective (Weil and Brady 2017). The drawbacks of soil-based application techniques include the negative impacts on the environment associated with high lime levels, combined with reduced absorption caused by poor ventilation from overwatering and the inability to transport all of the fertilizer to the roots because of their condition large volume in the soil, and soil contamination (Zokaee Khosroshahi et al. 2023). Injecting nutrients directly inside the tree's trunk is a promising technique that involves administering small quantities of nutrients with limited negative impacts on the environment. This method ensures that all injected materials are absorbed by the plants (Chakraborty et al. 2015). This approach allows for direct entry into the tree's vascular system. Following that, these substances are actively transferred from the base of the tree to the canopy (VanWoerkom et al. 2014; Berger and Laurent 2019). According to McVay et al. (2019) and Hu et al. (2018), endotherapy has been identified as a viable approach for tree nutrition, offering an effective alternative to traditional methods such as foliar treatment or soil-based approaches. In their study, Fernández-Escobar et al. (1993) demonstrated the successful introduction of Fe compounds into olive (*Olea europaea*) and peach (*Prunus persica*) trees through the injection method. Particularly, ferrous sulfate came as the most efficacious Fe compound in mitigating chlorosis, exhibiting a sustained impact over two consecutive peach seasons and a minimum of olive through three seasons. In a study carried out by ZaenEl-DeenE et al. (2018), it was found that injecting the trunk and applying it to the foliage exhibited greater efficacy in addressing micronutrient deficiencies compared to soil application. These treatments resulted in enhanced stimulation of parameters of growth, such as the width of leaves, length of leaves, area of leaves, and total chlorophyll concentration, as well as increased leaf mineral contents. Additionally, on calcareous and alkaline soils, Zokaee Khosroshahi et al. (2023) showed that the best way to deal with iron deficiency in peach trees is through trunk injection with $FeSO_4$.Improving the physical structure of the soil is another effective way to uptake plant nutrients. In this regard, feeding at depth provides the nutrients needed by the plant and improves the soil biological conditions (Watson 2002). Accordingly, deep placement can be used to increase the absorption of nutrients such as iron.

Furthermore, the application of organic mulches at ground level or the incorporation of mulch into the soil, through water retention, greatly enhances the soil's physical characteristics by decreasing its bulk density and enhancing its porosity (Liao et al. 2021). Furthermore, it has been observed that both inorganic and organic mulches exhibit usefulness in the preservation of soil moisture, alteration of soil temperature, mitigation of nutrient leaching from the root zone, and facilitation of nutrient release within the profile of soil (Sharma et al. 2005; Ahmad et al. 2007). According to Lee et al. (2005), the use of mulch results in enhanced root growth and biomass. As a result, it enhances the efficiency of water usage and the speed of photosynthesis (Tian et al. 2003; Zhou et al. 2009). Additionally, organic mulches increase the process of soil nutrient cycling, organic matter creation, decomposition, and temperature by decreasing soil evaporation and temperature (Sarkar et al. 2007; Nair and Ngouajio 2012; Namaghi et al. 2018).

The primary goal of this research is to employ multiple methods to enhance the effectiveness of nutrient uptake in plane trees in order to mitigate the occurrence of chlorosis. Specifically, this study aims to answer the question: can the combined application of trunk injection of iron, deep placement of nutrients, and the use of organic mulches significantly improve the nutritional status and health of plane trees, thereby reducing the symptoms of iron deficiency chlorosis? To address this question, we hypothesize that the integrated approach of nutrient injection, dig-hole fertilizer method, and organic mulches will result in increased accessibility and assimilation of iron and other essential nutrients, leading to a marked reduction in chlorosis symptoms and improved overall tree vigor. We can maximize the identification of chlorosis symptoms in plane trees by capturing the important period of stress response throughout the summer months (June, July, August, and September) when temperatures are at their highest. These months are especially important since they are when trees are under the most stress, which makes it possible to see how the trees react to rising temperatures. This innovative study combines three methodologies nutrient injection into tree trunks, dig-hole fertilizer (deep placement of nutrients), and the application of organic mulches to enhance nutrient uptake by trees and reduce iron deficiency chlorosis. By testing this integrated strategy, we aim to offer a comprehensive solution that improves the management of nutritional deficiencies in urban plane tree populations.

2 Materials and Methods

2.1 Experiment Design

The research design was executed as a factorial experiment on “Mirza Taher Street” in the urban environment of Isfahan, relying on a complete randomized block with four replications. Table 1 presents a selection of the physical and chemical properties of the soil sampled from a depth of 60–90 cm at the designated location. The treatments comprised two levels of endotherapy (injecting and non-injecting, with the non-injecting serving as the control) and four levels of fertilization technique (control, mulch, dig hole fertilizer, and mulch + dig hole fertilizer), as well as the interaction between these levels and endotherapy treatment. Initially, trees with comparable characteristics, including age (20–25 years), morphological traits, and irrigation system type, were chosen for the plan’s execution. The trees were injected using a technique known as low-pressure, high-volume endotherapy. The procedure involves the drilling of a hole into the tree trunk (Fig. 1A). The system consists of two components: a pressurized capsule that contains the nutritional solution, and an L-shaped connector that connects the tube to the tree trunk (Fig. 1B) followed by the alignment of the connector and the subsequent connection of the capsule to the trunk (Fig. 1C). The vascular system of the tree absorbs the injected solution, which is distributed uniformly throughout all of its tissues. The system is a patent of Fertinyect S.L. (Cordoba, Spain) and is distributed in Western Asia by Farinkesht Taban Company (Isfahan, Iran). A patented compound containing seaweed extract (2%) and iron amino chelate (2%) were all present in the capsules. Each tree received 3 capsules each containing 120 mL of the solution in May 2020.

Farinkesht Taban Company provided rooting and nutritional compounds (Fig. 1D). Two holes 50 × 50 cm in width and length and 100 cm in depth were excavated around the trees for the fertilizer treatment using the dig hole method (Fig. 1E). They were in the north and south trees. One of the holes was filled with the rooting compound (Fig. 1F), which consisted of an NPK complete fertilizer (30-5-15) (55%), mycorrhizal fungi such as *Rhizophagus irregularis* and *Funneliformis mosseae* are present at a concentration of 80 spores per g for each species The product also contains, potassium sulfate (11%), magnesium sulfate (11%), zinc sulfate (6%), and iron sulfate (6%). A mixture was prepared by combining 900 g of the rooting compound with 10 kg of manure, which was subsequently applied to the holes. A different nutritional composition was employed in the other hole. The formulation consisted of an NPK compound fertilizer (30-5-15) (37%), elemental sulfur (37%), Thiobacillus bacteria (4%), and supplementary components including potassium sulfate (7%), magnesium sulfate (7%), iron sulfate (4%), and zinc sulfate (4%) (Aalipour et al. 2019). The hole was filled with a mixture of 1350 g of this nutritional ingredient and 10 kg of manure.

Table 1 Physical and chemical characteristics of the soil in the area used in the experiment

Soil pattern	Soil acidity	Electrical conductivity of soil (dS m^{-1})	Lime (%)	Absorbable iron (mg kg^{-1} soil)
Heavy to very heavy	7.6	2.5	26.5	552

Wood chips were used for the application of the mulch treatment. The wood chips utilized in this study were derived from the fragmented remnants of tree bark and branches acquired during the process of pruning trees at Isfahan University of Technology (Fig. 1G). The mulch was laid by evenly distributing a layer of wood chips on the tree’s base, with a size that was roughly one-third to two-thirds of the tree’s drip line radius (Fig. 1H). For every tree, a layer of wood chips 10 cm thick was applied (Fig. 1I). The trees were subjected to irrigation at intervals of 7 days from April to October and 14 days from November to March, employing flood irrigation as the method of irrigation. The irrigation skipped during precipitations (data not presented).

We collected approximately 20 leaves from each tree, making sure to sample from all four sides and the middle of the crown, and conducted four replications for each treatment. We performed sampling for chlorophyll measurements during the last week of each month from June to September and reported the average chlorophyll content over these four months. We immediately wrapped the samples in aluminum foil and placed them in liquid nitrogen to preserve their integrity immediately after collection. The laboratory stored the samples at -80 °C until analysis. We also collected samples during the last week of August to measure nutrient concentrations, antioxidant enzyme activity, and other physiological traits.

2.2 Measured Parameters

2.2.1 Physical Health of the Tree

Every week from June to September, the physical health and degree of the greenness of each tree were measured using the Niu et al. (2007) approach. Every tree received a score between 1 and 10, which allowed for the calculation of the average physical health of the trees in each month.

Fig. 1 The procedure for administering injection treatment (**A**-**C**), applying dig-hole fertilizer (**D**-**F**), and mulching treatment (**G**-**I**) to the plane trees

As a result, the trees with yellow leaves and weak branches were represented by the lower number, while the trees with healthy, fully green foliage and expanding branches were represented by the higher number.

2.2.2 Concentrations of Chlorophyll and Carotenoid

The amount of carotenoid and chlorophyll in plant materials was measured using the Lichtenthaler technique (1987). A 0.2 g sample of new leaf tissue was mixed with acetone of 80% concentration, then strained and adjusted to a total volume of 10 mL. The mixture was subsequently measured for absorbance wavelengths of 663, 647, and 470 nm utilizing a spectrophotometer from Japan, specifically the Shimadzu UV-160 A model. The amount of chlorophyll and carotenoid in leaves is expressed in mg. g^{-1} FW of leaves was determined using the following Eqs. (1–4).

$$\text{Chlorophyll a} = (19.3 \times \text{A663}\ \ 0.86 \times \text{A647})\ \text{Volume}/100\ \text{Mass} \tag{1}$$

$$\text{Chlorophyll b} = (19.3 \times A647\ 3.6 \times A663)\ \text{Volume}/100\ \text{Mass} \tag{2}$$

$$\text{Total chlorophyll} = \text{Chlorophyll a} + \text{Chlorophyll b} \tag{3}$$

$$\text{Carotenoids} = 100\ (\text{A470})\ \ 3.27\ \left(\text{mg g}^{-1}\text{Chl a}\right)\ \ 104\ \left(\text{mg g}^{-1}\text{Chl b}\right)/227 \tag{4}$$

2.2.3 Relative Water Content

The López-Serrano technique (2019) was utilized to determine the relative water content (RWC) of the plant material. Fully matured leaves that had reached their full expansion were meticulously collected from the bottom of the leaf blade. To find out their fresh weight (FW), they were instantly weighed. Leaves were placed in purified water and allowed for 12 h at a moderate room temperature. The weight of the leaves was determined by turgid weight (TW), which was removed by drying the leaves with an excessive amount of moisture using a paper towel. The leaves were placed in an oven at 60 °C for 24 h to determine the weight when dry (DW). The RWC was used to establish Eq. 5 (López-Serrano et al. 2019).

$$\text{RWC} = \frac{(\text{FW} - \text{DW})}{(\text{TW} - \text{DW})} \times 100 \tag{5}$$

2.2.4 Total Soluble Carbohydrate

The total soluble carbohydrate content of plant material was determined utilizing the McCready approach (1950). The determination of total soluble carbohydrates was conducted utilizing the Anthrone method. 5 mL of 95% ethanol was employed to homogenize 0.2 g of leaf tissue, which was then centrifuged at 3500 rpm for 10 min at a temperature of 10 °C; the liquid above the sediment was moved to a different container. This procedure was replicated three times, with the second and third repetitions employing 70% ethanol. The 100 µL of extract obtained at the end of the third phase was combined with 3 mL of anthrone solution which consists of 0.15 g of anthrone mixed with 100 mL of 70% sulfuric acid and warmed to 95 °C for 15 min. The absorbance at 625 nm was measured with a Shimadzu UV-160 A spectrophotometer, following the chilling process. As a standard, glucose was utilized to determine the total soluble carbohydrates (McCready et al. 1950).

2.2.5 Proline Determination

The technique outlined by Bates (1973) was employed for measuring the proline levels in plant matter. In this method, 0.2 g of leaf samples were homogenized in a 10 mL solution containing 3% sulfosalicylic acid at 4 °C. Subsequently, the final product underwent centrifugation at 5000 rpm for 20 min after incubation. A solution was prepared by combining the supernatant (2 mL) with the following components: 30 mL glacial acetic acid along with 20 mL phosphoric acid with a concentration of 6 mol L^{-1}, and 1.25 g of ninhydrin. At a wavelength of 518 nm, the absorbance was determined (Bates 1973).

2.2.6 Determination of Electrolyte Leakage

Electrolyte leakage (EL) from plant materials was determined utilizing the Bajji method (2002). Following three washes with deionized water, the leaf segments were divided into portions of equal size (0.2 g per treatment) and immersed in 15 mL of distilled water. At the start of the rehydration phase, an initial electrical conductivity (Eci) evaluation was undertaken. The tubes containing the segments were subsequently placed in a 25 °C, dark chamber. Following that, Ecf measurements were obtained at various time intervals throughout the rehydration process, exactly at 0.5, 1.5, 3.5, 7.5, and 22.5 h. The samples were chilled to 25 °C after being subjected to 10 min of autoclaving at a temperature of 115 °C following the mentioned measurements. The overall electrical conductivity (Ect) of the samples was subsequently determined. Equation 6 was employed to calculate the expression for EL.

$$\text{EL}\ (\%) = \frac{(\mathbf{Ecf} - \mathbf{Eci})}{(\mathbf{Ect} - \mathbf{Eci})} \times 100 \tag{6}$$

2.2.7 Determination of Malondialdehyde

The Buege and Aust method (1978) was used to measure Malondialdehyde (MDA) in plant material by quantifying thiobarbituric acid reactive species (TBA). 0.2 g of leaves were macerated using a mixture of liquid nitrogen and 20% polyvinylpyrrolidone (PVPP) (w/v). The mixture was subsequently homogenized with 0.1% trichloroacetic acid (TCA) (w/v). Following this, the homogenate underwent centrifugation at a speed of 10,000 g for 10 min. After adding portions of the supernatant to a reaction mixture containing 0.5% TBA by weight and 10% TCA by weight, the specimens were then placed in an incubator at 95 °C for 30 min. To halt the reaction, the samples were cooled using ice. The spectrophotometer (Shimadzu UV-160 A, Japan) analysis measured the absorbance at 535 and 600 nm, with results reported as mol.g^{-1} of fresh weight.

2.2.8 Nutrient Concentrations

Five identical leaves were gathered for each treatment in August 2021. The leaves were meticulously rinsed with deionized water. After that, the leaves were dried for a certain amount of time at 65 °C to get a consistent weight. Following this, 0.5 g of the dry leaves were crushed into powder to analyze their mineral composition. Following a dry ashing process at 550 °C for a duration of 5 h, 2 M hydrochloric acid was used to extract the plant tissue material. For 20 min, the mixture was heated to 70 °C, yielding a light-colored solution. The residue was rinsed and filtered into a 50 mL flask using purified water and Whatman paper. After digestion, potassium (K) values in ppm were measured with a film photometer (model PFP7) (Murillo-Amador et al. 2007). Total phosphorus was determined using the vanadomolybdophosphate colorimetric method, which involved measuring absorption at an 880 nm Spectrophotometer wavelength (Shimadzu UV-160 A, Japan). The aqueous extract (0.2 g dried substance mixed with 10 mL purified water) was filtered, and the eluate was used to directly determine inorganic phosphorus (López-Cantarero et al. 1998). A Perkin Elmer model AA3030 atomic absorption instrument was employed to quantify the concentrations of iron and zinc.

2.2.9 Enzyme Activity

The plant samples were kept in a refrigerator at a temperature of -20 °C in hermetically sealed packets before extraction. Frozen samples were crushed with liquid nitrogen into a fine powder in order to conduct antioxidant enzyme assays. The powder obtained was subsequently subjected to extraction using a 0.1 mM phosphate buffer with a pH of 7.8 that was cooled to ice. This buffer solution consisted of 1 mM ethylene diamine tetra acetic acid (EDTA), 1 mM phenylmethane sulfonyl fluoride (PMSF), and 0.5% polyvinyl polypyrimidine (PVP). Spectrophotometric methods were used to quantify the function of ascorbate peroxidase (APX), catalase (CAT), and peroxidase (POD) enzymes in the apoplast. The measurement of APX activity was conducted by observing a rise in absorption at a wavelength of 290 nm. The 3 mL reaction mixture included 50 mM potassium phosphate buffer at pH 7.0, along with 0.5 mM ascorbic acid, 0.1 mM EDTA, 1.5 mM hydrogen peroxide (H_2O_2), and 0.1 mL of the enzyme. The process began with the introduction of H_2O_2. The activity of catalase (CAT) was assessed by observing the decrease in absorption at a wavelength of 240 nm. This measurement was conducted in a 50 mM phosphate buffer solution with a pH of 7.5, which also contained 20 mM H_2O_2. A single unit of CAT activity was operationally described as the amount of enzyme that can facilitate the action of conversion of 1 µmol of H_2O_2 per minute. The measurement of POD activity was conducted by observing the rise in absorbance at a wavelength of 470 nm. This was conducted in a solution that included 50 mM phosphate buffer with a pH of 5.5. The buffer solution also contained 1 mM guaiacol and 0.5 mM H_2O_2 (Sairam and Srivastava 2002; Li et al. 2015; Güneş et al. 2019).

2.3 Statistical Analysis

The information gathered from the trial was sorted in the Excel program, examined for normality, and log-transformed by the Shapiro-Wilk test if necessary. Statistix software ver. 8 was used for the ANOVA and mean comparison analysis based on the LSD test at the 5% probability. Statgraphics Centurion, Version XVI employed for conducting principal component analysis (PCA).

3 Result

3.1 The Effect of Endotherapy and the Fertilization Technique on the Physical Health of Plane Trees

The variance analysis results indicated that endotherapy alone did not have a statistically significant impact on the physical health of the trees from June to September on a month-to-month basis. However, during the same period, the fertilization technique had a significant effect on the trees' physical health. Furthermore, the interaction between endotherapy and the fertilization technique had a significant effect on the physical health of the trees in July, August, and September, with a probability level of 5% (Table 2). The fertilization technique showed that the trees had the highest physical health when treated with mulch + dig-hole fertilizer. This treatment increased the physical health of the trees by 26.5%, 35.6%, 43%, and 40% in June, July, August, and September, respectively, compared to the control treatment (Table 2).

The trees' health in July showed significant improvements in both the injection and non-injection groups when they were treated with either dig-hole fertilizer or mulch + dig-hole fertilizer. In addition, there was no statistically significant difference in the physical health of the trees in July when comparing the fertilization technique with the non-injection treatment (Fig. 2A). In August and September, the trees showed the highest level of physical health when treated with mulch and dig-hole fertilizer applied along the injection. This treatment resulted in a 55%

Table 2 Mean comparisons and analysis of variance of injection and fertilization technique, as well as their interaction on the physical health of the plane trees

Treatments		Physical health in June	Physical health in July	Physical health in August	Physical health in September
Endotherapy					
Injection		7.85 ± 0.17 a	7.11 ± 0.16 a	6.45 ± 0.15 a	6.26 ± 0.15 a
Non injection		7.10 ± 0.16 a	6.62 ± 0.16 a	6.19 ± 0.14 a	5.62 ± 0.14 a
Fertilization technique					
Control		6.51 ± 0.15 c	5.72 ± 0.14 b	5.22 ± 0.14 b	4.88 ± 0.14 c
Dig hole fertilizer		8.00 ± 0.20 ab	7.47 ± 0.17 a	6.93 ± 0.16 a	6.37 ± 0.15 ab
Mulch		7.15 ± 0.16 bc	6.50 ± 0.15 b	5.85 ± 0.14 b	5.69 ± 0.14 bc
Mulch + Dig hole fertilizer		8.24 ± 0.23 a	7.76 ± 0.17 a	7.46 ± 0.17 a	6.82 ± 0.16 a
Source of variation	df				
Endotherapy	1	n.s	n.s	n.s	n.s
Fertilization technique	3	**	**	**	**
Endotherapy × Fertilization technique	3	n.s	*	*	*
Error	21				
Coefficient of variation (%)		10.98	12.11	15.46	16.59

Means within the same treatment column that share a letter are not significantly different at a p-value of less than 0.05 based on the least significant difference test. ^{ns} indicates non significance, * indicates significance at a p-value of less than 0.05, and ** indicates significance at a p-value of less than 0.01. df: degree of freedom

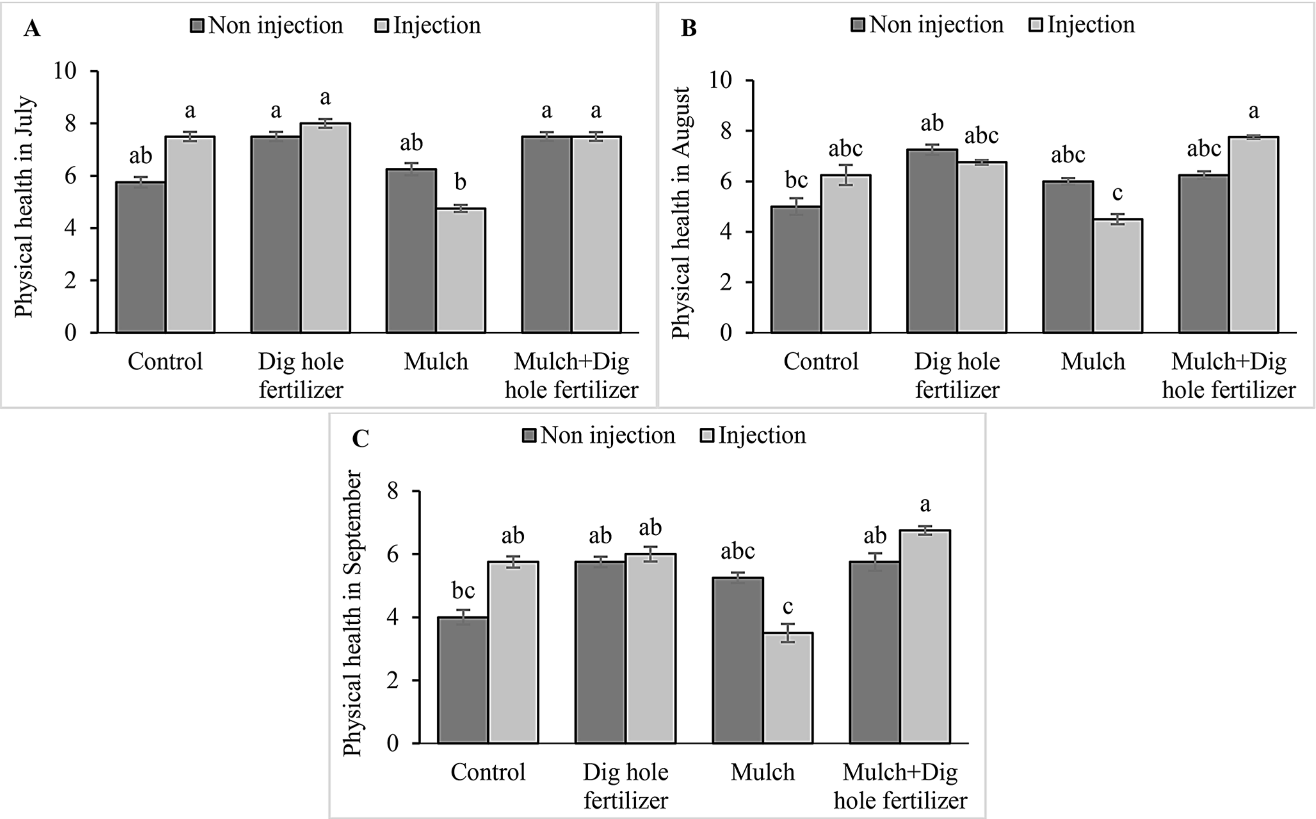

Fig. 2 The interaction effects of injection treatments and fertilization techniques on the physical health of plane trees from July to September. Treatments include non-injection and injection and fertilization technique treatment including control, dig hole fertilizer, mulch, and mulch + dig hole fertilizer. Significant differences ($p < 0.05$) between treatments were identified using the LSD test. Error bars represent the standard error of the mean

and 69% increase in health compared to the control group, which did not receive injection or fertilization (Fig. 2B, C).

3.2 The Effect of Endotherapy and the Fertilization Technique on Chlorophyll Pigments of Plane Trees

The variance analysis results indicated that endotherapy had a statistically significant effect on chlorophyll a, whereas it did not have any significant effect on other photosynthetic pigments. The fertilization technique had a statistically significant effect on chlorophyll b. The combined effect of endotherapy and the fertilization technique had a noticeable impact on chlorophyll a, b, and total chlorophyll content (Table 3). Based on the main effects analysis, the injection treatment resulted in a 28% increase in chlorophyll a compared to the non-injection condition. When it came to the fertilization technique, the dig-hole fertilizer treatment produced the highest levels of chlorophyll b, rising by 43% over the control (Table 3). The mulch treatment, when applied with injection, showed the highest concentration of chlorophyll a. This concentration was approximately 74% higher than that recorded in the control treatment, which did not include injection or fertilization (Fig. 3A). Indeed, the mulch treatment with injection resulted in the highest amounts of chlorophyll b and total chlorophyll. These levels were 35% and 29% greater, respectively, compared to the mulch treatment without injection (Fig. 3B, C). The carotenoid content exhibited a significant rise in both the injection and non-injection groups when exposed to mulch treatment (Fig. 3D).

3.3 The Effect of Endotherapy and the Fertilization Technique on some Physiological Characteristics of Plane Trees

Based on the main effects, the injection treatment resulted in a 5% rise in RWC and a 19% increase in proline content compared to the non-injected treatment. The EL and MDA content had a reduction of 15% and 39%, respectively, when treated with injections as compared to the non-injection group. During the fertilizing technique treatment, the RWC showed a 6% increase under the mulch + dig hole

Table 3 Mean comparisons and variance analysis of injection and fertilization technique, as well as their interaction on the photosynthesis pigments and physiological characteristics of the plane trees

Treatments		Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Chlorophyll total (mg g^{-1} FW)	Carotenoid (mg g^{-1} FW)	Relative water content (%)	Carbohydrate (mg g^{-1} FW)	Proline (mol g^{-1} FW)	Electrolyte leakage (%)	Malondialdehyde (mol g^{-1} FW)
Endotherapy										
Injection		3.23 ± 0.13 a	1.51 ± 0.06 a	4.75 ± 0.15 a	5.70 ± 0.18 a	76.10 ± 2.39 a	4.23 ± 0.14 b	0.27 ± 0.01 a	14.20 ± 0.28 b	0.090 ± 0.002 b
Non injection		2.62 ± 0.11 b	1.45 ± 0.05 a	4.98 ± 0.15 a	5.66 ± 0.18 a	72.10 ± 2.32 b	5.66 ± 0.19 a	0.23 ± 0.01 b	16.80 ± 0.39 a	0.148 ± 0.006 a
Fertilization technique										
Control		2.83 ± 0.12 a	1.20 ± 0.03 b	4.04 ± 0.13 a	5.21 ± 0.17 a	72.80 ± 2.33 ab	35.60 ± 1.74 a	0.27 ± 0.01 ab	18.20 ± 0.50 a	0.139 ± 0.005 a
Dig hole fertilizer		2.74 ± 0.11 a	1.72 ± 0.06 a	4.46 ± 0.14 a	5.83 ± 0.18 a	70.10 ± 2.30 b	35.20 ± 1.74 a	0.32 ± 0.01 a	15.20 ± 0.32 bc	0.130 ± 0.004ab
Mulch		3.22 ± 0.13 a	1.56 ± 0.06 a	4.79 ± 0.15 a	6.17 ± 0.17 a	76.10 ± 2.40 a	34.50 ± 1.72 a	0.24 ± 0.01 b	15.30 ± 0.32 b	0.1050.002 ± ab
Mulch + Dig hole fertilizer		2.91 ± 0.12 a	1.45 ± 0.05 ab	4.37 ± 0.14 a	5.51 ± 0.16 a	77.30 ± 2.41 a	36.00 ± 1.78 a	0.17 ± 0.01 c	13.30 ± 0.25 c	0.102 ± 0.002 b
Source of variation	df									
Endotherapy	1	*	n.s	n.s	n.s	*	*	*	**	*
Fertilization technique	3	n.s	*	n.s	n.s	*	n.s	**	**	*
Endotherapy × Fertilization technique	3	*	**	*	*	n.s	*	**	n.s	n.s
Error	21									
Coefficient of variation (%)		24	17.7	17.5	16.8	6.93	6.30	20.8	11.6	27.7

Means within the same treatment column that share a letter are not significantly different at a p-value of less than 0.05 based on the least significant difference test. ^{ns} indicates non significance, * indicates significance at a p-value of less than 0.05, and ** indicates significance at a *p*-value of less than 0.01. df: degree of freedom

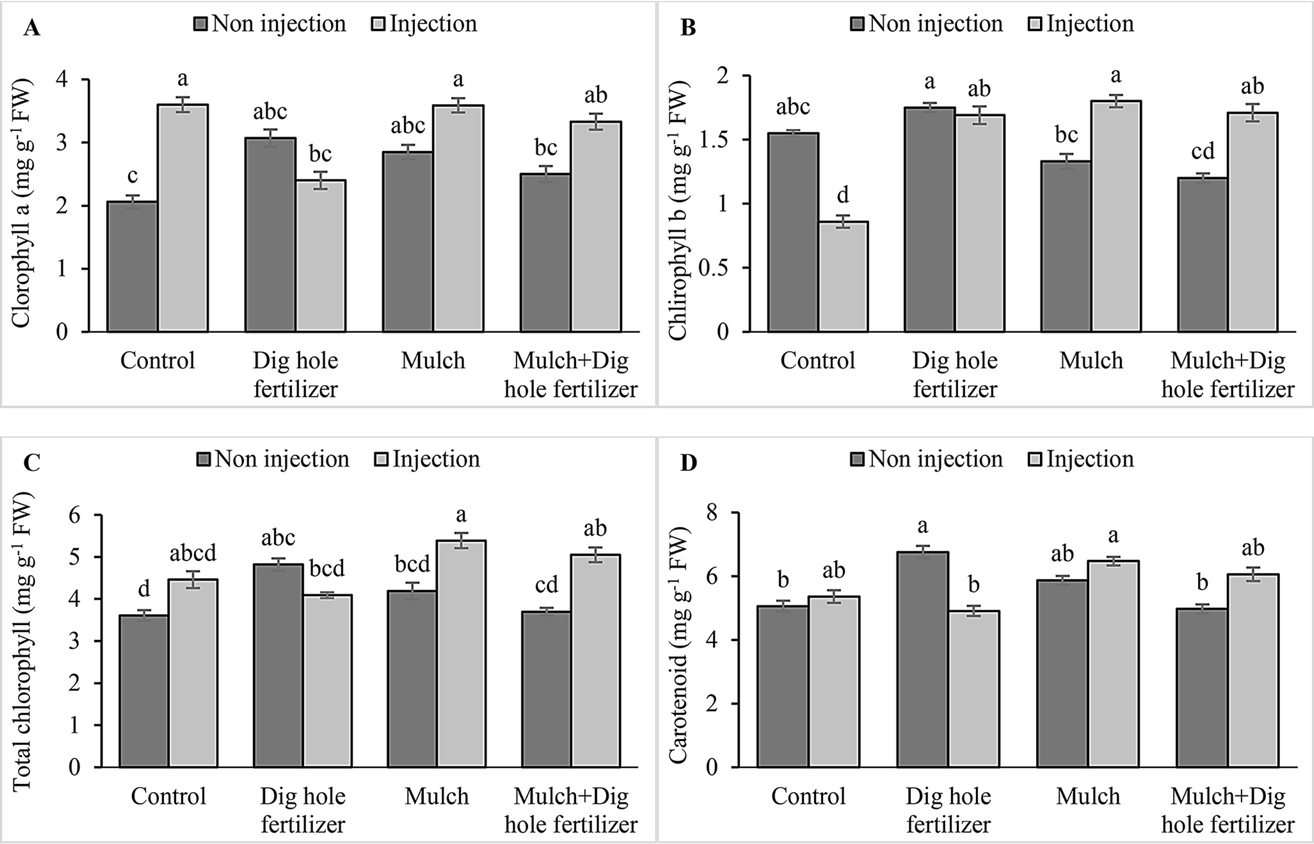

Fig. 3 The interaction effects of injection treatments and fertilization techniques on the photosynthetic pigments of plane trees in summer were analyzed. Treatments include non-injection and injection and fertilization technique treatment including control, dig hole fertilizer, mulch, and mulch + dig hole fertilizer. Significant differences ($p < 0.05$) between treatments were identified using the LSD test. Error bars represent the standard error of the mean

fertilizer treatment compared to the control. The dig-hole fertilizer treatment resulted in the highest carbohydrate content, exhibiting a 19% increase compared to the control. The mulch + dig hole fertilizer treatment exhibited the lowest levels of EL and MDA, which were 27% and 23%, respectively, lower than the values obtained from the control group (Table 3).

The carbohydrate concentration showed a significant increase when the mulch + dig hole fertilizer was applied using the injection treatment, compared to the dig hole fertilizer and mulch treatment. However, the non-injection treatment did not show any statistically significant change in carbohydrate concentration among the different fertilization techniques (Fig. 4A). The proline content did not show a normal trend. The dig-hole fertilizer treatment produced the highest concentration of proline, particularly when not injected. However, when administering the injection treatment, the mulch treatment exhibited the highest proline concentration (Fig. 4B).

3.4 The Effect of Endotherapy and the Fertilization Technique Nutrients Content of Plane Trees Leaves

The endotherapy treatment resulted in a significant increase in the leaves' iron (Fe) and phosphorus (P) contents. The injection treatment resulted in a 25% rise in Fe compared to the absence of injection. The mulch + dig hole fertilizer treatment had the greatest concentration of P, exceeding the control by an average of 12.5%. The dig-hole fertilizer treatment exhibited the highest concentration of zinc, showing a 21% increase in comparison to the control group (Table 4).

The interaction between injection and mulch showed the highest amount of zinc, rising by 28% compared to mulch under non-injection (Fig. 4C).

3.5 The Effect of Endotherapy and the Fertilization Technique on Enzymes Activity of Plane Trees

The main effects analysis revealed that the injection treatment resulted in a significant 41% increase in APX enzyme activity in comparison to the non-injection group. The application of mulch resulted in a 19% increase in POD enzyme

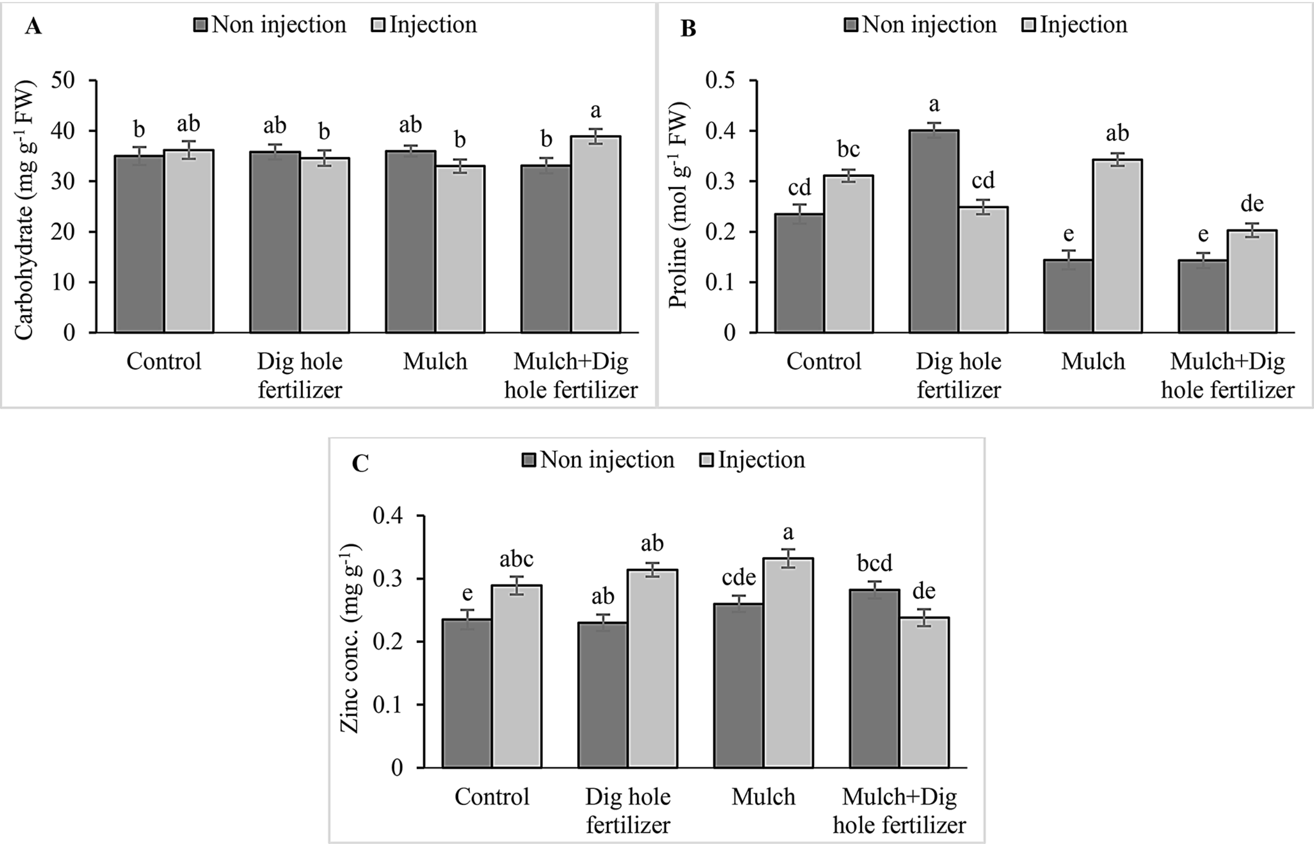

Fig. 4 The interaction effects of injection treatments and fertilization techniques on the carbohydrate, proline, and zinc content of plane trees in summer were analyzed. Treatments include non-injection and injection and fertilization technique treatment including control, dig hole fertilizer, mulch, and mulch + dig hole fertilizer. Significant differences ($p < 0.05$) between treatments were identified using the LSD test. Error bars represent the standard error of the mean

activity, as shown in Table 4. The interaction between the injection and fertilization techniques did not significantly affect enzyme activity (Table 4).

3.6 Relationship Between Traits

The PCA was used to assess the reactions and identify the most efficient treatment for minimizing chlorosis in plane trees by analyzing the overall correlation between traits and treatments (Fig. 5). We used the cosine of the angles formed by the vectors to illustrate the degree of correlation between traits. The PCA biplot utilizes the angles between the vectors representing traits (variables) to display the estimated amount of correlation between them. This biplot shows a positive correlation between variables forming acute angles (angles measuring from 0° to 90°) and a negative correlation between variables forming obtuse or straight angles (angles measuring from 90° to 180°). Variables that form a right angle have no correlation (angle measuring 90°). As the angles approach 0° and 180°, the magnitude of correlations increases, while the distance of the vectors from a trait to its starting point represents the extent to which each trait contributes to the PCA.

PCA with the applied treatments classified the traits into two distinct categories. The first group includes the interaction between injection treatment and fertilization techniques, control, dig-hole fertilizer, mulch, and dig-hole fertilizer + mulch. The category includes the physical health of trees as well as the concentration of photosynthetic pigments, nutrient content, and proline and antioxidant enzymes (POD and APX) (purple circle). The chlorophyll concentration in the leaves showed a positive correlation with the levels of nutrients and antioxidant enzyme activity. Similarly, the physical health of the trees during the summer months had a positive correlation with the levels of carbohydrates and nutrients. Additionally, we observed a negative correlation between these traits and EL and MDA levels. The control without injection treatment was classified into the second category and showed positive correlations with MDA, EL, and CAT (blue circle). Based on the PCA results, the interaction of injection treatment with dig-hole fertilizer + mulch resulted in the highest physical health of the plane trees. The nutrient content increased in the interaction

Table 4 Mean comparisons and variance analysis of injection and fertilization technique, as well as their interaction on the nutrient content and biochemical characteristics of the plane trees

Treatments		Potassium (mg g^{-1})	Phosphorus (mg g^{-1})	Zinc (µg g^{-1})	Iron (µg g^{-1})	Ascorbate peroxidase (Umg pr^{-1})	Catalase (Umg pr^{-1})	Peroxidase (Umg pr^{-1})
Endotherapy								
Injection		1.76 ± 0.06 a	0.26 ± 0.01 a	0.29 ± 0.01 a	10.60 ± 0.25 a	57.70 ± 1.87 a	398 ± 9.80. a	2187 ± 53.85 a
Non injection		1.76 ± 0.06 a	0.24 ± 0.01 b	0.27 ± 0.01 a	8.54 ± 0.23 b	40.90 ± 1.80 b	399 ± 9.80 a	2014 ± 49.46 a
Fertilization technique								
Control		1.31 ± 0.04 a	0.24 ± 0.01 c	0.26 ± 0.01 b	8.85 ± 0.23 a	49.70 ± 1.85 a	403 ± 9.80 a	2054 ± 49.94 ab
Dig hole fertilizer		1.85 ± 0.07 a	0.24 ± 0.01 bc	0.31 ± 0.01 a	10.10 ± 0.25 a	48.20 ± 1.84 a	462 ± 10.45 a	2287 ± 51.72 a
Mulch		1.89 ± 0.07 a	0.25 ± 0.01 ab	0.29 ± 0.01 a	10.50 ± 0.25 a	46.90 ± 1.83a	384 ± 9.70 a	2453 ± 61.96 a
Mulch + Dig hole fertilizer		1.98 ± 0.08 a	0.27 ± 0.01 a	0.26 ± 0.01 b	9.01 ± 0.024 a	52.50 ± 1.86 a	345 ± 9.12 a	1608 ± 42.50 b
Source of variation	df							
Endotherapy	1	n.s	*	n.s	**	**	n.s	n.s
Fertilization technique	3	n.s	**	**	n.s	n.s	n.s	*
Endotherapy × Fertilization technique	3	n.s	n.s	**	n.s	n.s	n.s	n.s
Error	21							
Coefficient of variation (%)		24.20	4.82	11.00	16.90	29.70	35.20	29.30

Means within the same treatment column that share a letter are not significantly different at a p-value of less than 0.05 based on the least significant difference test. ^{ns} indicates non significance, * indicates significance at a p-value of less than 0.05, and ** indicates significance at a *p*-value of less than 0.01. df: degree of freedom

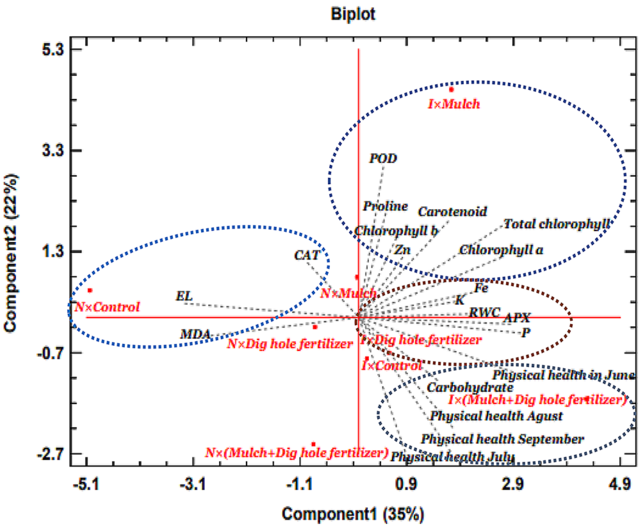

Fig. 5 The PCA graph of endotherapy and the fertilization technique application method on the health, biochemical, and some physiological characteristics of plane trees. N: non-injection, I: injection, P: phosphor, K: potassium, Fe: iron, Zn: zinc, RWC: Relative water content, MDA: malondialdehyde, EL: Electrolyte leakage, CAT: catalase, POD: peroxidase, APX: ascorbate peroxidase

between the injection and the dig-hole fertilizer treatment. In addition, the injection had a higher effect on the photosynthetic pigments, proline, POD, and Zn content when combined with the mulch treatment. The control treatment had the highest levels of MDA and EL, as seen in Fig. 5.

4 Discussion

This study investigated the effects of several nutrition techniques and soil amendments on reducing chlorosis in plane trees. The findings indicated that the control trees exhibited the poorest physical health compared to the trees that were treated with injection or fertilization techniques (Fig. 6A-D). Also, the application of injection treatment alone did not have a significant effect on the apparent health of the trees from June to September (Fig. 6E-H). However, the application of endotherapy treatment together with dig-hole fertilizer + mulch, had significant effects on enhancing physical health (Fig. 6I-L). The forces that influence trees' well-being in urban settings differ from those in their native habitats. It goes beyond the tree's nutritional condition. Several factors, including soil structure and quality, microclimate conditions, nutrient availability in the soil, and soil and air pollution, can influence the health of urban trees. The aforementioned factors have a significant influence on trees' physiological processes, growth, and overall health (Petrova et al. 2014). Our study showed that using mulch in combination with dig-hole fertilizer to improve soil quality greatly improved the physical health of the trees (Fig. 6M-P). Nikbakht et al. (2022) found that giving plane trees iron aminochelate improved their physical health in a study.

We can regard the concentrations of nutrients and pigments in the leaves as signs of the well-being and vigor

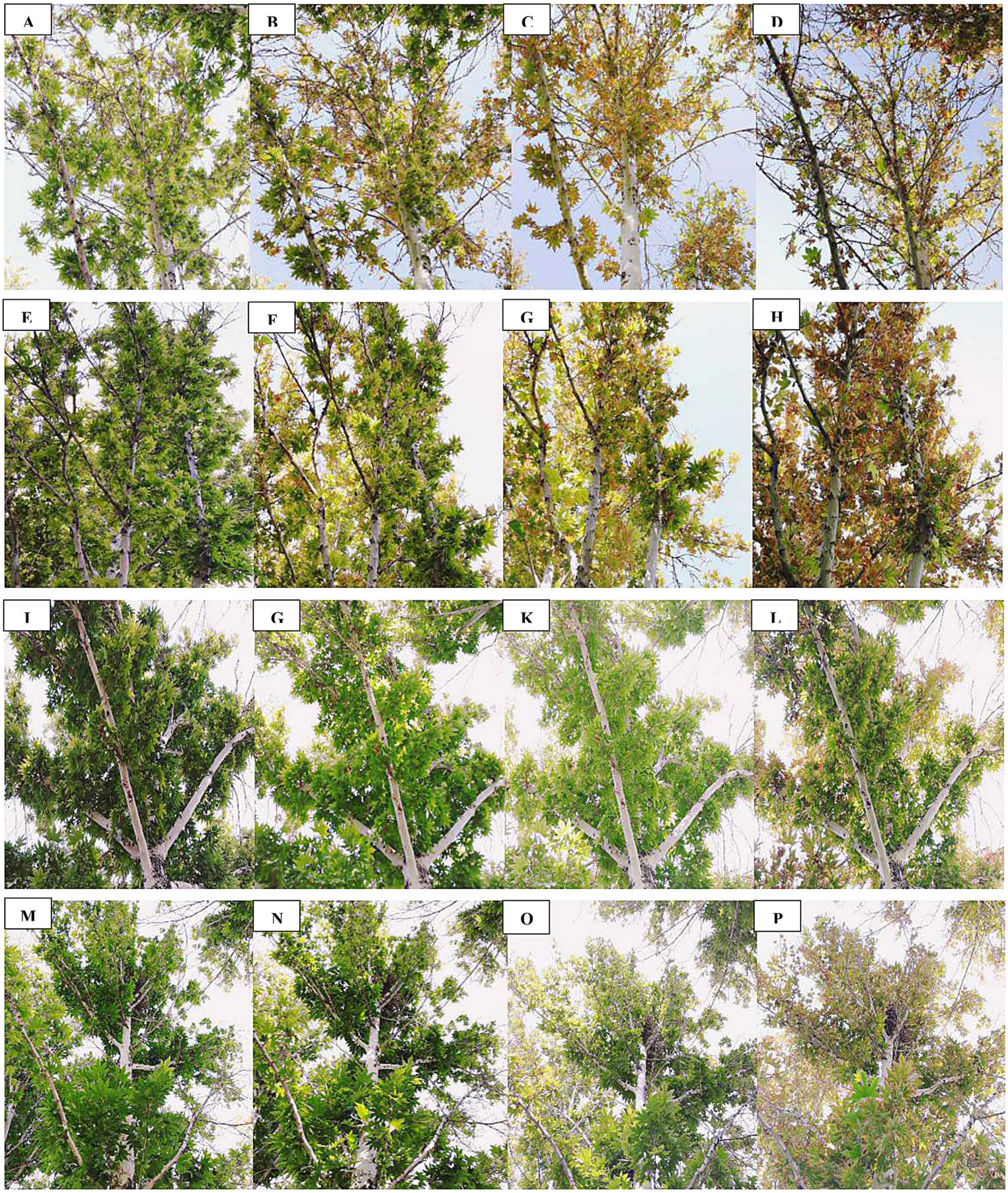

Fig. 6 The effect of control (without injection and fertilization technique) treatment (**A**-**D**), injection treatment (**E**-**H**), injection × (dig-hole fertilizer+mulch) treatment (**I**-**L**), dig-hole fertilizer+mulch treatment (**M**-**P**) on the physical health of plane trees, from left to right, in June, July, August and September

of plants, both functionally and physiologically (Hrdlicka and Kula 2001; Cekstere et al. 2008). A variety of factors can induce leaf chlorosis in plants. The primary factor causing leaf chlorosis has been established to be iron deficiency. The soil in the area may have an iron deficiency, which could be attributed to the limited availability of the transition from ferric to ferrous forms (Honetschlägerová et al. 2018). The significance of iron in chlorophyll production has been thoroughly described (Hänsch and Mendel 2009). Iron levels can control how quickly aminoacetylalanine (ALA) is created, which is needed for both chlorophyll and heme (Pushnik and Miller 1989). Iron is necessary to produce protochlorophyll from magnesium protoporphyrin (Marschner and Marschner 2012). Li et al. (2021) conducted a study where they analyzed the pigment concentration in both normal and discolored leaves. The findings demonstrated a significant decrease in all pigments, specifically chlorophyll a, chlorophyll b, and carotenoids, in leaves of betelnut palm *(Areca catechu* L.) experiencing iron deficiency. Conversely, as iron levels increased, the pigment contents exhibited an upward trend, highlighting the crucial role of iron levels in the synthesis of pigments in leaves.

The endotherapy treatment enhances the plant's ability to absorb mineral elements through its vessels, effectively improving the absorption of iron sources and alleviating tree chlorosis. Barney et al. (1984) conducted a study that demonstrated the effectiveness of injecting a 1% ferrous sulfate solution using a high-pressure system to eliminate chlorotic symptoms in 'red delicious' apple trees flourishing in poorly-drained calcareous soil. The increase in chlorophyll concentration demonstrated this effectiveness. In 1993, Fernández-Escobar et al. were the pioneers in utilizing the low-pressure injection technique to manage chlorosis in olive and peach trees. They discovered that the injection treatment significantly increased the chlorophyll content in the treated trees compared to the control group. Additionally, they found that iron sulfate had the most confirmed effect on reducing chlorosis. Our research findings, consistent with the aforementioned results, demonstrated that trunk injection significantly enhanced chlorophyll a level, resulting in a 28% improvement compared to the non-injection group. Ni et al. (2016) discovered that mulches did not impact the levels of chlorophyll b in their study on tea olive (*Osmanthus fragrans*). The chlorophyll a content rose by 36.3% in the Round Gravel treatment, by 46.3% in the Wood Chips treatment, and by 21.3% in the Manila grass mulch treatment. This suggests that mulch may enhance the photosynthesis rate in plant leaves under these specific conditions. Mulching improves chlorophyll content and plant growth by stimulating root activity and boosting soluble sugar levels. Additionally, it creates favorable circumstances for moisture and nutrient availability within the root area (Ni et al. 2016). Our research findings indicate that the combined effect of injection and mulch resulted in higher levels of chlorophyll b and total chlorophyll compared to the control group.

Farzi et al. (2017) demonstrated that the use of mulch resulted in increased RWC in olive (*Olea europaea* L.) while simultaneously reducing leaf and water osmotic potential compared to treatments without mulching. The higher RWC seen with mulching as compared to the no mulching treatment can be attributed to the enhanced retention of soil water, leading to lower evapotranspiration. The plants then absorb this retained water, leading to an increase in leaf water content. Our research findings indicate that the mulch + dig hole fertilizer treatment resulted in the highest level of RWC. When we fertilize the tree in a dig-hole, the roots become more effective at absorbing water and nutrients. This increased water absorption can also improve the leaf's RWC.

Iron deficiency decreases the functionality of Rubisco and other enzymes involved in photosynthesis, resulting in a decrease in the capacity to absorb CO_2 (Chen et al. 2004). Nevertheless, delivering the element directly to the tissues through trunk injection aids the plant in overcoming nutritional challenges resulting from soil alkalinity, such as in the soil in the present experiment. Enhancing plant iron availability results in elevated rates of photosynthesis and enhanced transportation of carbohydrates in plant tissues (Saleh et al. 2016). Chen et al. (2004) found that grapes (*Vitis vinifera* L.) with iron deficiency had reduced levels of non-structural carbohydrates in their source leaves. Contrary to the previously reported results, our study showed that the Fe trunk injection treatment did not have the ability to enhance the amounts of carbohydrates delivering soil nutrients through dig-hole, using beneficial microorganisms (Aalipour et al. 2019, 2023), and applying mulch to cover the soil effectively increased the carbohydrate content of the leaves. It demonstrates the importance of implementing an integrated management method rather than solely relying on trunk injection it may stem from the source of Fe, and other sources should be evaluated in the next experiments.

Manganese (Mn) and Fe deficiencies raise the levels of free radicals due to their crucial roles in the electron transport chain and enzymatic reactions. The production of osmotic regulators like soluble carbohydrates and proline acts as a protection method against free radicals (Hayat et al. 2012). Proline, serving as both a non-enzymatic antioxidant and an amino acid, typically experiences a rise in levels under various abiotic stresses caused by a mix of heightened production and slow breakdown in mitochondria, attributed to reduced proline oxidase activity and enhanced glutamate pathway enzyme activity (Cvikrová et al. 2013; Singh et al. 2019). The results of the present investigation showed that the injection treatment, combined with the application of mulch and dig-hole fertilizer, successfully mitigated a reduction in iron deficiency in trees, thereby lowering proline levels. According to research by Bardhan et al. (2021), adding nutrients at lower depths can improve nutrient use efficiency and promote deeper root growth. The results of the Aalipour et al. (2019) investigation were very similar to ours. They showed that adding AMF inoculation to the conventional fertilizer program was an efficient way to improve the iron content in plane tree leaves and addressed chlorosis disorder in plane trees in a way that was both environmentally and physiologically friendly. Additionally, their research indicates that drill-hole nutrition should be regarded as a typical strategy in calcareous soils to prevent nutritional diseases in urban environments. Tekaya et al. (2017) discovered that Rhizophagus irregularis colonization increased the amount of iron in the plants, suggesting a beneficial effect on the olive trees' iron nutrition. This might be the result of better iron transfer to the olive trees as well as increased iron release from the soil. It is widely recognized that some fungi produce iron-chelate compounds that can enhance iron availability in plants (Tekaya et al. 2016). From the findings described earlier, we can deduce that in our study, the use of mycorrhizal fungi in the dig-hole fertilizer treatment improves soil pH, resulting in improved nutrient uptake and the prevention of stressful conditions caused by nutrient deficiencies. As a result, the proline metabolism declines under these conditions.

It is well known that cell membrane lipid peroxidation produces MDA (Krantev et al. 2008). The amount of MDA in a living thing can tell you about the level of oxidative stress and cell membrane homeostasis in plants. Our research findings indicate that supplying iron sources to trees suffering from iron deficiency can decrease MDA levels by 40% by granting the tree access to iron. Kong et al. (2014) conducted a study that revealed that a lack of iron deficiency led to a notable rise in the MDA levels in peanut (*Arachis hypogaea*) leaves and roots, indicating the presence of oxidative stress. Liang et al. (2003) found that mulching treatments effectively reduced oxidative damage in rice (*Oryza sativa*) leaves. The mulching treatments demonstrated this reduction by reducing the MDA concentration, the final result of lipid peroxidation. Liang et al. (1999) proposed that the detrimental effects of elevated reactive oxygen species (ROS) on cell membranes can be mitigated by mulching, which effectively retains soil moisture, followed by improved leaf water content. Our research findings indicate that the use of mulch and dig-hole fertilizer effectively decreased the levels of MDA and EL in contrast with the control group. Plants subjected to endotherapy treatment exhibited an elevation in APX activity, a fundamental antioxidant enzyme that has a significant impact on safeguarding the cellular membrane.

According to this study, the trunk injection led to increased levels of phosphorus and iron in the leaves. Nikbakht et al. (2022) demonstrated that injecting plane trees with a 1% aminocholate solution led to a 165.98% increase in iron concentration compared to the control group. Providing iron to plants that are deficient in iron not only gives them enough iron for photosynthesis, but may also enhance the accessibility and movement of other nutrients like zinc, manganese, and phosphorus by reducing the acidity of the plant's sap (Taiz and Zeiger 1998). According to the PCA diagram results, the combination of dig-hole fertilizer and trunk injection treatment showed the highest phosphorus concentration in the leaves. Some microorganisms possess the capability to boost phosphorus levels in the soil through the manufacture of organic acids alongside a range of different enzymes (like phytase, nuclease, phosphatase, etc.) that can break down insoluble phosphate (Bashan et al. 2013). Since mycorrhizal fungi represent an essential element of the rooting chemicals in the dig-hole fertilizer treatment, they can be considered given the elevated phosphorus levels observed in the dig-hole fertilizer treatment (Collavino et al. 2010). Saleh et al. (2016) found that even when the soil contains high levels of iron in most calcareous soils, injecting iron fertilizer into the trunk of date (*Phoenix dactylitera*) trees is more effective than applying it to the soil (using dig-hole fertilizer). Plants have limited accessibility to this nutrient, maybe because of the influence of elevated pH on the formation of iron complexes that are not soluble in the soil (Brady and Weil 2008). Iron deficiency in plants leads to an increase in the buildup of ROS like superoxide radicals (O^{2-}) and H_2O_2 in their tissues, resulting in oxidative stress (Salama et al. 2009). Plants possess an enzyme-based antioxidant defense system that shields them from the build-up of ROS and fixes any oxidative harm (Das and Roychoudhury 2014).

Valipour et al. (2020) did a study that found that levels of CAT, APX, and GPX in the leaves of young quince (*Cydonia oblonga*) plants decreased when they were exposed to the both direct and bicarbonate-induced iron shortage. A study found a significant relationship between the actions of antioxidant enzymes and the concentration of iron in quince seedling leaves. This suggests a relationship between the constrained availability of iron and the decreased activity of these hemoprotein enzymes (Valipour et al. 2020). The PCA diagram demonstrated that the functions of POD and APX enzymes went up after the trunk injection treatment, as well as after the mulch and dig-hole fertilizer treatments. The results align with the discoveries of Valipour et al. (2020), who observed a correlation between the performance of antioxidant enzymes and the quantity of iron. Our study's findings demonstrated that administering iron to chlorotic trees via trunk injection resulted in increased activity of antioxidant enzymes.

5 Conclusions

This study aimed to explore the problem of chlorosis in plane trees, a growing concern in urban green spaces. The findings demonstrate that the integrated approach of trunk injection, deep nutrient placement, and organic mulches not only mitigates the symptoms of iron deficiency chlorosis but also enhances the overall physiological health of the trees. The findings support our hypothesis that the combined application of these methods significantly improves the nutritional status and physiological health of trees, as evidenced by increased chlorophyll concentrations and overall tree vigor. The significance of trunk injection treatments is underscored, particularly when combined with organic mulch, which facilitates increased chlorophyll levels and nutrient availability. This combination enhances the accessibility of essential nutrients, such as iron and zinc, directly to the tree's vascular system, thereby addressing chlorosis more effectively than traditional methods. Furthermore, the dig-hole fertilizer and mulch treatment has been shown to significantly improve soil structure, aeration, and moisture retention, which are critical for optimal root function and nutrient absorption. These mechanistic links suggest that the integrated strategy not only addresses immediate nutritional deficiencies but also promotes long-term soil health through enhanced microbial activity. The significant improvement in chlorophyll levels observed during peak stress months indicates that this approach effectively addresses the urgent nutritional needs of plane trees under environmental stress. This study provides a practical framework for urban planners, arborists, and landscape managers, emphasizing the importance of adopting integrated management strategies for urban tree health. Nutrient capsules cost about $2 each, totaling around $6 per tree when using three capsules. Additionally, utilizing mulch from pruned branches proved to be cost-effective, primarily incurring minimal expenses for shredding the wood. For the chalcoud treatment, each 25 kg package of the root-promoting and nutritional mix was about $10, resulting in an overall treatment cost of approximately $20 per tree. To further optimize the application of these treatments, additional research is warranted to refine the trunk injection techniques, assess the long-term effects of the combined treatments on chlorosis, and explore the precise mechanisms by which these practices influence nutrient uptake and tree health. By elucidating these interactions, we can better inform urban forestry practices and enhance the management of nutritional deficiencies in urban ecosystems.

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Declarations

Conflict of interest The authors declare no conflicts of interest.

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