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## Insights into Fusarium spp., Associated with Dry Root Rot of Citrus and its Management

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In recent years, citrus has become one of the major fruit crops in Pakistan for export. Citric acid, oxalic acid, malic acid, malonic acid and succinic acid are organic acids found in citrus fruits that provide calories and are easily digested as they are part of metabolic pathways in the human body. *Fusarium* spp., are primarily responsible for causing dry root rot in citrus, which is one of the most serious fungal diseases affecting citrus worldwide. Various citrus diseases, e.g., root rot, canker, damping-off, and wilt, have been linked to *Fusarium* species. The symptoms of dry root rot include larger roots decay and trunk below bud union without any oozing of gum, reduced vigor in the canopy, dull green leaves, twig dieback and inhibited new growth. *Fusarium* spp., is a complex species that can remain in the soil for a long time and is transmitted by wind, machinery, and water. Dry root rot and *Fusarium solani* and *Fusarium oxysporum* are commonly found causing this disease globally. Biocontrol agents, such as *Trichoderma* species (*harzianum* and *viride*), have been used to manage several phytopathogens, including the causal agent of dry root rot, *Fusarium*, to promote eco-friendly practices instead of using harmful chemicals in agriculture. Additionally, the management of dry root rot disease caused by *Fusarium* spp., necessitates the optimization of irrigation and fertilization inputs. Along with sanitation practices, regular scouting and monitoring of key pests and diseases play a crucial role in enhancing control methods and minimizing pesticide usage.

**Keywords:** Fusarium spp., citrus, dry root rot, Biological control, Integrated disease management, horticulture, disease diagnosis, plant health, soil-borne pathogens.

## INTRODUCTION

Citrus fruit is one of the major horticultural crops that is grown worldwide and is also considered one of the most highly demanded commodities in the world (Sagar et al., 2018). Citrus fruits, classified under the family Rutaceae, encompass a diverse range of fruits and hold the distinction of being the largest genus within any fruit family. Notable species within this genus include lemon, lime, sweet orange, sour orange, tangerine, grapefruit, citron, and shaddock.

Among these species, sweet orange stands out as the primary fruit crop, contributing approximately 70% to the overall global citrus production (Hussain *et al.*, 2021). Citrus fruits come in a variety of colors, sizes, and shapes and are delicious, appealing, and high in nutrients. Along with vitamins A and B, they are one of the best sources of vitamin C (Goldenberg *et al.*, 2018). They also contain 5-8% sugar and significant levels of minerals like calcium, phosphorus, and iron, all of which are important for maintaining healthy human vitality. Citrus is one of the most significant fruit crops

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in the world. The exact origin of citrus fruit is still under discussion, but it is assumed that citrus originated from Southeast Asia and spread worldwide under sub-tropical and tropical conditions where the climate and soil are favorable for yield and growth (Taylor et al., 2019). Citrus production is low due to many biotic and abiotic factors (Mubeen et al., 2024; 2015a, b). Fusarium spp., is the disease causal agent of Dry root rot of citrus (DRR), which is frequently spread in nurseries and orchards. Dry root rot, produced by Fusarium species, is one of the worst fungal diseases of citrus and can be a serious issue in many countries (Ploetz et al., 2007). Many economically significant crops, including citrus, vegetables, flowers, and field crops, may suffer from root rot due to Fusarium solani, which has a significant overall impact on production (Huang et al., 2021). Fusarium solani is an opportunistic pathogen that could also attack both people and animals and induce acute mycoses. The pathogen is a complex organism that can remain in the soil for a long time and is transmitted by wind, machinery, and water. On susceptible crops, the fungus produces a variety of destructive diseases, including wilt, gradual decline, and fruit rot. Dry root rot has been shown to cause a variety of symptoms, including wilt, gradual decline, fruit rot, and dieback over most citrus cultivars (Kaczmarek et al., 2019). Fusarium spp., the offenders behind citrus DRR, which is highly virulent, can be triggered by a variety of stresses (Guthman, 2019). These stresses include root wounds from insects, rodents, weeding operations or insects, inadequate root aeration, inappropriate fertilizing, conflict with grafting, and association with other diseases of citrus roots, e.g., Phytophthora spp. and Tylenchulus semi penetrans (Dreistadt, 2014). Water stress can also predispose the plant to infection by Fusarium spp., causing the disease to spread across the field (Marek et al., 2013). In the absence of stress, the pathogen can colonize roots, resulting in an infection that doesn't cause symptoms. It has been demonstrated that Fusarium spp. works by diminishing the tree's starch stores under extreme conditions. Additionally, several elements, such as inadequate drainage, excessive watering, and inadequate aeration, may raise the risk of Fusarium spp. infestations on citrus plants (Ventura et al., 2019). Root rot disease has been found to be more common in trees under environmental stress than in plants that are not under stress. In response to stressful circumstances, F. solani appears to be a predominant colonizer of citrus roots with lower starch stores in the tree. Symptomatic infections on citrus seedlings cause dry root rot (Ezrari et al., 2021). Fusarium species are generally found in citrus orchard soils (Yaseen et al., 2010). Fusarium DRR is a destructive and sporadic disease that occurs in orchards of citrus affected by other factors such as biotic and abiotic factors. Biotic factors include diseases like Phytophthora spp., and nematode infections, while abiotic factors include temperature, mechanical root injury, salinity, waterlogging (Khouja et al., 2008). Several Fusarium species

are suspected to be the causal agents of dry root rot disease, with Fusarium solani and Fusarium oxysporum being the most commonly isolated from affected citrus trees (Jaouad et al., 2020). F. oxysporum is a common and important agricultural pathogen, isolated from affected citrus roots and rhizosphere and widely dispersed around the world (Ezrari et al., 2022). The phytopathogenic fungus Fusarium semitectum and Fusarium equiseti are infrequently isolated from citrus roots but are commercially significant species linked to root rot and vascular wilt in various crops (Logrieco et al., 2003). F. equiseti can harm a variety of crops in the field and during storage, including maize, rice, and wheat (Nagaraja et al., 2016). All Fusarium spp. isolates tested were harmful to tomato and melon (Palmero et al., 2011). Fusarium spp., are very common pathogens causing post-harvest diseases. That cause rot on tomatoes and other perishable fruits and vegetables (Sinha et al., 2019). Ahsen et al. (2019) describe the evaluation of exotic citrus rootstocks against Fusarium spp., in Pakistan. Citrus is a major fruit crop grown commercially, and citrus rootstocks are significant in terms of output and disease tolerance or resistance. Employing resistant/tolerant rootstocks is an accurate way of preventing Fusarium-related risks to citrus production. DRR of citrus is caused by Fusarium spp. and is one of the most dominant causes of citrus plant death. The main objective of this study is to comprehensively investigate the pathogenic Fusarium spp., involved in causing citrus root rot, as well as to evaluate biocontrol agents such as Trichoderma spp. and nonpathogenic Fusarium species for controlling citrus root rot disease caused by Fusarium species.

Nutritional Aspects of citrus: Citrus fruits do not raise the acidity of the body. In comparison to stomach acid hydrochloric acid, these acids are quite mild (Silalahi, 2002). Potassium salts are the predominant type of acids (with Na or K cation). Potassium and sodium are alkaline metals, and their salts are expelled in the urine or perspiration (Kaur et al., 2018). Citrus fruits are low in fat and can be used as a snack in between meals. Consequently, we can avoid from cholesterol and saturated fats, which both increase the risk of heart disease. (Zelber-Sagi et al., 2017). Citrus fruits, unlike many other foods, are not a strong source of protein; hence they do not constitute a protein source in the diet from a nutritional standpoint (Jacobs et al., 2013). As a gift from nature, medicinal plants enable us to have healthy, diseasefree lives. A range of secondary metabolites from plants are a source of medications, fragrances, flavors, agrochemicals, pigments, food additives and biopesticides (Mirunalini et al., 2013). Health-promoting chemicals and phytochemicals are found in citrus. Citrus fruits and beverages includefiber, sugars, vitamin C, folate, potassium, calcium, niacin, thiamine, vitamin A, vitamin B6, phosphorus, copper, magnesium, pantothenic acid, riboflavin and many phytonutrients. These nutrients are important for



the body to work effectively, but some offer more protection rather than nutrition alone against chronic diseases.

Citrus industry in Pakistan and its significance: Pakistan has a diverse agroecological setting that supports the production of roughly 30 different types of fruits. Citrus, mango, dates. guava, and apple make up more than 75% of the total yearly production (Shahi, 2016). At this time, Pakistan is included in the top 11 citrus-producing countries in the world (Naseer et al., 2019). A total of 199,400 hectares are used for citrus cultivation, with an estimated 2.29 million tons produced annually (Abdulkadir et al., 2017). Pakistan's fruit economy is worth more than 65.5 billion Pakistani rupees (PKR), with 6.64 million tons produced and 5.4 billion PKR obtained from fresh fruit exports in 2004-05. Only citrus fruits account for one-third of the overall fruit export value (Tahir, 2014). Pakistan produces 3-4% of the world's citrus fruits and exports roughly 0.8 percent of the crop (Iqbal et al., 2009). Pakistan is the world's sixth-greatest producer of Kinnow and oranges, with 2.1 million tons produced (Siddique et al., 2018). Although consumption per capita is rising in emerging nations, citrus is primarily preferred in industrialized nations (Liu, 2003). According to FAO, consumption of fresh oranges is declining in developed nations and rising in newly industrialized nations like Mexico, India, Argentina, Brazil, and China. The expansion in mandarin production, which includes tangerines at the expense of fresh oranges, was one of the key market developments during the last two decades of the twentieth century (Ollitrault et al., 2012). Citrus juice consumption has risen as a result of technical advancements and improvements in quality (Corato, 2020). Additionally, 95% of citrus fruit is produced in Punjab, and around 70% of citrus is Kinnow (Ahmad et al., 2018). Citrus fruits account for around 40% of the total fruit production in Pakistan. Citrus fruit is grown in Pakistan's four provinces because of its good growing circumstances and ample water. Around 95% of the crop is produced in Punjab (Sharma et al., 2017). Over 70% of the citrus produced in Pakistan is produced by the major cultivar Kinnow Mandarin, which holds a monopoly in the country.

Major citrus diseases globally: Citrus species are endangered by a number of diseases, particularly those caused by viruses, bacteria, nematodes, and fungi, despite the fact that these crops are economically significant and widely distributed (Abd-Elgawad et al., 2020). The citrus fruit sector is plagued by these phytopathogens. One major issue that may restrict the yield of citrus around the world is citrus dieback. The root causes of citrus dieback have been determined to be several soil-borne diseases. The disease known as dry root rot is becoming another significant global problem due to the expansion and severity of citrus cultivation (Ezrari et al., 2022). Most significant Dry root rot, stem and root rot, vascular wilt, twig rot, twig blight and dieback are examples of pathogen groups that cause extreme symptoms. One of the main causes of citrus decline may be dry root rot, which is

more common when citrus plants are subjected to biotic and abiotic conditions. However, it is well known that citrus is susceptible to a variety of fungal diseases, such as dry root rot, which poses a severe risk to citrus plantations all over the world due to its rising prevalence under biotic and abiotic conditions.

Geographical distribution and global prevalence of Fusarium species: Yaseen et al. (2010) elaborate on citrus plant diseases such as Fusarium root rot and dry rot, which quickly kill plants when they are subjected to biotic or abiotic stress. Fusarium species were found in industrial citrus farms and nurseries in Egypt, Greece, Italy, and Tunisia. Based on visual and genomic traits, three Fusarium species (F. proliferatum, F. oxysporum and F. solani) were identified. The most frequently isolated species, however, F. oxysporum and F. solan were grouped into nine clusters based on analysis of the β-tubulin and α-tubulin Elongation Factor loci. Kurt et al. (2020) describe Fusarium dry root rot as one of the deadliest citrus diseases in Turkey. Fusarium solani has been identified through sequencing of ITS and tef-1 nucleotides. Ibrahime et al. (2019) demonstrated that serious diseases affecting many groves in Egypt include T. semi penetrans and F. solani, which are responsible for the dry root rot and slow of citrus trees. In Tunisia, studies were conducted to evaluate the seasonal variation and population of Fusarium spp. on three distinct citrus rootstocks (Troyer, Carrizo citranges and Sour orange) in three Tunisian citrus nurseries. Three Fusarium species from Tunisian citrus nurseries were identified based on visual and genetic characteristics: F. oxysporum, F. proliferatum and F. solani (Khouja et al., 2008). Citrus dry root rot has been shown to cause symptoms on most common citrus types in South Africa, Oman, Italy, Tunisia, Pakistan, Greece, Australia, Egypt, California, Florida, and Texas in the United States, including fruit rot, wilt, dieback, and gradual decline (Kurt et al., 2020). Fusarium solani is found to be the main pathogen responsible for dry root rot. Sankar et al. (2022) also elaborate that in India, Fusarium solani is a significant pathogen that triggers the citrus disease commonly known as dry root rot.

Disease symptoms and Morphological Characteristics: Fungi cause various destructive diseases e.g., wilt, fruit rot and slow decline on host crops. F. solani produces light purple inner vascular discolorations in citrus trees, leading to dry decay on fibrous roots, larger roots, and the trunk. Infected trees experience wilting, necrosis, chlorosis, leaf drop, and rapid death. Healthy plants can abruptly wilt and die, with black and rotted roots and brown vascular discoloration in the stem. F. solani infection is responsible to cause rots on scaffold roots and crown, with fibrous root rot associated with reduced growth (Kurt et al., 2020). Signs of root rot, necrotic roots, purple-colored wood, chlorosis, and dieback can lead to the decline of healthy trees, which could abruptly collapse and fall. The first signs of DRR are yellowing of the significant veins of the leaves and chlorosis.



Other symptoms include rotting, discoloration, decay, browning of wood and damage to the base of trunk and roots. wilting-like symptoms with browning of scaffold's crown and roots, gum leakage, and dieback until tree is entirely dead. Fusarium solani and F. oxysporum were consistently isolated from the primary and feeder roots of symptom-free rough lemon seedlings. Light and electron microscopy were used to investigate the infection process and histopathology of F. solani-infected roots. There is an indication that F. solani may infect citrus roots even in the absence of damage or stress and also that the fungus can exist in root tissues without causing apparent signs of disease. Dry root rot symptoms lead to decay of trunk and larger roots below bud union without oozing or any kind of gum, while the canopy of the plant has reduced vigor, the leaves turn a dull green color, new growth is restricted, and twig dieback occurs. On adult plants, the most obvious symptom is the lethal disintegration of the citrus trees (Rodrigo, 2000). For years, diseases can be symptomless, but under dry hot conditions, sudden collapse arises (Jaouad et al., 2020). Citrus dry rot attacks the root system, turning the affected root purple to grayish black. Symptoms include a reddish-purplish to grayish color. According to Kunta et al. (2015), brown discoloration extends into the trunk of the tree, stopping at the union of buds, and one or more major root structures are often blackened or dead. All kinds of rootstocks are vulnerable to DRR, but lemon fruit is most vulnerable to DRR (Graham et al., 1995).



Figure 1. Disease symptoms on Citrus plants and roots.

In 1988, Labuschagne and Kotze proclaimed that fungusinoculated seedling symptoms are identical to those on field trees (Ezrari et al., 2022). At an extreme stage of decline, canopy has scanty and thin appearance. Kurt et al. (2020) explains that one of the most fatal diseases affecting citrus in Turkey is Fusarium dry root rot. Some citrus orchards have experienced a dramatic deterioration since December 2015, resulting in vascular discolorations of light purple and fibrous roots dry decay. Off white-colored colonies formed ovoid, ellipsoid, or reniform microconidia with 1-2 cells. The macroconidia were slightly curved or straight, 3-5 septate, and hyaline.

Environmental Factors effecting disease incidence: Ezrari et al. (2021) described the optimal and marginal levels of Neocosmospora growth, F. solani, and other Fusarium species (F. oxysporum and F. equiseti) affiliated with citrus DRR, including the impact of environmental factors such as water potential (w) and temperature. The study also examined the effects of water potentials, incubation temperature, and their combination on the in vitro radial progression of development and lag stage of Fusarium species. Another study suggested that a range of environmental factors and soil conditions influence the emergence of the disease (Kumar et al., 2016).

Bio-compost and Biological Control: El-Mohamedy et al. (2013) demonstrated that plant diseases carried by pathogens residing in the soil can significantly reduce agricultural crop output and yield. Chemical fungicides and methyl bromide applications are hazardous to human health and contribute to ecological pollution. Current farming techniques strive to decrease or eradicate the use of fungicides by implementing eco-friendly safe control methods. In the rhizospheric soil of trees treated with bio compost, the Fusarium species population density was significantly reduced, while the population density of Trichoderma species was enhanced. This suggests that bio compost can be safely used commercially as a replacement for methyl bromide and fungicides for controlling soil-borne plant infections. It can be assumed that applying bio composted agricultural wastes will have the same impact as applying the fungicide Topsin-M. El-Mohamedy et al. (2016) discussed the serious diseases that attack several groves in Egypt, including Fusarium solani and Tylenchulus semi penetrans, which are the agents of the citrus diseases known as dry root rot and gradual deterioration. Using sour orange (Citrus aurantum) seedlings in a greenhouse, they assessed the efficacy of handling both diseases synergistically. The soil can be treated with biological agents and compost separately or jointly. The population densities of T. semi penetrans and the linear growth of F. solani were both decreased. With Bacillus subtilis, Trichoderma harzianum and T. viride, the linear growth was completely inhibited. By applying Bacillus subtilis, Trichoderma harzianum, or T. viride to the compost, the speed of nematode growth could be decreased respectively. Biological management is an effective control strategy that has proven its significant promise as a consistent



eco-friendly process for managing Dry root rot diseases. Biological control of *Fusarium* spp., with different antagonists and their mode of actions (Table 1).

Depending on an in vitro dual culture bioassay with *F. solani*, antagonist bacteria isolates were tested by Kurt *et al.* (2020).

Results suggested that antagonist bacteria varied greatly in their capacity to manufacture antimicrobial properties such as iturin, bacillomycin, fengycin, surfactin, and bacteriocin. Additionally, these antagonist microorganisms have the potential to be utilized as bio-fertilizer in sustainable

Table 1. Biological control of Fusarium spp. with different antagonists and their mode of action,

Disease	Pathogen	Antagonists	Mode of action and application	References
Dieback of avocado	Fusarium spp.	Bacillus spp.	<ul> <li>Isolates can produce volatile organic compounds (VOCs) like ketones, pyrazines, and sulfur-containing compounds.</li> <li>Mycelial growth of <i>Fusarium</i> spp. is inhibited and leading to morphological changes in the hyphae.</li> <li>Bacterial volatiles have been found to be effective against <i>Fusarium</i> spp. dieback.</li> </ul>	Guevara- Avendaño <i>et</i> <i>al.</i> , (2019)
Dry root rot	F. solani	Bacillus subtilis Pseudomonas fluorescens	<ul> <li>Reduce the incidence of dry root rot in treated citrus trees.</li> <li>Increase the density of citrus stands and yield.</li> <li>The high concentration of these two antagonists allows them to effectively adapt to new environments, enhancing their efficacy.</li> </ul>	Abd-Elgawad et al., (2020)
Dry root rot	F. solani	Penicillium citrinum T. pseudokoningii Aspergillus flavus	• Inhibition of <i>in vitro</i> mycelial growth of <i>F. solani</i> , the causal organism of citrus dry rot.	Khanzada <i>et al.</i> , (2016)
Potato dry root rot	F. solani	Rahnellaaquatilis	<ul> <li>The strain can produce siderophores and express genes for antibiotic compounds like phenazine (PHZ) and volatile compound HCN.</li> <li>Reduce disease severity and improve plant health.</li> </ul>	<u>Bahroun <i>et al.</i></u> (2018)
Fusarium root rot in corn, soybean, and Wheat	F. graminearum F. oxysporum	Burkholderia spp. Bacillus spp. Trichoderma spp.	• Inhibition of <i>Fusarium</i> pathogenic isolates growth and development.	<u>Parikh <i>et al.</i>,</u> (2018)
Fusarium wilt of banana	F. xysporum f. sp. cubense (Foc)	Pseudomonas spp. Trichoderma spp. Arbuscular mycorrhizal fungi Bacillus spp. non- pathogenic Fusarium strains	<ul> <li>Pseudomonas spp. achieved a 79% biological control efficiency, while endophytes and Trichoderma spp. reached up to 70%.</li> <li>Decreased efficacy (42–55%) with the use of arbuscular mycorrhizal fungi, Bacillus spp., and certain non-pathogenic Fusarium strains.</li> </ul>	Bubici <i>et al.</i> , (2019)
Fusarium wilt of cape gooseberry	F. oxysporum f. sp. physali	Bacillus velezensis Bs006	<ul> <li>The effectiveness of the antagonist was lower in sterile soil conditions compared to non-sterile conditions, and this effect was influenced by the pathogen concentration.</li> <li>High levels of bacterial supernatant can accelerate disease progression.</li> <li>The activity of PGPR is influenced by the presence of pathogens in the soil.</li> </ul>	<u>López-</u> <u>Bautista et al.,</u> (2020)
Fusarium crown and root rot disease of tomato (FCRR)	F. oxysporum f. sp. radicis- lycopersici	Pseudomonas spp. Bacillus spp.	<ul> <li>These strains produce various antibiotics, lytic enzymes, secondary metabolites, siderophores, hydrogen cyanide (HCN), and salicylic acid. They also have the ability to fix nitrogen and solubilize phosphate, as well as produce certain volatile compounds.</li> <li>Protects tomato plants from FCRR disease and enhances growth in greenhouses.</li> </ul>	Zhang et al., (2015)
Root rot in lettuce	F. oxysporum	Trichoderma harzianum Bacillus subtilis	<ul> <li>Enhanced growth (height and weight) in treated plants.</li> <li>Enhanced levels of photosynthetic pigments and primary metabolites in treated plants.</li> <li>Enhancing plant defense mechanisms.</li> </ul>	Alamri <i>et al.</i> , (2019)



agriculture. A previous study highlighted the role of T. harzianum, B. subtilis, Paecilomyces lilacinus and Streptomyces griseus as biocontrol agents against T. semi penetrans, a citrus nematode, and also demonstrated that the combination of Nemastop and T. harzianum is very effective in controlling T. semi penetrans populations. Meanwhile, the incidence of F. solani was decreased by B. subtilis or T. harzianum. Previous studies demonstrated that a variety of Fusarium species are responsible for this disease. The two Fusarium species most commonly linked to citrus decline are F. solani and F. oxysporum (Rehman et al., 2012). Numerous citrus diseases, comprising root rot, canker damping-off, and wilt, have also been linked to Fusarium species. Trichoderma strains have been found to significantly reduce the growth of the Fusarium isolates (Sukmawati et al., 2017). Trichoderma spp., such as Trichoderma harzianum and Trichoderma viride were proven to be competitive biocontrol agents against Fusarium spp., which are involved in causing DRR of citrus. These biocontrol agents can be successfully used to manage the dry root rot citrus caused by Fusarium oxysporum as an alternative to harmful chemicals that have hazardous effects on the health of humans and the environment (Sukmawati et al., 2017). El-Mohamedy, (2009) explain that two approved biocides (Rhizo-N and Plant Guard) and two bioagents (Bacillus subtilis NB and Trichoderma harzianum NB) were studied in a greenhouse with artificially infected soil for their effectiveness in eliminating Fusarium RRD on different citrus rootstocks. Kavitha, (2010) conducted a field study to determine the effectiveness of Trichoderma viride against the dry root-rot of acid lime (C. aurantiifolia) induced by F. solani. In reality, increasing temperatures, root activity, the constitution of the soil mix, irrigation strategies, and fertilizers caused a large rise in Fusarium spp. populations. The amount of feeder root infection and Fusarium population in the soil were shown to be positively correlated (r = 0.74). Very few citrus trees in the field have the specific signs of dry root rot. These findings indicate that dry root rot does not pose a danger to citrus farming in Tunisia, where practically all citrus types are grafted onto sour orange rootstock.

Conclusion: One of the main fruit crops in Pakistan is citrus, which has seen an increase in export in recent years. Fusarium spp., are mainly involved in causing dry root rot of citrus. Dry root rot, produced by Fusarium spp., is one of the worst fungal diseases of citrus and has been a serious issue in some nations. Fusarium spp., is a complex species that remains in the soil for a long time and is transmitted by wind, machinery, and water. The overcoming of constraints in the field depends on implementing effective disease management strategies, such as using fungicides and pesticides to control Phytophthora root rot. Biocontrol agents, such as Trichoderma have been tested as a means to manage this pathological issue and promote eco-friendly practices in the current era, instead of relying on harmful chemicals. Furthermore, it is suggested

that an integrated management approach for citrus root rot diseases must include reducing citrus rootstocks' reactivity to root rot pathogens and citrus nematodes.

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