The Management of Pistachio Gummosis by Biocontrol Strategies

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Abstract

Crown and root rot of pistachio trees is a serious disease in pistachio orchards that is induced by *Phytophthora* species. Due to the harmful effects of chemical compounds on the environment and human health, great emphasis has been placed on the biocontrol of plant diseases, in recent years. Some plant growth promoting microorganisms, such asrhizobacteria (PGPR) and fungi (PGPF), suppress plant pathogens by various direct and indirect mechanisms, including colonization, antibiosis, production of lytic enzymes and volatile compounds (VOCs), as well as induced systemic resistance (ISR). From among beneficial microorganisms, *Bacillus* and *Pseudomonas* strains and *Trichoderma* species have been used as biocontrol agents in many studies, due to their strong biocontrol effects on a wide range of plant pathogens. In recent years, many studies have been done on the biological control of crown and root rot (gummosis disease) of pistachio trees. In the current study, the biocontrol efficacy of soil microorganisms antagonistic to *Phytophthora*crown and root rot of pistachio trees has been evaluated.

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1. Introduction

Pistachios (*Pistaciavera*) are from among the major horticultural products around the world. They are the third largest non-oil commodity exported. Pistachios are of high significance among agricultural products due their high economic value, so necessary efforts must be made to maintain their global position. The pistachio yield can be affected by a great deal of abiotic and biotic stress. The plant disease is the major biotic stress that has a significant effect on the plant growth and yield. Over 30 pistachio diseases have been reported throughout the world, with soil-borne diseases having been considered as the most harmful factors. *Phytophthora* crown and root rot, Verticillium wilt, and root-knot nematodes are the major soil-borne diseases in Iran that inflict heavy economic losses every year [1]. From among these diseases, *Phytophthora* crown and root rot of pistachio trees, also named as pistachio gummosis caused by *Phytophthora* species, is of economic significance in Iran and some countries [2].

2. *Phytophthora* crown and root rot

Phytophthora crown and root rot of pistachio trees is one of the common diseases of pistachio trees that damages many fertile and non-fertile pistachio trees every year [3, 4]. The first species of *Phytophthora* was isolated from the crown and root of pistachio trees in Qazvin province in 1967. The fungus was identified as *Phytophthora citrophthora* [5]. Various species of that disease, including *Phytophthora megasperma*, *P. drechsleri*, *P. nicotianae*, *P. citrophthora*, and *P. cryptogea* have been identified in pistachio orchards in Iran [3, 6- 8]. The frequency and dispersion studies of the *Phytophthora* species have shown that *P. megasperma*, *P. drechsleri*, *P. cryptogea*, and *P. citrophthora* are the species frequently found in pistachio orchards [3- 7, 9]. From among these species, *P. citrophthora* is severely pathogenic [10]. Although many species, including *P. megasperma*, *P. drechsleri*, *P. cryptogea*, and *P. citrophthora* have been isolated from several pistachio orchards in Rafsanjan, *P. drechsleri* has been introduced as the most prevalent species of the disease in various regions of Rafsanjan city [11].

2.1. The disease cycle

Water is an important factor throughout all stages of the lifecycle and disease development in *Phytophthora* species. The induction of sporangium formation and zoospore release occur under the conditions of 100% humidity and the water potential of about 0MPa. Under warm and wet conditions, mycelia form sporangia. Mycelia of various mating types may grow together to produce sexual spores named oospores. Oospores are germinated and produce sporangia. Sporangia produce and release motile zoospores that swim in the soil water. Besides, zoospores can be carried along over large distances by the infected soil or water. Zoospores contact and infect roots and the trunk. They then adhere to the respective part and get encysted and germinated to produce germ tubes to penetrate into roots. Mycelia develop within the root system and initiate infection [9].

2.2. Symptoms

Crown and root rot accompanied by the exudation of sap seeping out of cracks in the bark, crown, and lower trunk are the symptoms of Pistachio Gummosis (20-30 cm above the soil surface). The part infected goes brown in the vicinity of the air. The underlying rotted tissue becomes brown or dark brown, with severe root rot due to the infection of the root system, resulting in the reduced uptake of water and nutrients and the sudden death of the tree [12]. This disease occurs at different stages of growth. The fungus infects feeder roots and results in the reduced uptake of water and nutrients, and by invading the vascular tissue (phloem), it affects the appearance of the tree, leading to the reduced growth, thinned canopy, chlorosis, early defoliation, and eventually death, within one year from infection [12]. Moradi et al. [13] reported that in severe cases of the disease, about 80% of the garden trees dried within a period of 5-10 years. The annual effects of the disease on the reduction of the pistachio yield have been reported to be about 2-12%.

3. The biocontrol of Phytophthora crown and root rot using biocontrol agents

Pistachio trees are susceptible to *Phytophthora* crown and root rot, throughout their lifecycle. This soil-borne pathogen is transmitted by infected seedlings, water, and soil. Several control methods, including resistant rootstocks as well as cultural, chemical, and biological controls have been recommended for the management of this disease [14, 15]. Using chemical compounds is one of the methods of protecting crops. Despite the strong and rapid effect of using chemical compounds as well as their easy utilization, they have significant disadvantages, including deleterious effects on the environment and non-target organisms. Cultural control is one of the oldest methods whose success is achieved in the long term, due to the need for long term planning and lack of sufficient knowledge of the bio-ecology of soil microorganisms. In addition, the mentioned methods affect one or a few properties of pathogens. Biological control is a natural method that unlike the chemical control method is safe and economical in fighting against pathogens. The use of fungicides is the most effective strategy for the management of *Phytophthora* crown and root rot [14, 15], but due to the destructive effects of the application of fungicides on microorganisms and useful soil micro-organisms, biocontrol can be an effective strategy to reduce dependence on chemical compounds [16]. Numerous studies have so far demonstrated the role of antagonistic microorganisms in reducing the incidence of *Phytophthora* crown and root rot.

3.1. Bacteria

Some antagonistic bacteria (such as *Pseudomonas*, *Bacillus*, and *Streptomyces*) have shown the ability to inhibit fungal growth. In a study, it was reported that *Streptomyces* isolates inhibited the growth of *Phytophthora drechseleri* in culture plates and controlled crown and root rot of pistachio seedlings under greenhouse conditions by the production of antibiotics with fungicidal properties against *P. drech*seleri [17]. Saberi-Riseh et al. [18] reported that many isolates of *Pseudomonas* and *Bacillus* collected from pistachio rhizosphere showed antagonistic effects on *P. citrophthora* in vitro. These isolates inhibited *P. citrophthora* growth by the production of antifungal metabolites, including antibiotics, volatile metabolites, HCN, and siderophores. Several studies showed that *Pseudomonads fluorescent* and *Bacillus subtilis* strains were able to inhibit mycelia growth and control crown and root rot of pistachio trees caused by *Phytophthora drechsleri*, by inducing the plant defense system as well as producing multiple secondary metabolites and plant growth regulatory materials [19- 21]. Hajabdolahi et al. [22] reported that from among 400 bacterial isolates collected from the soil and rhizosphere of pistachio regions in Kerman, 19 isolates were able to inhibit *P. pistaciae* growth in vitro. Moradi et al. [23] also reported that from among a total of 321 isolates collected from pistachio orchards, 13 isolates identified as *Bacillus subtilis* showed promising results as biocontrol agents against *Phytophthora pistaciae.* They showed that the inoculation of pistachio seedlings with *B. subtilis* reduced the mortality rate of pistachio seedlings in greenhouse experiments by 80%.

3.2. Fungi

Antagonistic fungi such as *Trichoderma* species have been widely studied for over 20 years as mycoparasites that are potent bilological agents against a number of plant pathogenic fungi, with their considerable potential in biological control proven [24]. Recent studies have shown the effects of *Trichoderma* species on *Phytophthora* spp. control. Fani et al. [25] reported that *Trichoderma harzianum* isolates inhibited the growth of *Phytophthora melonis* obtained from the soil surface in vitro and in vivo, collected from Kerman, Yazd, Khorasane Razavi, and Semnan provinces.

The co-inoculations of *Trichoderma harzianum* strains and *P. melonis* increased growth factors and decreased the mortality rate of seedlings. In another study, the effects of 27 isolates of *Trichoderma harzianum, T. crassum, T. koningii, T. aureoviride, T. asperellum, T. brevicompactum, T. longibrachiatum*, and *T. virens* were examined on the growth and zoospore production of *Phytophthora drechsleri*. These isolates demonstrated inhibitory effects on *P. drechsleri* by secreting extra-cellular and volatile compounds [26].

4. Biocontrol mechanisms of beneficial microorganisms

4.1. Colonization

The root colonization ability is a crucial factor for biological control agents and is required for other biological control mechanisms. Successful root colonization by antagonistic microorganisms depends on the plant's age, cultivar, root exudate compounds, and environmental conditions [27]. Beneficial plant bacteria and fungi that succeed in colonizing plant roots and promote plant growth are called Plant Growth Promoting Rhizobacteria (PGPR) and Plant Growth Promoting Fungi (PGPF) [28].

4.2. Antibiosis

Antibiotics are low-molecular-weight metabolites (LMWMs) that lead to the death and growth inhibition of microorganisms by affecting their vital systems. A group of useful bacteria and fungi, such as *Bacillus*, *Pseudomonas,* and *Trichoderma* induce the death and growth inhibition of plant pathogens by producing antibiotics (antifungal compounds). The functional mechanism of antibiotics is called antibiosis [29]. Antifungal metabolites, including phenazines, pyrrolnitrin, 2,4-diacetylphloroglucinol (DAPG), and pyoluteorin are produced by rhizobacteria [30]. Shahidi Bonjar et al. [17] reported that the application of Streptomyces isolates controlled *P. drechsleri* by exerting antifungal effects, with fungicidal properties, on pathogen mycelia. Besides, the inhibitory effects of *Pseudomonas* on the mycelia growth of *P. drechsleri*, by producing antibiotics, had been previously reported by Daroodi et al. [19].

4.3. Parasitism

Biological control agents cause the lysis of pathogenic fungal cell walls by producing extracellular hydrolytic enzymes, such as chitinases, glucanases, proteases, and lipases [31]. Although chitin is the main component of most phytopathogenic fungi cell walls, the chitinase effect of probiotic bacteria and fungi disintegrates the cell walls of fungal phytopathogens [32]. Besides, proteolytic enzymes and toxins produced by phytopathogenic fungi are inactivated by *fluorescent pseudomonads*, with their pathogenicity reduced [33]. It has been reported that extra-cellular secretions, such as β-1,3 glucanase and cellulose produced by *Trichoderma* isolates play a significant role in inhibiting *P. drechsleri* growth and zoospore production [26]. Fani et al. [25] examined the antagonistic properties of *Trichoderma* species in suppressing *P. melonis* growth in vitro and in vivo. They observed that *Trichoderma* species inhibited the mycelial growth of *P. melonis* by different mechanisms, including the secretion of extracellular enzymes.

4.4. Volatile compounds (VOCs)

Volatile compounds are low-molecular-weight metabolites, of low polarity and a high vapor pressure [34]. These compounds that are released by antagonistic bacteria and fungi can be used as signaling molecules for inter-species and intra-species communications. They have been reported to have exerted inhibitory effects on the mycelial growth rate, the enzymatic activity, and spore germination of phytopathogenic fungi [35]. The role of volatile compounds produced by *Trichoderma* species, *Bacillus* spp., and *Pseudomonas* spp. has been verified in the suppression of *Phytophthora* spp. The causal agent of the crown and root rot of pistachio trees has also been reported [19, 20, 23, 25, 26].

4.5. Induced systemic resistance (ISR)

The biological approach to enhancing the plant’s defense ability by biotic and abiotic agents is termed as "Induced Resistance". Induced Resistance is triggered in plants by stimulating the plant’s defense system, thereby leading to the activation of defense responses against various types of pathogens [36]. After the colonizing of the roots by the plant’s useful bacteria and fungi, defense responses are increased in the plant. This type of resistance is known as "induced systemic resistance" (ISR) that protects the plant in dealing with future infections. ISR occurs through jasmonic acid (JA) and ethylene (ET)-mediated signaling pathway [37]. The induced systemic resistance is accompanied by the accumulation of phytoalexin and phenolic compounds as well as the enhancement of the defense enzymes, such as phenylalanine ammonialyase (PAL), chitinase, b-1,3-glucanase, peroxidase (PO), and polyphenol oxidase (PPO) [37]. The study results demonstrated that the bacterial isolates of *Pseudomonas fluorescens* strain VUPF5 and *Bacillus subtilis* strain 96 reduced the occurrence of crown and root rot of pistachio seedlings caused by *P. drechsleri* in the greenhouse experiment. These strains triggered ISR in pistachio seedlings by increasing antioxidant enzymes, such as PO and PPO that play an important role in reducing the oxidative stress of reactive oxygen species (ROS) due to the pathogen activity and also by the accumulation of phenolic compounds with toxicity effects on pathogens [21]. Several strains of *Pseudomonas fluorescens* (CHA0, VUPF760, VUPF5, VUPF506, and T17-4) have been shown to exert biocontrol effects on inhibiting pistachio gummosis and increasing resistance in pistachio seedlings by enhancing enzymatic activities and the levels of phenolic compounds [20].

5. Conclusions

There has been great emphasis placed on the biocontrol of plant diseases, in recent years. Plant growth promoting rhizobacteria (PGPR) and fungi (PGPF) exert biocontrol effects on phytopathogens through multiple mechanisms. It has been demonstrated that PGPR bacteria have the ability to colonize plant roots, stimulate plant growth, and reduce the incidence of diseases. These bacteria are environmentally friendly, induce soil fertility, suppress pathogens, and remove abiotic stresses. However, there are some challenges concerning antagonist applications in soil, including bacterial placement, adaptation and viability. The biocontrol effects of the antagonistic bacteria and fungi isolated from pistachio growing regions on *Phytophthora* spp. demonstrated that they have the potential to be used as biological control agents in reducing or inhibiting the incidence *of Phytophthora* crown and root rot. Although the application of such antagonist bacteria and fungi has demonstrated that they have successfully reduced the disease incidence in vitro and greenhouse studies, using them in orchard conditions is difficult due to the lack of favorite conditions required for their adaptation and placement in saline and dry soils. More studies are required to understand the effects of PGPR bacteria and fungi to inhibit crown and root rot of pasticcio trees and their application under orchard condition.

Conflict of interest

The authors declare no conflict of interest.

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