Loranthus europaeus Jacq. Infection Alters Leaves Morphology and Physiology of Persian oak (*Quercus brantii* Lindl.)

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Leafy mistletoes are semi-parasitic plants that have negative impacts on their hosts, but there is inadequate knowledge about the impacts of *Loranthus europaeus* Jacq. on Persian oak trees (*Quercus brantii* Lindl.). This study examined the impacts of *L. europaeus* Jacq. on morphological and physiological characteristics of Persian oak tree leaves in western Iran. A forested area infested with *L. europaeus* Jacq. was selected to study. Five infected and five healthy Persian oak trees were selected. Leaf samples were collected from the southern side of infected and healthy tree crowns to quantify and compare the leaf area, leaf weight, and concentrations of leaf minerals. The area and weight of leaves and the amount of leaf K, P, and Ca in healthy trees were statistically more than in infected trees. Leaf N, Mg, Mn, Zn, and Fe contents were not significantly different between healthy and infected trees. The higher amount of leaf area and weight in healthy trees suggested that they had a better growing status, which increased their leaf size and weight. The higher concentrations of K and P in healthy trees indicated their greater growth and resistance to stresses such as mistletoe. Thus, mistletoe causes physiological disorders and decreased growth in oak trees.

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Keywords: Infected trees; Loranthus europaeus; Mistletoe; Leaf nutrients; Leaf size; Persian oak

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INTRODUCTION

Leafy mistletoes are semi-parasites plants that survive on trees and shrubs. These plants rely on the other plants known as host for establishment, growth, and survival. This reliance includes removal of water and minerals from the host's wooden vessels (Zuber 2004; Yan *et al.* 2016).

There are three species of mistletoe in Iran, including *Loranthus europaeus*, *Loranthus grewinkii*, and *Viscum album*; the primary two species belong to the family of Loranthaceae (Mozaffarian 2008). The *Loranthus* Jacq. genus has approximately 600 species, with 450 to 500 species that are distributed in the tropical region (Zebec and Idzojtic 2006). Most of its species are present within the sort of semi-parasites on angiosperms, with a few on gymnosperms (Zebec and Idzojtic 2006). The sole European species of this genus is *Loranthus europaeus*. It is a deciduous species, and its fruits ripen in late autumn. The seeds are propagated by birds. This species often lives on oak, sweet chestnut, common beech, birch, maple, hornbeam, walnut, and olive trees (Zebec and

Idzojtic 2006). In Iran, it is distributed across the Zagros forests in west of Iran and lives only on Persian oak trees (Mozaffarian 2008). Because the highest distribution and abundance of tree species in the Zagros forests belongs to the Persian oak, this semi-parasitic plant can find a wide range of distribution within the Zagros forests. The activity of *L. europaeus* plays a key role in the branches or crowns dieback of oak trees (Ghaedi and Nikbakht 1994; Hosseini 2009). As a result, the activity of this plant in a large area of Zagros forests in western Iran can cause great damage to oak trees and these forests.

Because *L. europaeus* is a semi-parasitic plant, it absorbs many minerals and water from its host, and thus can play a great role in the physiological disorders and imbalance of elements within the infected tree or its infected branches (Watson 2001; Briggs 2003; Hosseini *et al.* 2008). Studies have shown that these plants often have more nutrient accumulation than their host tissues (Karunaichamy *et al.* 1999; Grieve 2005). Accordingly, the hypothesis of this study is that the concentrations of mineral elements in infected trees are less than those of healthy trees. Hence, consumption of nutrients by mistletoe can reduce leaf growth in infected trees. It is expected that leaf area and weight in infected branches will decrease compared to uninfected branches or trees. Based on the existing hypotheses, this study investigated the impacts of *L. europaeus* on the eight mineral elements including N, P, K, Ca, Mg, Fe, Zn and Mn within the leaves as well as the leaf area and weight of infected Persian oak trees.

Physiological studies on mistletoes and their relationships with hosts have been conducted (Hosseini *et al*. 2008; Zweifel *et al*. 2012), but no study has been done on *L. europaeus* within the oak forests of western Iran. Moreover, some studies have been done at the branch level and have compared the infected branches with uninfected ones in the same tree (Hosseini *et al*. 2008; Lo Gullo *et al*. 2012; Logan *et al*. 2013). Few studies have been carried out at tree level and compared infected trees by mistletoe with uninfected trees (Miller *et al*. 2003; Sanguesa-Barreda *et al*. 2012). To understand the status of minerals in infected oak trees by *L. europaeus*, the present study investigated changes at the tree level in an oak forest in western Iran. The infected Persian oak trees with mistletoe were compared to oak trees without mistletoe. For this, tree leaf morphology as well as leaf physiology in terms of N, P, K, Ca, Mg, Mn, Zn, and Fe concentrations were studied.

Considering the extent of *L. europaeus* contamination across the Zagros forests, the results of this study provide a better understanding of the effects of this mistletoe on Persian oak trees and could be useful in the management of mistletoe. The study goals were: 1) to determine the differences between infected and uninfected oak trees in terms of leaf area and leaf weight and 2) to identify the most changeable leaf minerals of host tree affected by mistletoe.

EXPERIMENTAL

Study Area

This study was completed in the Hyanan forested area on the southern slope of Manesht Mountain, located in the north of Ilam province and in the west of Iran (3727205 N and 0635765 E, UTM) (Fig. 1). The general aspect of the study area is south, and its general slope is gentle. It is located at an altitude of 1850 to 1950 m above sea level. This area was severely infested with *Loranthus europaeus* Jacq., growing only on Persian oak trees (*Quercus brantii* Lindl.). Oak trees in the study area existed in two forms: standard and coppice; standard trees were selected for the study. The average of annual temperature at the study area was 16.5 °C, and the average of annual precipitation was 590 mm. The mean height of trees was nearly 7 m, which often grew individually in the stand. The stand density was approximately 155 trees/ha. The mean area of tree crowns was nearly 25 m^2, and the mean dbh of trees was 26.4 cm. There were an average of three mistletoes on each oak tree, and the maximum infection was 11 mistletoes on one tree.

Fig. 1. Location of the study area - Manesht mountain

Data Collection

Leaves of Persian oak trees were collected one time in the year, in August. The samples were collected randomly from five infected Persian oak trees and five healthy oak trees. All trees were situated in the central part of the forested area. The selected trees had nearly the same status in terms of DBH and whole height. Leaf samples were taken from the southern side of the tree crown at a middle height of the crown. Oak leaf samples were divided into two types. The first type was done to investigate the effect of mistletoe on the morphology of oak trees leaves (leaf area, leaf weight), and 12 mature leaves were taken from each tree. The collected leaves were weighed immediately, and their area was measured with a leaf area meter device. The second type of leaf sampling was done to investigate the effect of mistletoe on the physiology of oak trees leaves (leaf mineral elements; N, P, K, Ca, Mg, Mn, Zn, Fe), which 100 mature leaves (including petiole) were taken from each tree to have enough material for nutrient analyses. Leave samples were placed into nylon bags and transferred to the laboratory with minimal delay to prevent further changes in the quality of samples. Both of leave sample types were simultaneously taken from each tree.

Nutrient Analyses

The leaf samples of the second type were dried in an oven at 65 °C for 48 h and then ground to a fine powder using a Wiley mill (Emami 1996; Hosseini *et al*. 2008). Digestion of ground samples was performed (wet digestion technique) in a balloon with compounds of H_2SO_4, salicylic acid, H_2O_2, and selenium powder, and the extract of samples was obtained (Emami 1996). The digest was filtered with Whatman paper no. 42 (Hosseini *et al*. 2008). In the final stage, the concentration of nitrogen was measured by the Kjeldal method, phosphorus by Olsen method using a spectrophotometer (Jenway model 6505, Stone, UK), potassium by flame photometry with a flame emission spectrometer (Jenway model Clinical PFP7), and calcium, magnesium, iron, zinc, and manganese by atomic absorption spectrophotometer (Shimadzu 6550, Kyoto, Japan) (Emami 1996; Hosseini *et al*. 2008).

Statistical Analysis

The Kolmogorov-Smirnov test and Leven test were used to check the normality and homogeneity of variance of the data, respectively. As the data were not repeated measures of the same leaves, a T-test was used for statistical comparison between infected oak trees and uninfected oak trees in terms of leaf area, leaf weight and leaf nutrients concentrations. All statistical analysis was done using SPSS software (IBM, Armonk, NY, USA).

RESULTS AND DISCUSSION

Analysis of the area, weight, and nutrients concentrations of Persian oak infected tree leaves showed that there were significant differences between infected oak trees and healthy ones, which indicated the negative effects of *L. europaeus* on Persian oak trees, although some of nutrients were not statistically different (Tables 1 and 2). The findings were consistent with the negative effects of mistletoe on the host, as has been shown in many prior studies (Ghaedi and Nikbakht 1994; Hosseini *et al.* 2008; Rigling *et al.* 2010; Logan *et al.* 2013; Marias *et al.* 2014). Examination of leaf area and leaf weight showed their reduction in infected Persian oak trees in comparison to healthy oak trees (Table 1, Fig.S1).

Table 1. Mean Values (n = 5 trees) of Leaf Area and Leaf Weight in Infected Persian Oak Trees and Uninfected Trees

Leaf traits	Mean±SD			
	Infected tree	Uninfected tree	t	Sig.
Leaf area (cm^2)	28.45±4.36	39.90±9.81	2.38	0.044
Leaf weight (g)	6.39±1.29	9.62±2.44	2.61	0.031

Independent-samples T-tests were used to examine statistical differences between infected oak trees and uninfected trees for each Leaf trait. Differences were considered significant at $p < 0.05$.

Table 2. Mean Values (n = 5 trees) of Leaf Nutrients in Infected Persian Oak Trees and Uninfected Trees

Leaf nutrients	Mean±SD			
	Infected tree	Uninfected tree	t	Sig.
N (%d.w.)	1.667±0.138	1.739±0.1	0.938	0.376
P (%d.w.)	0.212±0.044	0.37±0.074	4.116	0.003
K (%d.w.)	0.552±0.027	0.782±0.158	3.211	0.030
Ca (%d.w.)	0.643±0.158	0.939±0.240	2.30	0.050
Mg (%d.w.)	0.189±0.033	0.185±0.059	0.120	0.907
Mn (ppm)	0.0058±0.0012	0.0085±0.0048	1.22	0.258
Zn (ppm)	0.0027±0.0016	0.0035±0.0013	0.787	0.454
Fe (ppm)	0.0661±0.0126	0.0587±0.0088	1.073	0.314

Independent-samples T-test were used to examine statistical differences between infected Persian oak trees and uninfected trees for each mineral element. Differences were considered significant at $p < 0.05$.

Changes in leaf area and weight of infected oak trees are a reflection of the physiological changes in the oak tree in response to the mistletoe infection. Mistletoes uptake the water and minerals of infected trees through the xylem vessels of their infected branches (Zuber 2004; Yan *et al*. 2016), which cause stress in the infected trees, especially in their infected branches, and reduce the area and weight of their leaves (Zuber 2004; Yan *et al*. 2016). Moreover the cell structure of leaf tissue is defective due to the lack of minerals, consumed by mistletoe, and the leaf cells are not well nourished and strengthened in terms of mineral supply, and as a result, the leaves of infected trees lose their area and weight (Hosseini *et al*. 2008). After the host branches are infected with mistletoe, changes occur in their leaf area due to the reduction in their growth efficiency (Karunaichamy *et al.* 1999). These reductions are often due to the competition between mistletoe and host tree branches for water and mineral uptake (Karunaichamy *et al.* 1999). The reduction of leaf size and weight has been reported (Hosseini *et al*. 2008; Rigling *et al*. 2010; Gebauer *et al*. 2012; Sanguesa-Barreda *et al*. 2012; Logan *et al*. 2013; Yan *et al*. 2016; Bilgili *et al*. 2018). For instance, Rigling *et al*. (2010) found that due to decreasing in water availability, the needle length of infected branches by mistletoe decreased compared with uninfected branches.

The concentration of potassium, phosphorus, and calcium in the leaves of infected oak trees was significantly lower than that of uninfected trees, but the concentrations of nitrogen, magnesium, manganese, zinc, and iron did not show significant difference between Persian oak infected trees and uninfected trees (Table 2, Fig. S2). In addition to its numerous roles in the physiological activities of the plant, potassium plays an important role in the plant's defense against invasive and stressful factors, so that its concentration is increased to enhance the resistance of affected plant and protect it (McWilliams 2003; Hosseini *et al.* 2008). In these conditions, the host tree creates a defense mechanism of increasing potassium concentration within its cells and vessels, which resists the invading agent to by maintaining the durability of the attacked branches (McWilliams 2003). In this study, the concentration of potassium in infected Persian oak trees was lower than that of healthy oak trees, which is due to the greater uptake of potassium by mistletoe from host tree.

The rate of water transpiration in mistletoe is higher than that of the host plant (Mathiasen *et al.* 2008), which reduces the efficiency of water use by the host (Sanguesa-Barreda *et al.* 2012; Bilgili *et al.* 2018). The high rate of transpiration is a mechanism for mistletoe to take up sufficient nutrients with water from the xylem of the host (Lamont 1983). Mistletoe removes nitrogen from the host to produce its own biomass (Zuber 2004). Nitrogen is potentially the most limiting nutrient for the growth and transpiration of mistletoe (Gebauer *et al.* 2012). Therefore, the lack of significant difference in nitrogen content between mistletoe-infected trees and healthy trees is probably due to the efforts of infected trees to prevent mistletoe from absorbing more nitrogen in order to hinder its growth and development. Of course, the similar nitrogen concentrations for mistletoe (*L. europaeus*) and its host tree (*Quercus pubescens*) may indicate that both species have the same assimilation rate (Gebauer *et al.* 2012). However, Lamont (1983) found that the nitrogen content of *Loranthus* leaves is more than that of host leaves. This is probably due to the role of season, because in the study of Gebauer *et al*. (2012) the nitrogen content of *Loranthus* leaves was more in the spring and autumn seasons. The findings of the current study were related to the summer season.

Of course, the behavior of nutrients plays a role in their variability. Potassium, phosphorus, and calcium had a descending trend, and iron had an ascending pattern in infected trees. In the study of Hosseini *et al*. (2008), also, the pattern of element changes in the *Carpinus betulus* and *Parrotia persica* trees infected with mistletoe was different depending on the type of element. Therefore, the activity of *L. europaeus* on the oak trees causes disturbances in the values of its mineral elements due to the selective uptake of nutrients (Lamont 1983; Karunaichamy *et al.* 1999; Grieve 2005; Hosseini *et al.* 2008). This means that mistletoe does not absorb the mineral elements of the host tree equally, but instead consumes them in different amounts (Karunaichamy *et al.* 1999; Grieve 2005; Hosseini *et al.* 2008). As a result, the amounts of these elements do not exist uniformly in the infected trees, and there is an imbalance between the elements. While not showing significant differences between groups, the amounts of nitrogen, zinc, and manganese were slightly lower in infected trees than in healthy trees. Another reason for element fluctuations can be due to the performance of the mineral elements themselves (Hosseini *et al.* 2008). In this way, the elements, through the interactions they have with each other, can increase or decrease the concentration of each other and as a result have different amounts together (Hosseini *et al.* 2008). For example, changes in the amounts of zinc had a decreasing status in infected oak trees, whereas the amount of iron in infected trees was increased.

CONCLUSIONS

1. Given the fact that mistletoe relies on its host for water and minerals, *L. europaeus* has a negative effect on the growth of host Persian oak trees.
2. There were significant changes of area, weight, and some nutrients (P, K, Ca) in infected Persian oak by *L. europaeus*, except for the leaf N, Mg, Mn, and Zn contents.
3. In every host tree there are fluctuations of the minerals due to the factors discussed in the article, but the pattern of element's variability between infected and healthy trees is different depending on the host tree species as well as the site conditions of each tree species and its way of life. Therefore, conducting such studies for one species of mistletoe or for one species of host tree cannot achieve all the necessary findings in this field. Thus, analogous studies should be done on all host species and on all mistletoe species.
4. Given the performance of *L. europaeus* (competition with host trees for mineral uptake) in creating physiological imbalances of nutrients within oak trees, it is suggested that the control management of mistletoe in oak forests should be done carefully. However, considering the benefits of this plant in terms of ecology, medicine and *etc.*, its control measures should not lead to its complete elimination.

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Competing Interests

The authors have declared that no competing interests exist.

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APPENDIX

Supplementary Materials

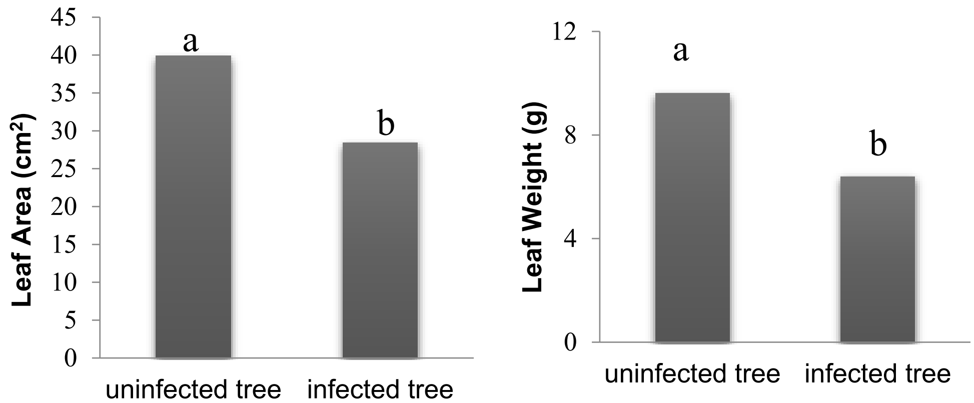
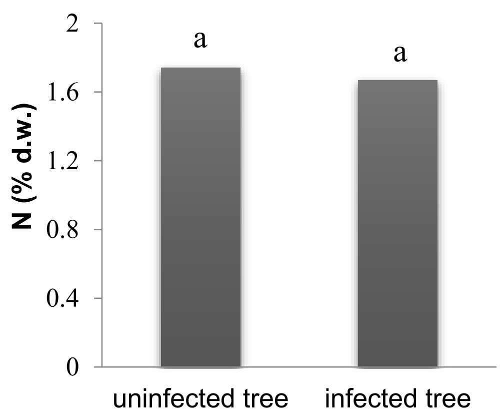
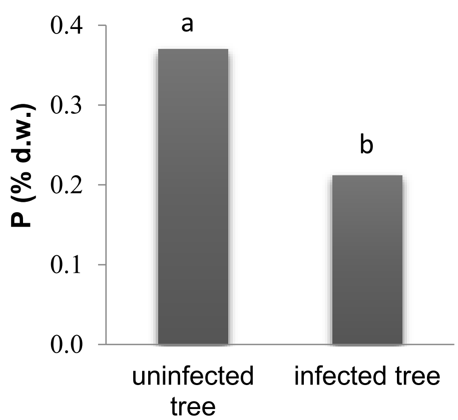


Fig. S1. Mean values of area and weight in the leaves of uninfected and infected Persian oak trees





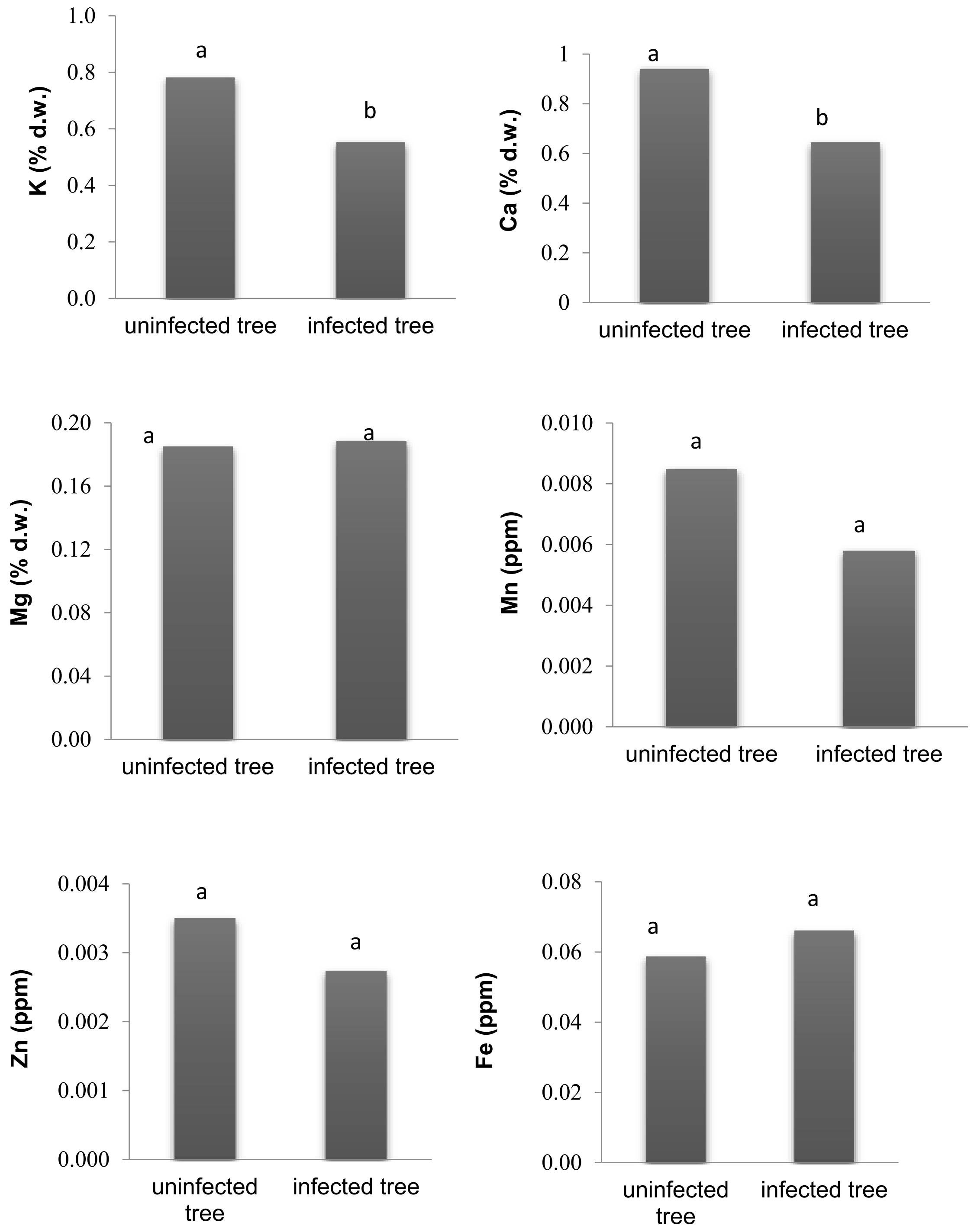


Fig. S2. Mean values of mineral elements (N, P, K, Ca, Mg, Mn, Zn, Fe) in the leaves of uninfected and infected Persian oak trees