



Impacts of pine mistletoe on the physiological traits and plant nutrients of Anatolian black pine

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Abstract Mistletoes and other parasitic plants pose serious problems for forests because of the impacts on their host trees. This emphasizes the importance of understanding these relationships in the light of changing environmental conditions and forest management. This study investigates the effects of pine mistletoe (*Viscum album* subsp. *austriacum* Wiesb.) on the physiological traits and plant nutrients of Anatolian black pine (*Pinus nigra* Arnold subsp. *pallasiana* (Lamb.) Holmboe). Fifteen uninfected (control) [thin class control (TC) and medium class control (MC)], and fifteen moderately infected [thin class infected (TI) and medium class infected (MI)] Anatolian black pine trees were sampled from both thin and medium diameter classes. The study revealed that mistletoe-infected trees exhibited significant reductions in chlorophyll content and mineral nutrients, alongside increases in proline, malondialdehyde

(MDA), and hydrogen peroxide (H_2O_2), particularly in the medium-diameter class. These findings suggest that mistletoe infection caused a notable decline in photosynthetic efficiency and nutrient absorption, affecting plant health. The principal component analysis showed a clear distinction between infected and uninfected trees, further confirming the detrimental impact of pine mistletoe infection on Anatolian black pine.

Keywords Pine mistletoe · Anatolian black pine · Physiology · Plant nutrient

Introduction

Forests face numerous challenges, both from natural and anthropogenic sources. Among these challenges, the impact of parasitic plants such as mistletoes stands out due to their complex interactions with host trees and the broader forest environment (Muche et al., 2022; Wahyuningtias et al., 2021). Mistletoes, classified under Santalales, rely on haustoria to extract water and nutrients from their hosts while simultaneously contributing to photosynthesis (Wahyuningtias et al., 2021).

In Türkiye, *Viscum album* manifests in three subspecies: *V. album* ssp. *album* L., *V. album* L. ssp. *abietis* (Wiesb.) Abromeit, and *V. album* L. ssp. *austriacum* (Wiesb.) Vollman, each associated with distinct host trees ranging from conifers to broadleaved

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species (Üstüner, 2018). The presence of these mistletoes can significantly affect the vitality of their hosts, leading to reduced growth rates, decreased wood production, and increased susceptibility to additional stressors such as fungal infections and insect pests (Bilgili et al., 2018; Szmidla et al., 2019; Teshome et al., 2020; Walas et al., 2022). Furthermore, they can alter the growth of leaves and the wood structure in its hosts (Ozturk et al., 2019).

As climate change and other abiotic factors intensify, forests are increasingly vulnerable to stressors that increase the impacts of biotic agents (Ertuğrul & Komut, 2020; Gea-Izquierdo et al., 2019; Sakici et al., 2023). The interplay between mistletoe and their hosts highlights the broader implications for forest management and ecosystem stability, underscoring the need for a deeper understanding of these interactions in the face of global environmental changes (Sakici et al., 2023).

In Türkiye, coniferous trees are the main hosts of mistletoe (Sakici et al., 2023). In 2020, coniferous forests made up 48% of Türkiye's total forest area (OGM, 2021). Vulnerability to mistletoe damage among coniferous forest trees is particularly pronounced in the Anatolian black pine, a key species in Türkiye's coniferous forests, which covers approximately 18% of the country's forest area (Ayan et al., 2021; OGM, 2021; Sivacıoğlu & Ayan, 2010). One of the main hemi-parasitic plant species in Anatolian black pine forests is pine mistletoe.

Earlier studies on the effects of pine mistletoe on forest trees in Türkiye were mostly conducted on Scots pine. They explored how its growth (Sönmez, 2014), radial growth (Bilgili et al., 2018), wood anatomical traits (Göl et al., 2018), branch wood and needle anatomy (Ozturk et al., 2019), the relationship between leaf area and sapwood area (Bilgili et al., 2020) and basal area increment (Sakici et al., 2023) changed under pine mistletoe infection. However, only one study was carried out specifically on the effects of pine mistletoe on the basal area increment of Anatolian black pine. Thus, the purposes of the present study were (i) to determine whether there were significant differences in physiological traits and plant nutrients between infected and uninfected Anatolian black pine trees of both diameter classes, (ii) to examine whether pine mistletoe effects physiological traits and plant nutrients of Anatolian black pine, and (iii) to explore correlations between infected

and uninfected trees for each of physiological traits and plant nutrients independently in both diameter classes.

Materials and methods

Study area

The study area is located in Eldivan Mountain in Çankırı-Türkiye (40°14'27''–40°34'34''N; 33°22'23''–34°10'03''E) (Anonymous, 2019). The seven-month vegetative period runs from April (10 °C) to October (11 °C). The lowest and highest temperatures are –17.5 °C in February and 37.0 °C in August, respectively. In this district, 510.9 mm of precipitation falls on average per year. According to Akkaya and Çakır (2022), there is an average yearly relative humidity of 65%. The Tertiary Oligo-Miocene gypsum series starts with a thick and red colored base conglomerate, followed by light-colored clays and marls with gypsum beds. In several locations, the upper layers of the gypsum series include the Miocene (Anonymous, 1998; Ketin, 1962). The district is in the Iran-Turanian flora region, one of the three big flora areas in Türkiye. The woody species in the area are *P. nigra* subsp. *pallasiana*, *Quercus cerris* L., *Q. robur* L., *Q. pubescens* Willd., *Corylus avellana* L. var *avellana*, *Populus tremula* L., *Acer campestre* L., and *Salix alba* L. (Davis, 1971). The investigation was carried out in an Anatolian black pine afforestation area that was plagued with mistletoe. The study area (Fig. 1) has a slope of 15%, a north-eastern aspect, and an average elevation of 1150 m above sea level. The closure in the study area was estimated to be between 0.5 and 0.6.

Sampling strategy

The sampled trees within the study area were classified into two categories based on their diameter measurements: the thin (10.9–19.9 cm) and the medium diameter classes (20–35.9 cm). The average age of thin- and medium-diameter trees is 46–58 years, the average height is 9–12 m and the average diameter is 0.18–0.24 m.

The six-class dwarf mistletoe rating system (DMRS) was used to assess the mistletoe density and quantity (Hawksworth, 1977). Accordingly, the

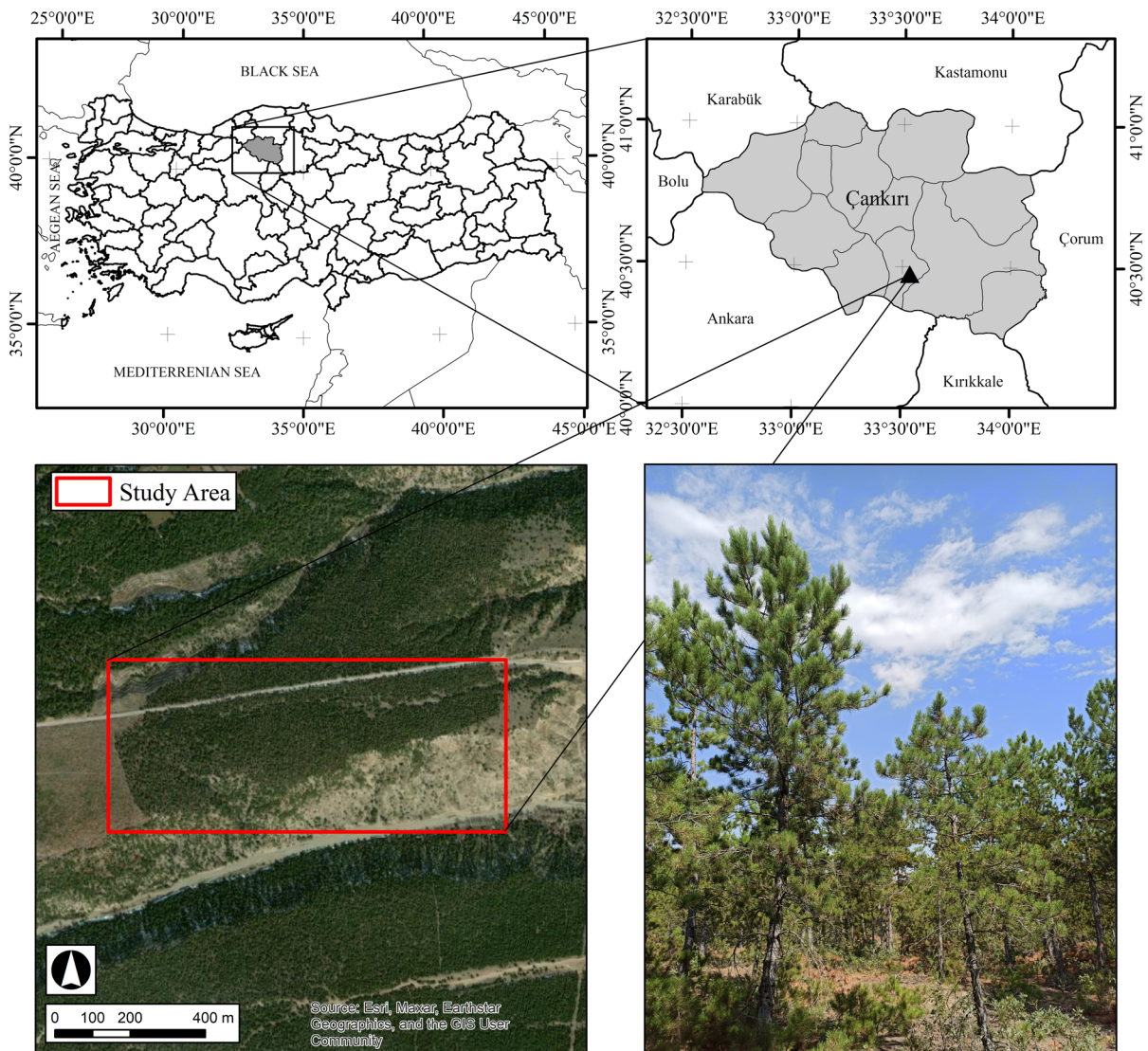


Fig. 1 Location of the study area

crown of the tree is divided into three equal parts. For each of the three parts, values of 1 and 2 are given for mistletoe presence on the branches of 50% or less, and more than 50%, respectively. The overall value is calculated by adding the values specified for the three parts of the tree crown. The classification of infection is based on its degree: 0 denotes no infection, 0.1–2.0 mild infection, 2.1–4.0 moderate infection, and 4.1–6.0 severe infection.

In the thin-diameter class, 28% of the trees had no infection, 23% had a mild infection, and 49% had moderate infection based on the measurements

in the study area. In the medium-diameter class, 15% of the trees had no infection, 13% had a mild infection, 21% had a moderate infection, and 51% had a severe infection. 15 uninfected (control) and 15 moderately infected Anatolian black pine trees were sampled from both thin and medium diameter classes. A total of 60 trees were sampled. Branches from the middle of the tree canopies with full southern exposure were selected for needle sampling. One-year-old, fully extended needles were randomly selected from these branches.

In August 2023, about 5 g of fresh leaf samples were taken from each tree for physiological analyses. After sampling, they were covered with aluminum foil and placed on dry ice. Until the analysis, fresh leaf samples were stored at -40°C . Additionally, in late September 2023, about 250 g of leaf samples from each tree were collected for the analyses of plant nutrients. Following collection, these leaf samples were dried in an oven at 70°C for five days.

Physiological analyses

The 0.25 g of fresh leaf samples were divided into tiny pieces and then homogenized in 10 mL of 90% v/v acetone. The extract was filtered, and a spectrophotometer (UV/VIS-1201, Shimadzu Corp., Kyoto, Japan) was used to measure the absorbance at 645, 663, and 470 nm. Based on Lichtenthaler (1987), the concentrations of carotenoid (car) and chlorophyll (chl) were determined.

The 0.25 g of fresh leaves were homogenized in 10 mL of 3% sulphosalicylic acid and filtered through Whatman No. 2 filter paper to measure the proline concentration (Bates et al., 1973; Yücedağ et al., 2021).

Lipid peroxidation in fresh leaves was measured at 532 and 600 nm by spectrophotometer (Heath & Packer, 1968) and the values were calculated in terms of MDA using the following equation.

$$\text{MDA (nmol mL}^{-1}\text{)} = [(A_{532} - A_{600})/155000] \times 106$$

Using a spectrophotometer, fresh leaves were examined for the amount of H_2O_2 at 415 nm (Mukherjee & Choudhuri, 1983; Teranishi et al., 1974). The standard curve created using H_2O_2 was used to calculate the measured conversion values.

Mineral nutrients

The dried leaf samples were dissolved in 10 N nitric acid (HNO_3) after being processed for six hours at 500°C in a muffle furnace using the dry-ashing method. The resulting extracts were then filtered and kept in plastic sample jars until the day of the mineral nutrient analysis (Miller, 2004). Phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), copper (Cu), manganese (Mn), iron (Fe), and zinc (Zn) concentrations were measured

by ICP-OES (Optima®2100 DV, Perkin-Elmer, Waltham, MA, USA). Nitrogen (N) concentration was ascertained using Kjeldahl's (1883) technique.

Data analysis

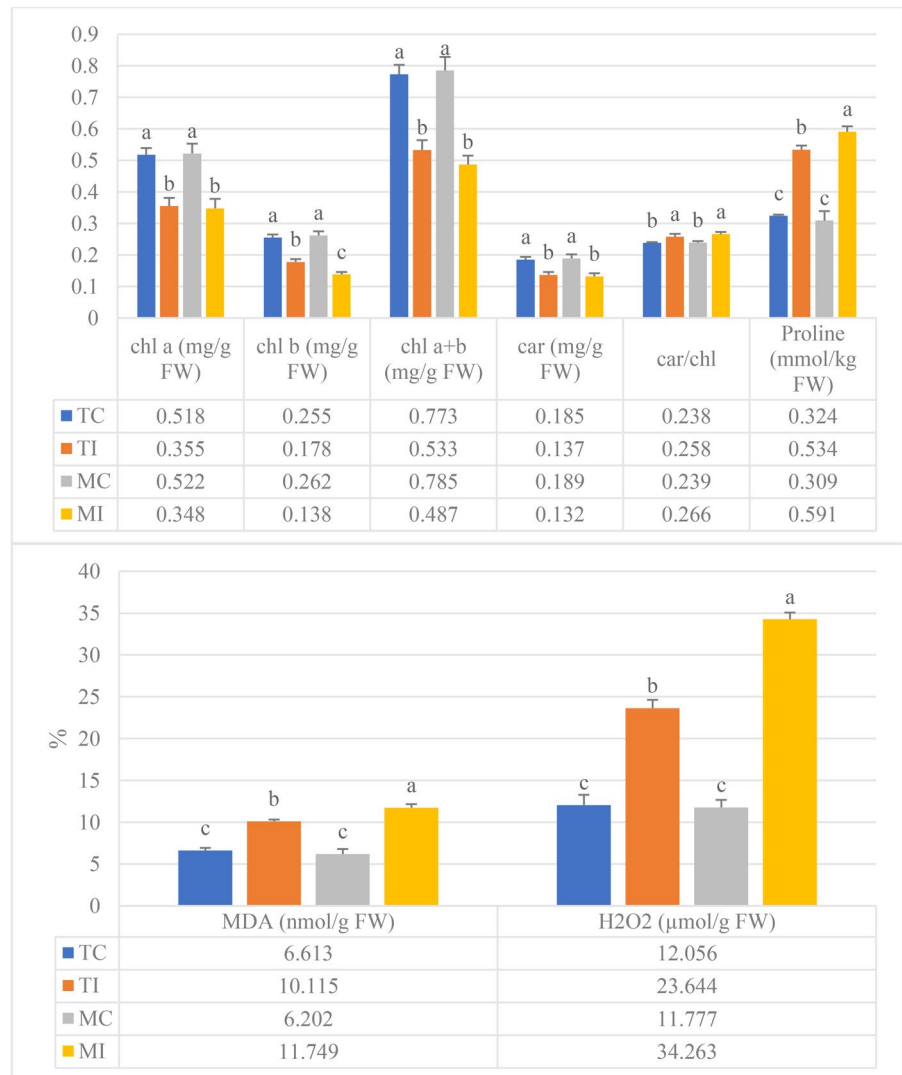
The Levene and Kolmogorov–Smirnov tests were used to verify the data's variance equality and normal distribution, respectively. To ascertain whether or not the means of the four treatments (thin and medium classes with no infection and moderate infection) were equal, an ANOVA was done. If there was a significant difference between the treatments, the Duncan multiple comparison test was used to identify treatments that were homogeneous or distinct ($p < 0.05$). The correlation between infected and uninfected trees for any trait in each of the diameter classes was analysed using Pearson's correlation coefficients. These statistical analyses were carried out utilizing the SPSS program. Principal Component Analysis was used to reduce the dimensionality of the dataset while preserving the important information expressed in terms of variable variance (Savy et al., 2022).

Results

The ANOVA revealed statistically significant differences in the physiological traits apart from car/chl among the diameter classes with infection and non-infection ($p < 0.001$). In comparison with the control, infected trees exhibited the lowest reduction (8%) in car/chl and the highest reduction (32%) in chl *a* in the thin diameter class, and the lowest reduction (11%) in car/chl and the highest reduction (47%) in chl *b* in the medium diameter class. On the other hand, infected trees had the lowest increase (53%) in MDA and the highest increase (96%) in hydrogen peroxide in the thin diameter class, and the lowest increase (90%) in MDA and the highest increase (191%) in H_2O_2 in the medium diameter class. In the two diameter classes, the reduction in chl *a* and *b*, chl *a* + *b* and car were virtually close compared to the control, but the medium diameter class had a higher proline, MDA, and H_2O_2 increase than the thin diameter class (Fig. 2).

The analysis of variance showed significant differences across treatments for all plant nutrients at

Fig. 2 Physiological traits of infected and uninfected trees across different diameter classes (thin and medium)



the 0.001 probability level except for Ca ($p = 0.001$). All plant nutrients were generally less in the infected trees than in the controls. Infected trees showed the lowest reduction (11%) in Fe, Cu, and Zn the highest reduction (40%) in K in the thin diameter class, the lowest reduction (8%) in Fe, and the highest reduction (32%) in Mg in the medium diameter class (Fig. 3).

In the thin diameter class, positive correlations were found for chl *a* ($p = 0.028$; $R^2 = 0.3255$), chl *a* + *b* ($p = 0.007$; $R^2 = 0.445$), Ca ($p = 0.046$; $R^2 = 0.2634$) and Mn ($p = 0.010$; $R^2 = 0.4105$) between infected and uninfected trees. In the medium diameter class, proline ($p = 0.004$; $R^2 = 0.474$), car/chl ($p = 0.037$; $R^2 = 0.0486$), and Mn ($p = 0.044$; $R^2 = 0.276$) of control trees were positively correlated

with those of infected trees (Fig. 4). In both the thin and medium diameter classes, no correlation was observed between infected and uninfected trees concerning other explored traits.

Two primary groups were identified using a principal component analysis (PCA) based on the means of all explored traits: the first group consisted of controls (TC and MC) from both diameter classes, while the second group consisted of infected trees (TI and MI). Of the overall variation, the first two PCA coordinates accounted for 91.6% and 4.8%, respectively (Fig. 5). These groups were in line with the findings of the Duncan tests for every explored trait.

Fig. 3 Plant nutrient concentrations in infected and uninfected trees across different diameter classes (thin and medium)



Discussion

The current study unveiled that there were notable differences in physiological traits and plant nutrients, aside from car/chl and Ca, between the diameter classes with infection and non-infection. Pine mistletoe-infected trees generally exhibited higher levels of proline, MDA, and H_2O_2 but lower levels of plant nutrients, car, and chl *a*, *b*, and *a* + *b*.

In the present study, Pine mistletoe-infected trees showed the highest reductions in chl *a* and *b* in the thin and medium diameter classes, respectively. Researchers reported that the levels of chl *a* and *b* in leaves from mistletoe-infected *Tilia cordata* Mill. (Skrypnik et al., 2021) and *Abies cilicica*

Carr. (Ozturk et al., 2022) tree branches were considerably lower than those in leaves from uninfected trees. Similarly, uninfected *P. sylvestris* L. trees consistently had a higher chl content than the mistletoe-infected ones (Mutlu et al., 2016). Moreover, Amutenya et al. (2023) indicated that the photosynthetic rates of uninfected *Boscia albitrunca* (Burch.) Gilg & Benedict trees were considerably greater than those of the mistletoe-infected ones, indicating a reduction in photosynthetic rate in host plants caused by the parasite. In contrast to these results, Üstüner (2019) stated that mistletoe influenced the level of chl *b* but had almost no effect on the amount of chl *a* in both *Amygdalus* spp. and *Prunus armeniaca* L.

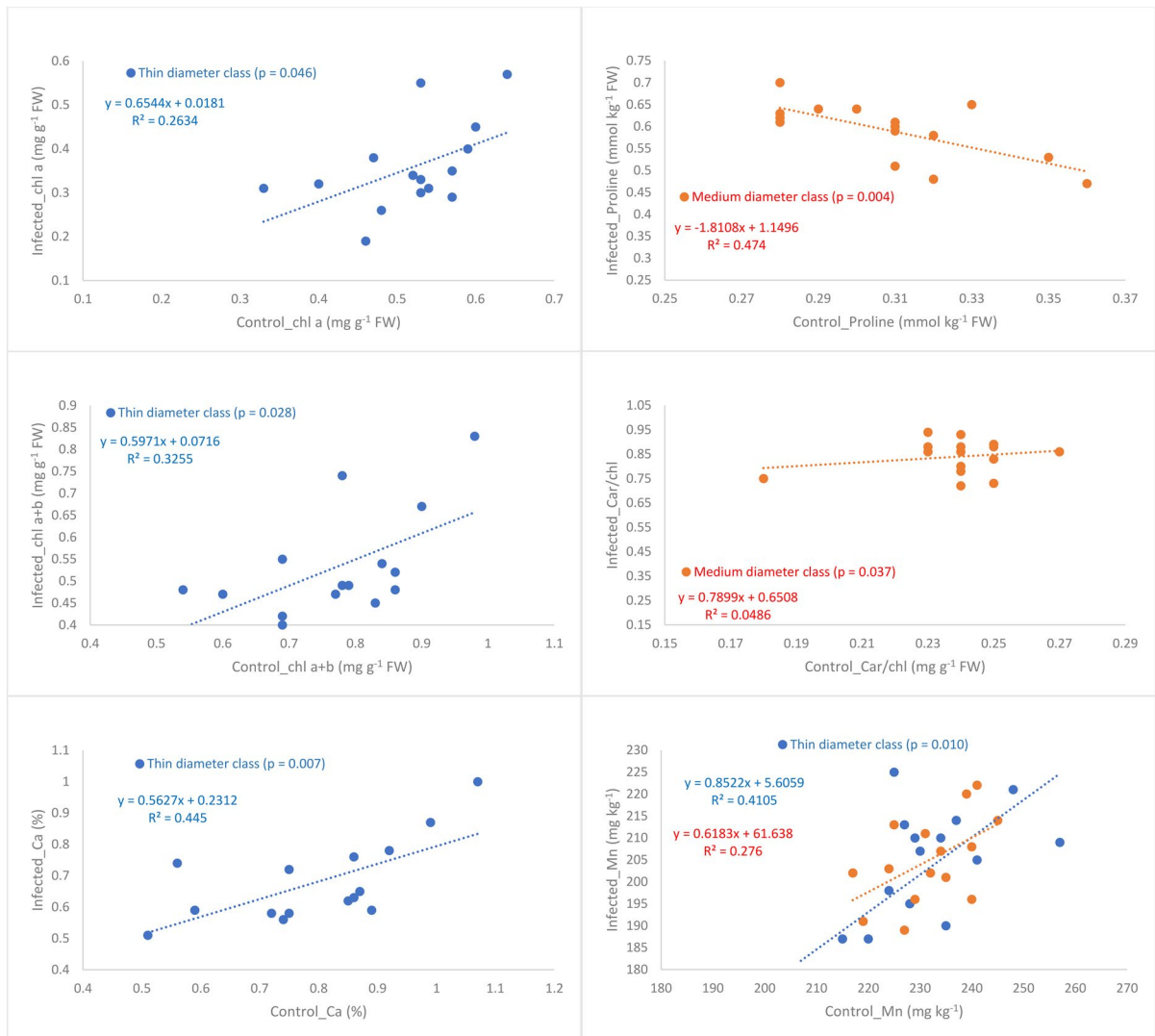


Fig. 4 Correlations between infected and uninfected Anatolian black pine trees in both thin and medium diameter classes for chl *a*, chl *a* + *b*, Ca, Proline, car/chl, and Mn

According to the present study, pine mistletoe-infected trees showed the highest increase in hydrogen peroxide in both diameter classes. Likewise, both low and high levels of mistletoe infection in *T. cordata* branches' leaves showed noticeably greater levels of hydrogen peroxide (Skrypnik et al., 2021). Interestingly, while reductions in chl *a*, *b*, and car were comparable across the diameter classes relative to controls, the medium diameter class exhibited a higher increase in proline, MDA, and H₂O₂ levels. This suggests that trees with medium diameter class may experience a more pronounced oxidative

response to infection, potentially due to greater metabolic demands or differences in nutrient allocation.

In the present study, pine mistletoe-infected trees consistently had lower nutrient levels than controls, with the thin diameter class showing minimal reductions in Fe, Cu, and Zn and maximum reduction in K. These results were consistent with those reported by Mutlu et al. (2016), who found that needles of uninfected *P. sylvestris* trees had greater levels of P, K, Ca, Mg, Fe, Cu, Zn, and Mn than those of mistletoe-infected trees. Scalon et al. (2021) also indicated that N, P, and K were lower in leaves of mistletoe-infected

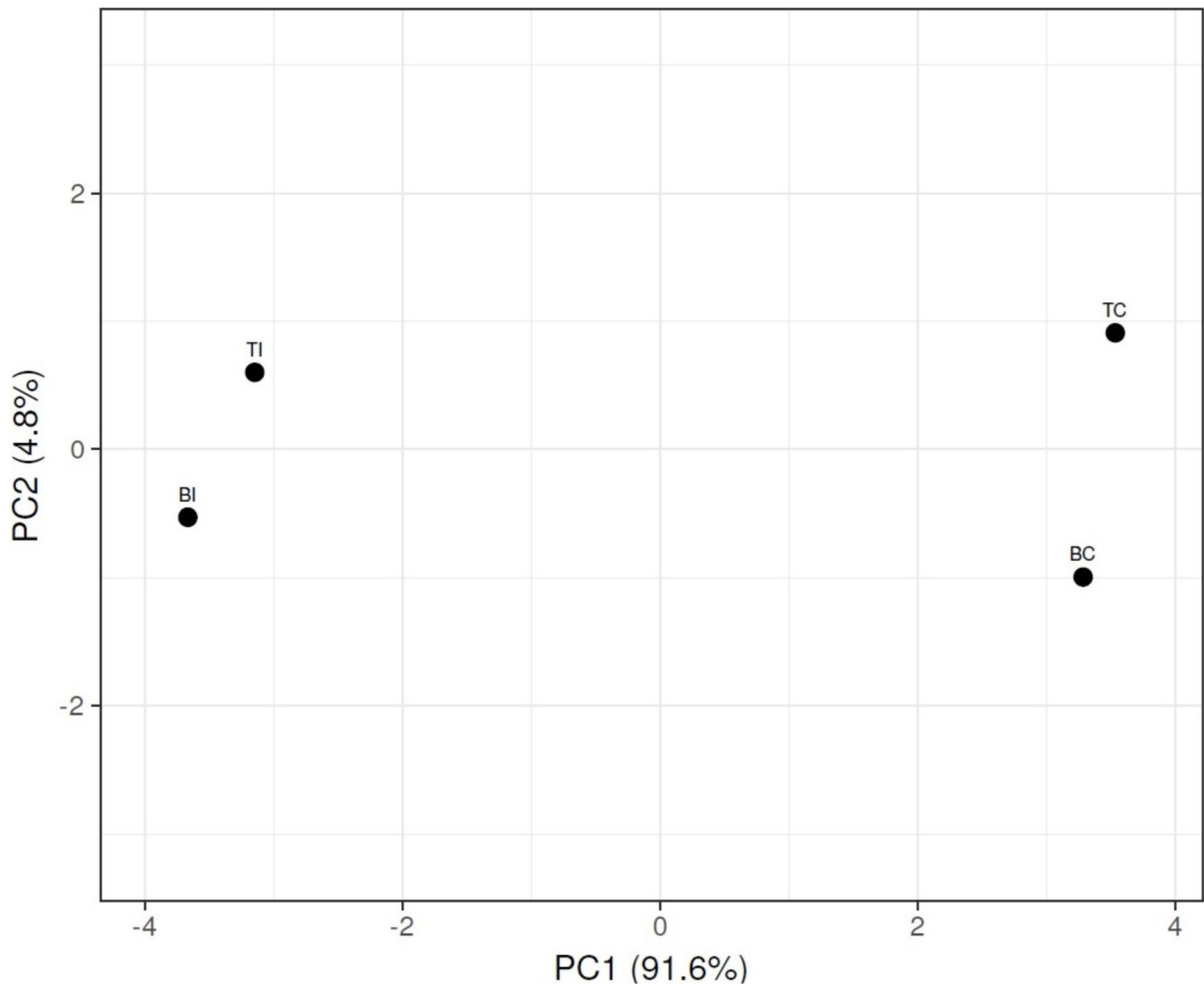


Fig. 5 The results of principal component analysis (PCA) of control and trees based on eight physiological traits and nine plant nutrients of Anatolian black pine

Miconia albicans (SW.) Triana and *Byrsonima verbascifolia* (L.) DC. trees. Yan et al. (2016) suggested that the removal of mistletoe increased N availability, which in turn caused the growth rates of *P. sylvestris* trees to increase. Contrary to these results, Hosseini et al. (2008) reported that mistletoe-infected *Carpinus betulus* L. and *Parrotia persica* C. A. Mey. trees had higher levels of K, Mn, and Zn but less N than uninfected trees.

The results indicated that in the thin diameter class, significant positive correlations were observed between infected and uninfected trees for several key traits, including chl *a*, chl *a+b*, Ca, and Mn. These correlations suggest that even under infection stress, some physiological functions related to these

nutrients may remain relatively intact, highlighting a potential resilience in these traits among infected trees. In the medium diameter class, proline, car/chl ratio, and Mn also showed positive correlations between infected and uninfected trees. Proline accumulation, often associated with stress responses, suggests that infected plants may be actively managing oxidative stress or osmotic pressure, which can be critical for survival. However, the lack of correlation for other traits in both the thin and medium diameter classes indicates that infection may disrupt physiological processes more severely in certain aspects, preventing the expected relationships from forming. This variation underscores the complexity of plant responses to infection and suggests that while

some traits may be resilient, others may be significantly compromised. Future research could further explore the mechanisms behind these correlations and the implications for plant health and ecosystem dynamics.

The principal component analysis (PCA) revealed a clear distinction between two primary groups based on the means of all explored traits: the control trees (TC and MC) formed one group, while the infected trees (TI and MI) constituted the second. This separation underscores the significant impact of infection on physiological traits across diameter classes. The distinct grouping suggests that infected trees are experiencing a collective physiological shift, likely due to stressors associated with infection. Understanding these differences is crucial, as they may have implications for management strategies in affected populations. Further investigation into the specific traits driving this separation could provide deeper insights into the mechanisms of infection and inform future research on plant resilience and adaptation.

In conclusion, this study highlights significant variations in physiological traits and plant nutrients between pine mistletoe-infected and uninfected trees across different diameter classes. The infected trees showed higher oxidative stress indicators such as proline, MDA, and H_2O_2 while exhibiting lower levels of essential nutrients, chl, and car. These findings suggest that mistletoe infection causes a notable decline in photosynthetic efficiency and nutrient absorption, affecting plant health. The medium-diameter class exhibited a stronger oxidative response, potentially due to higher metabolic demands, indicating that infection responses may vary based on tree size. The principal component analysis emphasized a clear distinction between infected and uninfected trees, further confirming the detrimental impact of pine mistletoe infection on Anatolian black pine.

Implementing control measures, such as pruning infected branches, could mitigate the negative effects on plant health, potentially improving growth rates and nutrient levels. More studies should explore the physiological mechanisms behind varying plant responses to pine mistletoe infection, with particular attention to nutrient allocation and oxidative stress management.

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Data Availability All data in this study are included in this published article.

Declarations

Competing interests The authors did not receive support from any organization for the submitted work. The authors have no competing interests to declare that are relevant to the content of this article.

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