

## Strategies to Manage Pistachio Dieback Diseases

Mohadeseh Hassanisaadi (PhD)<sup>1</sup>, Roohallah Saberi Riseh (PhD)<sup>1,2\*</sup>, Masoumeh Vatankhah (PhD Student)<sup>1</sup>

<sup>1</sup> *Departement of Plant Protection, Faculty of Agriculture, Vali-e-Asr University of Rafsanjan, Rafsanjan, Iran*

<sup>2</sup> *Pistachio Safety Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*

Information	Abstract
<p><b>Article Type:</b> Review Article</p>	<p>Pistachio (<i>Pistacia vera</i> L., Anacardiaceae) is an economically important nut crop cultivated worldwide, but the devastating dieback disease threatens its production. As an important disease in pistachio, it is characterized by the wilting and death of branches, often leading to tree decline and eventual death. Several biotic agents, including fungal pathogens like <i>Botryosphaeria dothidea</i>, <i>Dothiorella viticola</i>, <i>Phaeoacremonium parasiticum</i>, <i>Paecilomyces formosus</i>, <i>Paecilomyces variotii</i>, as well as bacterial agents such as <i>Xanthomonas translucens</i>, have been identified as causal agents of pistachio dieback. Furthermore, abiotic factors, such as high salt levels, insufficient nutrient supply, and improper orchard watering, can increase plants' vulnerability to this disease. This review article highlights the importance of pistachio cultivation and the symptoms associated with dieback trees. Also, this article investigates the role of abiotic and biotic agents in dieback occurrence. The article discusses nonbiological strategies, such as chemical control using fungicides and bactericides, cultural methods like pruning and sanitation, and biological strategies involving antagonistic bacteria and fungi. As the disease's complexity and diversity of causative factors make long-term control difficult, future research should focus on integrated disease control techniques that target many disease cycle stages to preserve pistachio crops worldwide.</p>
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<p><b>Corresponding Author:</b> <b>Roohallah Saberi Riseh</b>  <b>Email:</b> r.saberi@vru.ac.ir  <b>Tel:</b> +98-3431312041</p>	

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## 1. Introduction

*Pistacia vera* L. (Anacardiaceae) is a significant nut crop extensively grown in Iran, Turkey, other Mediterranean countries, and the United States. Commercial cultivation of pistachio nuts started in the late 1970s in California and quickly became a major commodity in the San Joaquin Valley [1]. At present, pistachio is considered a crucial commodity in several nations, including Iran. In recent decades, the cultivation of pistachios has been greatly affected by unfavorable environmental conditions that are not conducive to pistachio production. Factors such as water scarcity, high temperatures, soil salinity, intensified agricultural techniques, and the aging of plantations have all contributed to the occurrence and spread of diseases in pistachio trees [1, 2]. The unfavorable environmental and nutritional conditions weaken the trees, rendering them susceptible to disease fungi [2]. Pistachio dieback is a devastating disease that affects pistachio trees worldwide, causing significant economic losses to growers. In 1997, Edward et al. from Australia first reported this disease [3]. The management of pistachio dieback is crucial for the sustainability and profitability of pistachio cultivation. Dieback can lead to significant yield losses, reduced tree longevity, and increased production costs due to the need for replanting and disease control measures. Moreover, the disease can spread rapidly within orchards, posing a threat to pistachio trees' overall health and productivity. The disease was estimated to affect over 85% of the pistachio crops in Iran. It can decrease crop production by up to 90% in poorly maintained orchards [4, 5].

This review article aims to offer a comprehensive overview of current strategies and best practices for managing pistachio dieback diseases. It covers the identification and characterization of the pathogens responsible for these diseases, as well as various management approaches, including cultural practices, chemical control, and biological control. Additionally, the article discusses how to integrate these strategies into a holistic disease management program to ensure the long-term sustainability of pistachio orchards.

### Symptoms of pistachio dieback

The symptoms of dieback disease include the wilting of clusters, leaves, and buds, along with necrosis and darkening of the bark and wood. Cankers and dieback are also common indicators of this disease. Cankers develop from wounds or necrotic areas in the bark of stems or twigs and spread outward from the infection site in all directions. However, they tend to grow more rapidly along the main axis of the stem, branch, or twig, affecting both the bark and wood. Diseased tissues change color from dark brown to black. Cankers typically appear depressed beneath the bark's surface and may exude a resinous gum. Surrounding some cankers, adjacent healthy tissues may swell and appear elevated due to the formation of callus tissue as a response to the infection. The bark in the affected area often has a rough and fractured texture. If a canker encircles a twig, branch, or stem, all tissues above the canker will wither and eventually die. In some cases, symptoms may be associated with the discharge of resin. Ultimately, these symptoms can lead to the death of the tree [2, 6-8]. Fig. 1 shows the symptoms of pistachio dieback.



**Fig 1.** The symptoms of pistachio dieback. Necrotic areas in the bark of stems and twigs, color change of the disease's tissue from brown to black. These symptoms are caused by the biotic agent *Liberomyces pistaciae* sp. nov. [9].

### Factors associated with the dieback of pistachio trees

The dieback of pistachio trees has been linked to various abiotic and biotic causes.

#### Abiotic factors aggravating the dieback of pistachio trees

Exposure to high salt levels, nutrient deficiencies, and improper orchard watering may increase plants' vulnerability to this disease [2]. The scarcity of water in dry and semi-arid regions, soil salinity, and unfavorable temperature conditions are obstacles that have hindered the expansion of cultivated land for this plant in various regions. The primary reason contributing to reduced plant development in salty soils is the combination of low water potential and the detrimental impact of specific ions such as chlorine, bicarbonate, boron, and sodium. An improper balance in nutrient content further exacerbates these issues [10]. These conditions can lead to many problems for plants, such as the dieback of trees. Additionally, Chitzanidis et al. [11] Stated that the deficiency of potassium fertilizers aggravates dieback in pistachio.

### Biotic agents associated with the dieback of pistachio trees

In addition to abiotic stressors, various fungal and bacterial agents were isolated and identified from infected tissues. *Xanthomonas translucens* pv. *pistaciae* has been identified as a bacterial pathogen responsible for causing pistachio dieback, as documented in certain reports [12-14].

Rumbos [15] discovered the fungus *Eutypa lata* in dried pistachio tree branches for the first time in Greece, identifying the pistachio as a new host for this fungus. Aminae and Ershad reported in 1987 that the cause of this complication was a fungus called *Paecilomyces variotii* [16]. In 1998, Swart and Blodgett [17] revealed that *Botryosphaeria dothidea* is a causal agent of dieback and canker of pistachio in South Africa. In this vein, Alizadeh et al. [7] conducted a study on the cause of the dieback disease in pistachio plants in Iran and reported the causative culprit as *P. variotii*. Additionally, in 2012, it was found that *B. dothidea* is associated with pistachio branch cankers in Australia [18]. Also, in 2015, Mohammadi et al. [19] reported that *Phaeoacremonium parasiticum* was first used as a dieback disease

agent on pistachio cv. Fandoghi. They could also isolate *pH. aleophilum*, *ph. cinereum*, *Ph. viticola*, *B. dothidea*, and *Dothiorella viticola* from dead trunks. *Lasiodiplodia exigua* and *Neofusicoccum hellenicum* were found in damaged pistachio shoots in Arizona and Greece in 2015. When observed in the natural environment, these fungi could create sores on pistachio branches [20]. In 2018, Vitale et al. [9] reported *Liberomyces pistaciae* as the causal agent of pistachio canker and dieback in Italy. In a study by Heidarian et al. [4], 277 strains of *Paecilomyces* were isolated from pistachio samples, and 23 fungal isolates were recovered from other plant species. Additionally, five isolates were obtained from the air in pistachio orchards affected by the disease. All 305 isolates were initially identified as *P. variotii* based on their appearance. However, further physiological studies revealed that the majority (299 isolates) belonged to *P. formosus*, with only three isolates confirmed as *P. variotii*. For more definitive results, a subset of 62 isolates underwent phylogenetic analysis based on DNA variation in the ITS region,  $\beta$ -tubulin, and calmodulin genes. This analysis showed that all isolates formed a clade with *P. formosus*, a species complex comprising the three former species *P. formosa*, *P. lecythidis*, and *P. maximus*.

In this vein, in a more recent study, Torabi et al. [2] Identified the presence of *Paecilomyces* in the shoots of pistachio trees in Kerman, Iran, which exhibited indications of dieback and canker. The morphological characterization revealed that the pathogen isolated was *P. variotii*. The isolated *Paecilomyces* was identified as *P. formosus* by physiological differentiation and molecular identification using ITS and  $\beta$ -tubulin genes.

### **Pistachio dieback in Iran and other countries**

In Iran, Aminae and Ershad first reported Pistachio dieback caused by *Paecilomyces* in Kerman, with *Paecilomyces variotii* as the pathogen agent in 1989 [21]. Other researchers have also studied the causes of this disease. They identified fungal agents, including *Cytospora sp.*, *Coniothyrium sp.*, *Stemphylium sp.*, *Neofusicoccum magniferae*, *Acremonium sp.*, *Phaeoacremonium sp.*, and *Ulocladium sp.*, in pistachio branches showing dieback symptoms, and confirmed that these fungi are pathogenic [19, 22]. In 2018, Heydarian et al. [4] conducted a study sampling pistachio orchards in different regions of Iran. They obtained 227 *Paecilomyces* strains from 567 symptomatic pistachio samples. Initially, all the isolates were identified as *P. variotii* based on morphology. However, further phylogenetic and physiological studies revealed that almost all of them belonged to the *P. formosus* species, with less than 1% identified as *P. variotii* according to these criteria. They estimated that 85% of Iran's pistachio orchards had disease symptoms, with an average contamination of 35%.

Ozan et al. [23] observed dieback symptoms in around 7% of pistachio trees in a commercial orchard located in southeastern Turkey. They isolated 15 pieces of wood from diseased branches of several trees. Morphological characteristics matched with *P. maximum*. However, phylogenetic studies proved to have a 99% match with *P. formosus* sequences. Furthermore, in 2022, Ören et al. [24] isolated *Fusarium* as a dieback agent in pistachio orchards in Turkey for the first time. Morphological characteristics and phylogenetic analysis confirmed that the isolated samples were attributed to *F. proliferatum*, causing

dieback symptoms in approximately 5% of pistachio orchards.

Additionally, pistachio dieback is a significant issue in other countries and the United States, particularly in the major pistachio-producing regions such as California. López-Moral et al. [25] reported that this disease is caused by several fungi, such as *Botryosphaeria dothidea*, *Lasiodiplodia pseudotheobromae*, *Neofusicoccum mediterraneum*, *N. parvum*, *Eutypa lata*, *Eutypa* sp., *Diaporthe* sp., *Diaporthe neotheicola*, *Cytospora* sp., and *Phaeoacremonium minimum*. They reported this disease as emerging in the novel Spanish pistachio-growing areas. Nouri et al. [1] conducted research in 2015-16 to examine cankers and dieback signs, focusing on orchards at least 15 years old. Their phylogenetic analyses revealed symptoms associated with 11 fungal species, namely *Colletotrichum karstii*, *Diaporthe ambigua*, *Cytospora pistaciae*, *Cytospora californica*, *Cytospora joaquinensis*, *Cytospora parapistaciae*, *Didymella glomerata*, *Diplodia mutila*, *Neofusicoccum mediterraneum*, *Phaeoacremonium canadense*, and *Schizophyllum commune*. Nouri et al. [1] reported that *N. mediterraneum* and *Cytospora* spp. were the dominant and disease-causing species linked to canker diseases in pistachio trees in California.

### **Strategies for managing pistachio dieback Nonbiological strategies**

Pistachio dieback poses a significant threat to pistachio orchards, making the selection of effective management strategies essential for mitigating the impact of this disease. The disease primarily affects trees that are under environmental stress and weakness, while it tends to be less common in well-maintained gardens with adequate nutrition and watering.

Therefore, good garden management practices and appropriate agricultural techniques can substantially help in managing and controlling this disease. Following sound nutritional principles, particularly by using fertilizers that contain potassium, calcium, and zinc, can significantly reduce the incidence of dieback. The nutritional condition of pistachio trees plays a vital role in their overall health and their susceptibility to dieback disease. A deficiency in essential nutrients weakens a tree's natural defenses, making it more vulnerable to infections. This can result in symptoms such as wilting, branch dieback, and canker lesions. Deficiencies in key nutrients like potassium, calcium, and zinc can impair a plant's ability to produce energy, synthesize proteins, and maintain cell structure—all of which are crucial for resisting pathogens. Adequate levels of these minerals can reduce the duration of dieback by up to 63% [5]. Mozaffari et al. conducted a ten-year evaluation of pistachio orchards and found that the incidence of dieback in pistachios was associated with electrical conductivity (EC), sodium (Na), potassium (K), the Na/Ca ratio, and the Na/K ratio. In their experiments, they also examined the effects of different fertilizers, gypsum, zinc sulfate, and potassium sulfate, on dieback occurrence. Their results indicated that the application of potassium, calcium, and zinc significantly reduced the incidence of dieback in pistachios. Furthermore, due to the variety of pathogens responsible for pistachio dieback, it is crucial to implement sanitation measures such as pruning and removing diseased plant material, particularly during the dormant season, to lower pathogen inoculum levels in the orchard. Since chemical pesticides are relatively inexpensive and highly effective, the management of plant diseases currently primarily relies on spraying these chemicals. To alleviate the damage,

growers commonly spray copper oxychloride late in winter and after pruning in autumn or winter [2]. Jalalabadi et al. [26] found that Benomyl and Rovral TS are particularly effective against *P. variotii*. Benomyl is a systemic fungicide that disrupts fungi's cell division by inhibiting microtubule assembly. This prevents the fungi from forming the structures necessary for growth and replication, effectively stopping the spread of the disease. In the context of dieback disease, which is often caused by fungal pathogens, Benomyl can effectively prevent the disease's progression by targeting the causative fungi. When the plant absorbs benomyl, it can protect itself from various fungal diseases, including those that cause dieback [27]. Rovral TS, also known as iprodione, is a contact fungicide in the dicarboximide class. Its mechanism of action involves inhibiting the germination of fungal spores and blocking the growth of the fungal mycelium. It disrupts the production of fungal DNA, RNA, and protein, which are essential for the pathogen's development and survival. Applying Rovral TS to plants affected by dieback disease can help control the spread of the fungal pathogen responsible for the disease [28]. Their results revealed that the orientation of the branches (horizontal vs. vertical) also seems to affect the outcome, suggesting that the fungicide application method is an important factor in disease management. They also stated that Elite fungicide's poor performance is not an effective choice for controlling this disease.

### Biological strategies

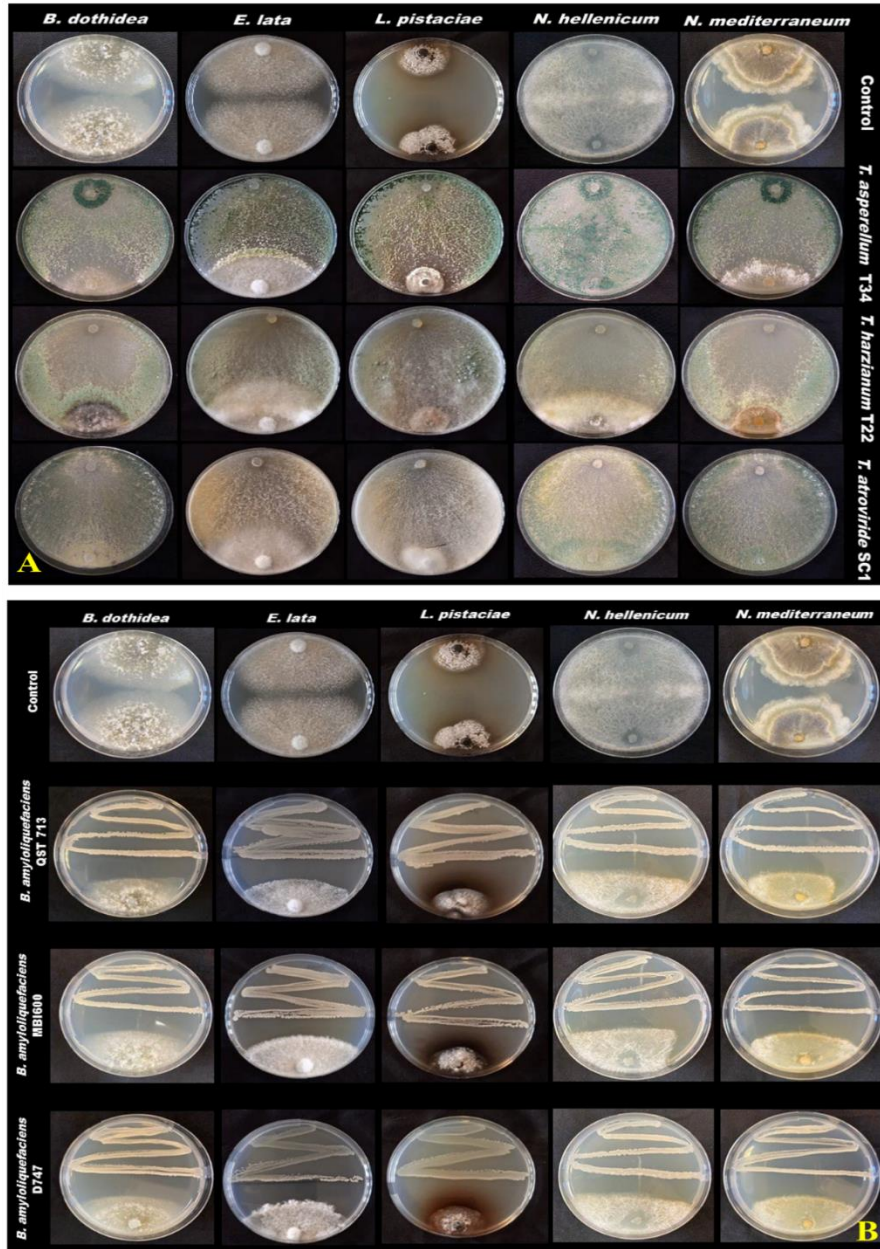
Fungicides have significant drawbacks, including their toxicity and the risk of pests developing resistance. However, consumers are increasingly concerned about food safety and environmental sustainability. This underscores the need for alternatives to these agricultural

chemicals. Consequently, the use of microbial plant protection products, based on biological control agents and metabolites, is becoming more popular. Nevertheless, there are only a few reports on the biocontrol of the biotic agents responsible for dieback disease. In a study, Pollard-Flamand et al. [29] investigated the potential of *Trichoderma* species isolated from grapevines in controlling *Diplodia seriata* and *Neofusicoccum parvum*. The results showed that the *Trichoderma* species had inhibitory effects against these dieback agents. These isolates performed as well as or better than commercial chemical products. A study used bacterial strains (*Bacillus subtilis*, *Erwinia herbicola*, *Serratia plymuthica*, and actinomycete isolates) with significant potential to control *Eutypa lata*, suggesting their use as biological control agents to manage dieback. In this study, one *B. subtilis* isolate, two *E. herbicola* isolates, and one actinomycete isolate exhibited the highest antagonism up to 70-100% [30]. As reported by Salowi in 2010 [31], a disease caused by *Xanthomonas translucens* in Australia was effectively managed using *Bacillus subtilis* and *Pseudomonas* sp. In a recent study, researchers investigated the use of actinomycetes, particularly *Streptomyces* species, which are known for their ability to suppress diseases caused by *P. formosus* through the production of bioactive compounds and enzymes. Out of 52 actinomycete isolates that were tested, two strains of *Streptomyces misionensis* (BH4-1 and BH4-3) demonstrated significant antagonistic activity. The results indicated that these *S. misionensis* strains were effective in controlling pistachio dieback caused by *P. formosus* when compared to the control group [2].

In the more recent study in 2022, in Italy, biological control *Neofusicoccum hellenicum*, *Leptosillia pistaciae*, *Cytospora pistaciae*, and

*Eutypa lata* were investigated against various strains of *Trichoderma* and *Bacillus amyloliquefaciens* (Fig. 2). Through in vitro testing, it was determined that all biocontrol agents examined were able to suppress the development of the pathogen effectively.

Nevertheless, in vivo trials conducted on detached twigs showed little efficacy, except for therapies targeting *N. hellenicum* and *L. pistaciae*.



**Fig 2.** In vitro antifungal activity of various strains of *Trichoderma* against pistachio dieback disease agents (A); In vitro antibacterial activity of *Bacillus amyloliquefaciens* against pistachio dieback disease agents (B) [32].

### **The mechanism of biocontrol agents**

Biological control agents can suppress pistachio dieback disease through various mechanisms. Understanding these mechanisms is essential for creating conditions that effectively support the activity of biocontrol agents. These mechanisms are categorized as direct and indirect mechanisms. Direct mechanisms involve the release of compounds such as antioxidants, lipopeptides, antibiotics, hormones, biosurfactants, volatile compounds, and cell wall-degrading enzymes. [33-35]. Höfte claimed that antimicrobial metabolites such as bacteriocins, pyrrolnitrin, polyhedrin, dialkyl resorcinols, and phloroglucinols are involved in the biological control of plant pathogens [36]. Also, Lajis in 2020 [37] reported that bacilli bacteria prevent the formation of spores or the synthesis of the pathogenic cell wall by producing several bacteriocins, such as Subtilin A, Subtilin B, Amylosin, Thuricin, Amylocyclicin, and Amylolysin, thereby suppressing the pathogen's survival. Indirect mechanisms of plant biocontrol agents include colonization, competition for food and space, and induction of the host's defense system. Biocontrol agents, by occupying the plant's ecological niche, restrict the resources accessible to pathogens, therefore diminishing their capacity to invade and induce disease. Based on competition, this technique effectively inhibits the proliferation and dissemination of infections, aiding in the biological management of plant diseases [38]. Additionally, biocontrol agents can stimulate systemic resistance in plants, resulting in the buildup of physical barriers and the host's initiation of diverse biochemical and molecular defense mechanisms. This mechanism encompasses signaling pathways incorporating phytohormones, phytoalexins, and defensive

enzymes, including phenylalanine ammonia-lyase, chitinase, PR-proteins, and phenolic chemicals [39, 40].

### **Integrated management strategies**

Considering both nonbiological and biological factors, integrated management is the most effective solution against dieback diseases in pistachio. This approach entails a multifaceted strategy that combines cultural, biological, and chemical methods to combat these detrimental conditions effectively. Furthermore, by utilizing practices such as proper pruning techniques, sanitation measures, and optimized irrigation schedules, growers can create an environment less favorable for disease development. The incorporation of biological control agents, such as beneficial microorganisms or bio-fungicides, further enhances disease suppression while minimizing environmental impact. Additionally, the careful use of targeted chemical treatments at critical stages can offer immediate control of pathogens. This integrated approach not only mitigates the challenges posed by canker and dieback diseases but also promotes the long-term health and sustainability of orchards [2].

### **Challenges and limitations of dieback management**

Managing dieback in pistachio trees presents a complex array of challenges and limitations rooted in both the biological nature of the disease and the practical realities of agricultural practices. Dieback is characterized by the decline and death of shoots, deformation of leaves, and necrosis of the trunk and vascular tissues, posing a significant threat to pistachio crops globally. The disease's causes are often multifaceted, involving bacterial and fungal pathogens. Additionally, exposure to high salt levels, insufficient nutrient supply, and improper orchard watering may increase plants' vulnerability to this disease. One of the primary

challenges in managing pistachio dieback is the application of chemical control measures, such as fungicides, which are often limited by concerns over environmental impact, human health risks, and the development of pathogen resistance. This has increased interest in biological control methods, offering a more sustainable approach. However, the efficacy of biological agents can be variable and is influenced by numerous factors, including compatibility with the host plant, the mode of application, and the prevailing environmental conditions.

One challenge is the lack of effective cultural practices to prevent the disease. Although it has been suggested that pruning infected wounds can help limit the disease's spread, it remains unclear how effective this measure is, and it may not be feasible on a large scale. Furthermore, the economic constraints faced by growers, such as the costs of implementing new control strategies and potential yield loss due to disease, complicate dieback management even further. In conclusion, managing dieback in pistachio trees is difficult due to the complex nature of the disease, the limitations of current control methods, and the practical considerations of agricultural production. Addressing these challenges requires a comprehensive approach that includes accurate pathogen identification, the development of effective and sustainable control methods, and the implementation of practical cultural practices, all while considering the economic realities faced by pistachio growers.

## **2. Conclusion and prospects**

Effectively managing pistachio dieback diseases requires a proactive and integrated approach. It is crucial to understand how

environmental factors, such as high salt levels, nutrient deficiencies, and irrigation practices, interact with fungal and bacterial pathogens. Combining chemical controls, cultural practices, and biological strategies, such as using biocontrol agents, is essential for mitigating dieback in pistachio trees. Despite challenges like fungicide resistance and delayed symptom expression, the future of dieback management looks promising. Advancements in genetic resistance breeding, precision agriculture technologies, and sustainable farming practices offer new opportunities. Additionally, integrating plant probiotic bacteria and natural polymers for encapsulation shows great potential. Collaboration among researchers, growers, and industry stakeholders is vital to addressing the complex challenges posed by pistachio dieback and ensuring the long-term sustainability and productivity of pistachio orchards worldwide..

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## **Conflict of Interest**

The Authors declare no conflict of interest.

## **Ethical Review**

This study does not involve any human or animal testing.

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