Adv. Hort. Sci., 2019 33(2): 171-177 DOI: 10.13128/ahs-23326

AHS
Advances in Horticultural Science

Relationship between chlorosis, photosynthesis and the nutrient content of plane trees in the presence of chemical and organic fertilizers

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Key words: mycorrhizal fungi, nutrient acquisition, organic matter, symbiosis, urban trees.

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Citation:
AALIPOUR H., NIKBAKHT A., ETEMADI N., 2019 - *Relationship between chlorosis, photosynthesis and the nutrient content of plane trees in the presence of chemical and organic fertilizers*. - Adv. Hort. Sci., 33(2): 171-177

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 31 May 2018
Accepted for publication 10 January 2019

Abstract: Chlorosis disorder is a major problem affecting the growth and physiological processes of many trees including plane trees (*Platanus orientalis* L.). This experiment was conducted to study the relationship between leaf chlorosis disorder and the nutritional status and some important physiological characteristics of plane trees. The experiment was arranged in a randomized complete block design with six replications and four treatments including control, manure (M), manure + fertilizer (20-5-10) (MF), and manure + fertilizer + mycorrhizal fungi (MFA) (*Glomus intraradices* + *G. mosseae*). The results showed that although all treatments significantly improved the nutrients content, soluble carbohydrates content, photosynthesis rate and chlorophyll content in the leaves, they mostly reached their peak in the mycorrhizal inoculated plants. Nitrogen (N), phosphorus (P) and zinc (Zn) were increased in the AMF amended trees compared to the control plants. The photosynthesis rate was enhanced by all the mixtures at least by 60% compared to the control. The most Chlorosis (17.5%) to the leaves recorded on the control plants, while leaf damage dropped to less than 2.9% at mycorrhizal treatment leading to the improved nutritional balance in the plane trees. The results proved the effectiveness of including mycorrhizal inoculation to the common fertilization practices to prevent leaf chlorosis in the plane trees.

1. Introduction

Plane tree *(Platanus orientalis* L.) is among the most common ornamental and street trees planted in the urban landscape in Iran and some Mediterranean countries (Anselmi *et al.*, 1994; Khorsandi *et al.*, 2016). They are known for their longevity and wide distribution in the temperate zones.

However, the chlorosis as an important physiological disorder in the plane trees has affected a majority of them in Iran in recent years (Khorsandi *et al.*, 2016). The problem is a common physiological disorder affecting many plants around the globe. It is especially a major problem in the calcareous soils and soils with high pH (Wallace, 1982).

The chemical properties of the soil and the adequate supply of nutrients are major factors affecting natural plant growth and extension (Cekstere and Osvalde, 2013), therefore important factor in nutrient uptake is the availability of the nutrients in the soil. Most trees cultivated in the alkaline and calcareous soils are exposed to the incidence of chlorosis which is reported to be basically due to Fe deficiency (Mortvedt, 1986). Several factors can contribute to leaf chlorosis including nutritional disorders and a disorder in the chlorophyll biosynthesis. Indeed, the lack of some nutrient elements such as nitrogen (N), zinc (Zn) and expecially iron (Fe), lead to the chlorosis in plants (Godde and Dannehl, 1994). Moreoever, following the lack of sufficient chlorophyll, the affected plant will not be able to operate photosynthesis process, resulting in stunted growth (Miller *et al.*, 1984).

Arbuscular mycorrhizal fungi (AMF) are obligate biotrophs that colonize the roots of the most land plants and increase host nutrient acquisition (Desiro *et al.*, 2014) and it is claimed that virtually all trees acquire nutrients through symbiotic mycorrhizal fungi (Brundrett, 2009). Mycorrhizal inoculation is documented as a method to the improve nutrient uptake in many plants (Lehmann *et al.*, 2014; Varga, 2015; Young *et al.,* 2015). AMF are effective symbionts for plants, and their symbiotic relationship can increase plant growth (Vafadar *et al.,* 2014). Moreover, there is a lack of information on symbiosis relationship between the plane tree and AMF fungi.

In the present study, we added AMF to the common fertilization program of the plane trees in the urban landscape to study the following items. Firstly study the effect of mycorrhizal association on the trees response and then, observe how nutrient content and different physiological processes are associated with the leaf chlorosis disorder. To the best of knowledge, this is the first report attempting to discover the correlation between different plane tree physiological processes and leaf chlorosis disorder under AMF inoculation.

2. Materials and Methods

Experimental site and treatments

The experiment was conducted during 2013-2014, on the campus of the Isfahan University of Technology in Isfahan (32°39' N, 51°40' E; 1600 m), Iran. The site is characterized as having an arid climate with cold winters, 122.8 mm average annual rainfall and 23.4°C average annual temperature. Twenty-four uniform 15-year-old plane trees (*P. orientalis* L.) were selected.

The experiment was a randomized complete block design (RCBD) with four treatments. Treatments included control, manure (M), manure + fertilizer (water soluble 20-5-10 N-P-K compound fertilizer with 12.8% sulfur, 1.3% magnesium oxide, NovaTec Solub, Compo, Germany) (MF), and manure + fertilizer + mycorrhizal fungi (MFA). Six replications were prepared for each treatment. The plants were inoculated with two AMF inoculations including *Glomus intraradices* and *G. mosseae* (both of them have been transferred to new genera, so Index Fungorum considers them now as *Rhizophagus intraradices* (N.C. Schenck & G.S. Sm.) and *Funneliformis mosseae* (Nicolson & Gerd.) (Schüßler and Walker, 2010). The AM fungi were provided by the Institute of Soil and Water Research, Tehran, Iran. Inoculum was comprised of a mixture of spores (80 spores g^{-1} for *G. intraradices* and 80 spores g^{-1} for *G. mosseae*). The mixtures of filling materials were placed into 0.5 × 0.5 m holes, depending on the treatment in early spring. This technique provides a nutrients in a zone in and around each hole. With the first wetting, the nutrients are released from the fertilizer into the soil and the manure slowly lower the pH of the soil surrounding the hole. Over a period of time, a zone of soil around each hole is modified to be lower in pH and rich in micronutrients in approximately the correct proportions. Two identical holes were drilled around each tree about one meter away from the tree trunk and filled up with the corresponding mixture. During the process, we avoided drilling into large buttress roots. In M treatment, trees received 5 kg of manure per hole mixed with the soil of the drilled hole. In MF treatment, 100 g of fertilizer per hole was added to the manure. Trees of MFA treatment received the AMF inoculums by adding 250 g of mycorrhizal inoculums into each hole mixed with manure and fertilizer (500 grams of inoculum per each tree in total). The control group did not receive any treatment (two identical holes were drilled). The trees were irrigated once a week. Some chemical and physical properties of the soil and cow manure are presented in Table 1.

Measurements

Various morphological and physiological parameters were measured 5 months after treatment. The

Table 1 - Some chemical and physical properties of the soil and cow manure used in research

Factors	Texture	pH	EC (dS m^{-1})	Organic matter (%)	N (%)	P-available (mg kg^{-1})	K-exchangeable (mg kg^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})
Soil	Clay	7.9	1.53	1.15	0.15	140	235	1400	21
Manure	-	8.02	15.23	20.4	3.07	791	2030	12300	194

mineral contents of the plant leaves were determined in the second year of the experiment. Plant samples were oven-dried at 65°C for 48 h and then were ground to determine their mineral composition. The determination of the total N in the leaf samples was based on the Kjeldahl method (Baker and Thompson, 1992). The extraction of P, K, Fe, and Zn from the plant tissue material was performed by using 2 M hydrochloric acid (HCl) after dry ashing at 550°C for 5.5 h. The concentrations of Fe and Zn were determined by atomic absorption spectrophotometer (670 Shimadzu, Kyoto, Japan) (AOAC, 2006). P concentration was determined by vanado molybdate phosphoric acid method with a spectrophotometer (UV-160A UV-Visible Recording Spectrophotometer, Shimadzu, Tokyo, Japan) (Cottenie, 1980). LCI Portable photosynthesis and transpiration rate analyzer (Li - 6400; LICOR, Lincoln, NE, USA) was used to measure the net photosynthesis rate (A) between 09.30-11.30 h on 10 fully expanded current-season leaves situated at the mid-canopy height. The soluble sugars were measured according to the phenol-sulfuric acid method (Dubois *et al.,* 1956). The extraction of the leaf chlorophyll pigments was carried out using 100% acetone according to Lichtenthaler (1987).

For evaluation of the leaf chlorosis extent, 100 leaves from each tree were selected randomly and both leaf surfaces were scanned by a scanner (Canon i-SENSYS MF4010, Canon Inc., Korea). The leaf chlorosis was determined by digital image processing using MATLAB software. A range of color was defined for the leaf chlorosis in the program and total leaf area was examined pixel by pixel by the software and the percentage of pixels which was defined as chlorotic areas were calculated by the software (Rathod *et al.*, 2013).

Data analysis

Data were assessed for normality and log-transformed used to make data conform to normality when necessary prior to analysis. Non-homogeneity data were observed in leaf chlorosis, being the data transformed with the formula

$$\arcsin \sqrt{(\text{leaf chlorosis}/100)}$$

to obtain homogeneity. The experimental data were statistically analyzed by the analysis of variance (ANOVA). The significance of the differences between treatments was estimated using the least significant difference (LSD) test at P≤0.05, and graphs were drawn using Excel 2010. Statistical correlation was calculated by Pearson's correlation coefficient (r). This test was used to measure the strength of a linear association between the leaf chlorosis and other variables including nutrients content, photosynthesis rate and chlorophyll and soluble sugar contents. The value r = 1 means a perfect positive correlation and the value r = -1 means a perfect negative correlation. The experimental data were statistically analyzed with Statistical Analysis Systems (SAS) software, version 9.1 and Statistics, version 8.0.

3. Results

AMF inoculation increased all nutrients content including P, N, Fe and Zn in the leaves of the treated trees (Table 2). N, P and Zn reached their peak value only when the fertilizer mix amended by mycorrhizal inoculums. P and Zn increased by 424% and 425%

Table 2 - Influence of arbuscular mycorrhizal (AM) fungi and other treatments on nutrient uptake of plane tree (*Platanus orientalis* L.)

Treatment	Nutrient			
	N (g kg^{-1})	P (g kg^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})
Control	17.47 d	1.85 c	54.93 b	4.82 d
Manure	19.14 c	5.25 b	148.99 a	15.09 c
Manure + Fertilizer	19.65 b	7.31 b	146.71 a	17.32 b
Manure + Fertilizer + AMF	20.39 a	9.71 a	170.14 a	25.31 a

Means in the same column followed by the same letters are not statistically different at P≤0.05 by the Least Significant Difference test (LSD).

respectively, compared to the control plants. All treatments (regardless of the composition of the mixture) successfully enhanced Fe and N contents in the leaves compared to the control plants (Table 2).

All treatments significantly improved the soluble carbohydrates content in the leaves; however, it reached the peak in the mycorrhizal inoculated plants. The soluble carbohydrates content increased by 35.44% and 13.82% compared to the control of non-fertilized treatment and non-inoculated plants, respectively (Fig. 1).

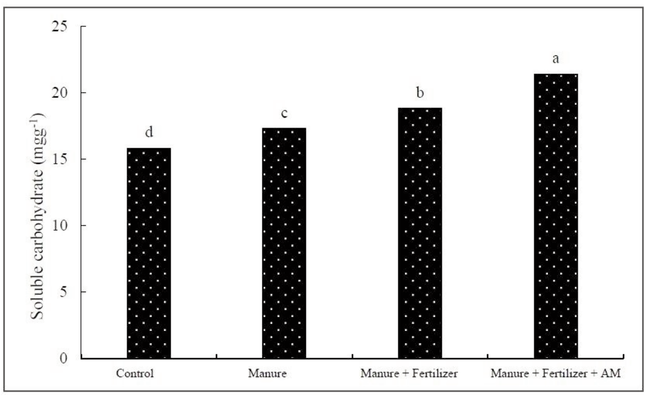

Fig. 1 - Influence of arbuscular mycorrhizal (AM) fungi and other treatments on soluble carbohydrate content in plane tree (*Platanus orientalis* L.). Means are separated by LSD test at P≤0.05.

All treatments increased the photosynthesis rate at least by 60% compared to the control, although no significant difference was observed between the treatments (Fig. 2). The same trend was observed in the case of chlorophyll content (Fig. 3), where it increased at least by 32% compared to the control.

Leaf chlorosis was influenced dramatically by the treatments (Fig. 4). The most leaf chlorosis was recorded in control non-fertilized treatments. Leaf chlorosis reduced to less than 2.9% on inoculated trees by AMF, while 17.5% of leaves tissue were affected by chlorosis in the control plants. The AMF inoculated plants showed an increase of 13.44% compared to the plants that received manure + fertilizer.

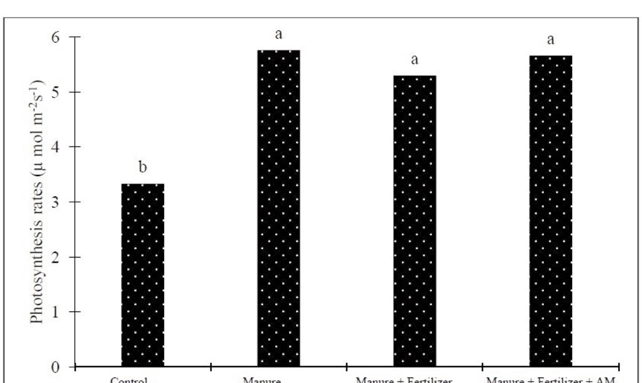

Fig. 2 - Influence of arbuscular mycorrhizal (AM) fungi and other treatments on photosynthesis rates in plane tree (*Platanus orientalis* L.). Means are separated by LSD test at P≤0.05.

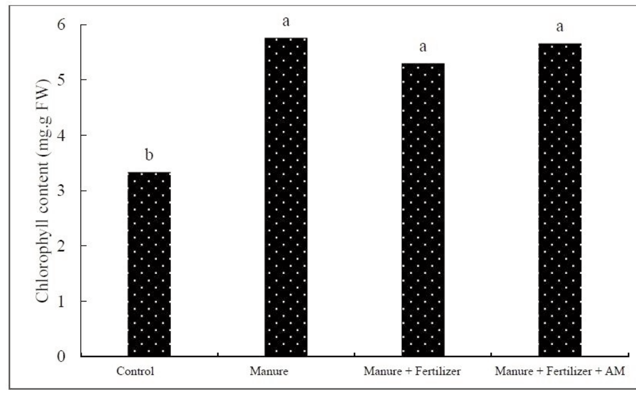

Fig. 3 - Influence of arbuscular mycorrhizal (AM) fungi and other treatments on chlorophyll content in plane tree (*Platanus orientalis* L.). Means are separated by LSD test at P≤0.05.

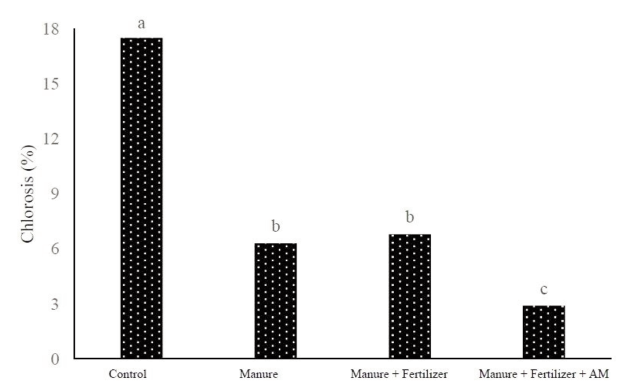

Fig. 4 - Influence of arbuscular mycorrhizal (AM) fungi and other treatments on chlorosis damage in plane tree (*Platanus orientalis* L.). Means are separated by LSD test at P≤0.05.

A strong relationship was found between leaf chlorosis and Fe, N and Zn contents (Fig. 5).

A significant and linear relationship was also found between chlorosis and the chlorophyll content and net photosynthesis in the plane tree leaves (Fig. 6). Leaf chlorosis in the plane trees resulted in a dramatic and linear decline in the soluble carbohydrate in the leaves (Fig. 6).

4. Discussion and Conclusions

Two explanations can be presented for increasing Fe content under any treatments (except for the con-

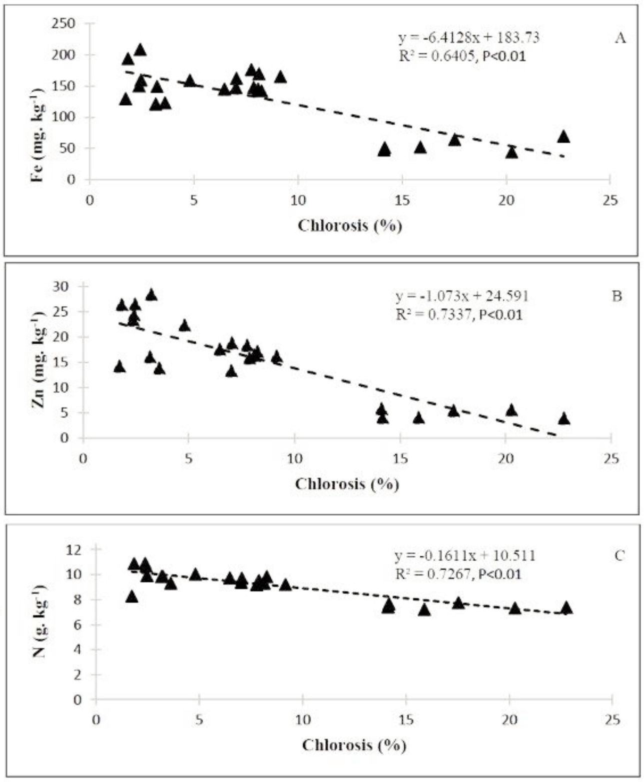

Fig. 5 - Relationship between chlorosis with Fe (A), Zn (B) and N concentration (C) in plane tree (*Platanus orientalis* L.).

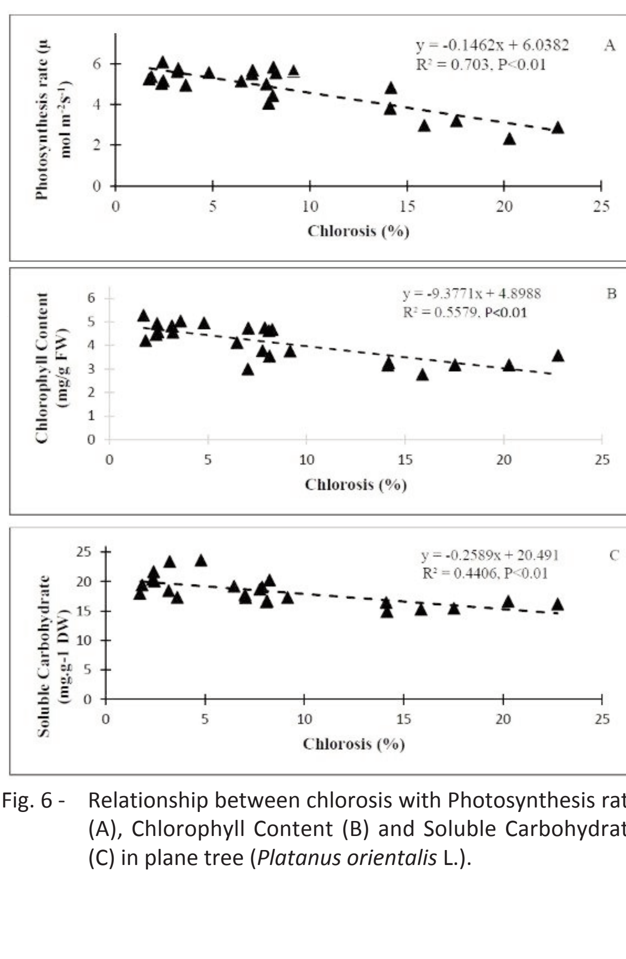

Fig. 6 - Relationship between chlorosis with Photosynthesis rate (A), Chlorophyll Content (B) and Soluble Carbohydrate (C) in plane tree (*Platanus orientalis* L.).

trol) in this experiment (Fig. 1). Firstly, as the soil of the site was a calcareous and compacted soil, any treatment increasing the air flow in the soil could improve Fe content in the plant (Lucena, 2003). Secondly, using the manure itself can increase Fe content in the plants (Mortvedt, 1986). It is documented that the manure provides micronutrients including Fe and improves the structure of the soil (Lucena, 2003). Our results at least partly confirm the findings that combining Fe fertilizers with the organic matter is more favorable in terms of Fe uptake than Fe sources applied alone (Mortvedt, 1986). It has been reported that the application of $FeSO_4$ (4-8 kg tree^{-1}) mixed with manure, cotton seed cake, or other organic substances in 8 to 10 holes in the soil around the crowns of apple trees (*Malus sylvestris* Mill.) resulted in marked correction of Fe chlorosis (Zheng-Qing and Cang-Zhen, 1982). It is also well established that as a result of the decomposition of organic matters in the soil, compounds such as humic acid (HA) and fulvic acid (FA) are produced in the soil (Nardi *et al.,* 2002). These acids are well known as naturally-occurring chelating agents (Mortvedt, 1986; Nardi *et al.,* 2002). There are many reports showing enhanced micronutrients uptake by the plants receiving HA, FA (Nikbakht *et al.,* 2008; 2014) or organic matter (Atiyeh *et al.,* 2002). It is shown that inoculation turfgrass (*Lolium prenne* L.) with AMF receiving HA not only improved plant growth but also showed more elevated nutrients content in the leaves than in non-inoculated (control) plants or plants receiving only HA (Nikbakht *et al.,* 2014).

Researchers believed that the role of AMF in NO^{-3} transport to the root surface is significant (Subramanian and Charest, 1999; Javaid, 2009). They especially insist that the role of AMF is of value and importance in nitrate uptake in Mediterranean and (semi-) desert ecosystems which are characterized by calcareous soils.

N, P and Zn uptake reached their peak value when the fertilizer mixture amended by AMF (Table 2). These results confirm the well-documented effect of AMF inoculation on nutrients uptake (Brundrett, 2009; Varga, 2015; Young *et al.,* 2015). A strong relationship between leaf chlorosis and Fe, N and Zn contents implies that the chlorosis is not only because of Fe deficiency in the plant, but also other nutrients including N and Zn play they own role (Fig. 5). It indicates that leaf chlorosis in the plane trees was not simply due to Fe deficiency. It is well documented that Fe is an essential element for many vital processes in a plant including photosynthesis, respiration, N fixation, chlorophyll and hormone synthesis; Fe is also a constituent of heme proteins (cytochromes, catalase, and peroxidase) (Briat and Lobreaux, 1997). As a result, affected plants by Fe deficiency suffer severe metabolic and structural disorders (Javaid, 2009). There are also some reports indicating that the major cause of Fe deficiency is the very low solubility of Fe oxides in the soil (Mortvedt, 1986). It shows the importance of the fact that the role and priority of each element in the plane tree chlorosis remain to be investigated further.

Fe deficiency depresses the synthesis of chlorophyll, which results in the decrease of photosynthetic products, which in turn affect plant growth (Wang *et al.,* 2008). As a result of carbohydrates synthesis reduction in chlorotic leaves, which slows the movement of K^+ from the leaf to the phloem vessels, a decline in the production of biomass is reported (Maldonado-Torres *et al.,* 2006). These explain why we found a relationship between chlorosis and chlorophyll content, net photosynthesis and soluble carbohydrate content of the leaves (Fig. 6). Moreover, increased photosynthetic capacity by AMF is in agreement with the results of the previous study by Birhane *et al*. (2012). It seems this process has improved nutrition, leading to higher photosynthetic rates (Vafadar *et al.*, 2014). To the best of our knowledge, no similar information has yet been provided for interaction effect of fertilizers and AMF inoculation on plane trees and its relationship with the leaf chlorosis disorder.

This study demonstrated that AMF inoculation added to the common fertilizer program served successfully as a biological and environmental-friendly method to overcome chlorosis disorder of the plane trees. In addition, the findings of this study suggest that in calcareous soils drill hole nutrition should be considered as a standard method to prevent nutritional disorders in the urban landscape. The results also revealed that Fe is not the only nutrient participating in the leaf chlorosis of plane trees. It suggests further investigations to study the weight and importance of each nutritional element in chlorosis disorder of the plane trees.

In this study we mainly focused on the effect of improved media around the plane trees, rather than specific effect of AMF. Indeed, this study was one of the primary trail in a series of experiments we want done later. In the later, we specifically will studied the AMF effect on the plane trees. Our experiment creates a paradigm for future studies of relationship between plane trees and microorganisms.

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