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Pathobiome: Driven Innovations in Sustainable Olive Disease Management

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Abstract

The olive industry is facing considerable pressure from emerging diseases caused by various pathogens, including *Xylella fastidiosa*, *Verticillium dahliae*, and *Fusarium oxysporum*. This has led to increased interest in developing environmentally friendly, sustainable management strategies for these diseases through biological control and resistant rootstocks. The recent researches have emphasized the need to know the pathobiome, the complex community of microorganisms that coexists with the plant and affects its health and production. Pathobiome microorganisms may be beneficial and detrimental to the plant. In addition, the pathobiome is important to understand in order to create new varieties of olive, resistant to diseases. Although there are serious challenges, the current developments in the field of sequencing technologies and bioinformatics tools are aiding in overcoming these challenges. It is important to develop sustainable and environmentally friendly methods, which take into consideration both the plant and the associated microorganisms, so as to ensure effective disease control measures and the formulation of new disease-resistant olive varieties. The review paper will inform about the importance of the pathobiome in the establishment of effective disease management strategies and indicate challenges and opportunities in the field.

Keywords: pathobiome; microbiome; pathogens; olives; sustainability; disease

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1. Introduction

The olive industry is a significant part of the world economy. It is estimated that there are about 10 million hectares of olive orchards that yield more than 20 million tons of olive products annually (FAOSTAT, 2021). The industry is, however, experiencing major challenges in the form of new diseases that are a result of different pathogens. As an example, the bacterium *Xylella fastidiosa* causes the Olive quick decline syndrome (OQDS), which can kill trees in a few years (Picciotti et al., 2021). There is a growing need to develop safe and sustainable control approaches, including biological control and resistant rootstocks, to control such diseases because chemical treatments have many limitations (Zicca et al., 2020).

Recent research has emphasised the need to know the pathobiome, the complex of microorganisms that interacts with the plant and determines its health and productivity (Berg et al., 2020). These microorganisms can be utilised as bio-control agents and can have either positive or negative effects on plants. *Pseudomonas fluorescens* and *Trichoderma harzianum*, for instance, have been shown to alleviate the symptoms of *Phytophthora* root rot and *Verticillium* wilt in olive trees (Montes-Osuna & Mercado-Blanco, 2020). Koch's postulates remain the foundational criteria for establishing a causal link between a specific microbe and a disease (Moriarty et al., 2020). However, modern microbiology increasingly views them as a starting point rather than a universal rule, especially in the context of complex systems like the pathobiome. The pathobiome is defined as a holistic approach to disease that moves beyond the traditional "one pathogen-one disease" model (Koch's postulates). It views disease

as the result of complex interactions between a host, the environment, and a diverse community of microorganisms, including bacteria, viruses, fungi, and parasites.

The pathobiome microorganisms can have an effect not only directly on the plant but also indirectly on the production of volatile organic compounds (VOCs), which are also a part of the defence mechanisms of plants and can attract or repel other organisms. The VOCs of bacteria have been detected using metagenomic and volatilomic methods and applied as a form of biocontrol agent to the pathogens of olive trees (Vaccalluzzo et al., 2022). *Bacillus subtilis* and *Bacillus thuringiensis*, for instance, have been found to inhibit the growth of *Xylella fastidiosa* by emitting volatile organic compounds (VOCs) (Sathishkumar et al., 2019). The BeXyl project (2022–2026) is the leading European initiative applying the pathobiome concept. Scientists are developing synthetic microbial consortia to engineer the olive pathobiome, introducing stable groups of beneficial endophytes that directly outcompete *Xylella fastidiosa* for space and nutrients within the xylem (BeXyl Project, n.d.).

New developments in bioinformatics systems and sequencing technology are contributing to the resolution of these issues. For instance, high-throughput sequencing technology has enabled the analysis of the whole microbial community within a particular sample, giving a more detailed view of the pathobiome (Berg et al., 2020). (Mercado-Blanco et al., 2018) The discovery of a few microbial strains associated with disease-resistant olive varieties is noteworthy. The strains can be used to create new olive varieties that are resistant to particular pathogens. By introducing genes that produce

antimicrobial peptides, genetic engineering techniques like CRISPR-Cas9 have also been used to increase olive trees' resistance to *Xylella fastidiosa* (Deng et al., 2021). Moreover, they can be used to define the patterns and relations in the pathobiome data, with the assistance of machine learning algorithms and other computational tools (Catalano et al., 2022). The objective of this study is to discuss the challenges and opportunities in this field, as well as the significance of the pathobiome in the creation of efficient strategies for dis-ease management.

2. Pathogens and diseases affecting olive trees

The olive tree is prone to several bacteria (*Xylella fastidiosa*), fungi (*Verticillium dahliae*), viral organisms (olive latent virus), nematodes (*Pratylenchus* spp.) and oomycete pathogens (*Phytophthora cinnamomi*) that have serious effects on the production of the olive tree (Table 1)

Annexure (A)

3. Economic impact and geographical distribution of olive

In the past two years, numerous olive-growing areas, particularly in the Mediterranean, have been experiencing growing economic and geographic stress due to devastating diseases, with *Xylella fastidiosa* being the most serious menace (EPPO, 2024). Nowadays, over 450 vulnerable plant species are included on updated lists, which indicates their impressive versatility and ability to propagate in a wide range of habitats (Cavaliere et al., 2025). The Apulia region of Italy has suffered the most, as over 21 million olive trees have been affected (European Parliament, 2024). On a wider European scale, estimates suggest that *Xylella* would result in a loss of production of approximately EUR5.5 billion and an

additional EUR0.7 billion in exports each year, unless effectively contained, making it one of the most economically crippling plant diseases to have hit Europe (European Parliamentary Research Service, 2024).

4. Beneficial microorganisms associated with olive trees

The Mycorrhizal fungi are symbiotic fungi, including *Pseudomonas*, *Enterobacter*, and *Bacillus* species, which develop symbiotic relationships with plant roots to enhance the nutrient uptake and stress resistance of plants. (Porrás-Soriano et al., 2009). Many bacterial species have been identified in the rhizosphere and phyllosphere of olive trees and are important for the production of antimicrobial compounds, stimulation of plant growth, and induction of systemic resistance to pathogens (Balthazar, 2022). Inoculation with *Glomus mosseae*, *Glomus intraradices*, or *Glomus claroideum* has been reported to enhance plant growth and nitrogen, phosphorus, and potassium uptake in saline and non-saline nursery and subsequent irrigated desert orchards (Porrás-Soriano et al., 2009; Dag et al., 2009), respectively. A study on the olive tree roots that had been inoculated with *Rhizophagus irregularis* (Dede & Güven, 2023) has shown higher photosynthetic rate, nutrient uptake, and carbohydrate concentration in the leaves and roots.

Recent work on *Streptomyces staurosporininus* Z.55.4.3 and *Streptomyces eurocidicus* Z.56.2.2 have shown high antagonistic activity against pathogenic bacteria (Tekaya et al., 2017). It has also been demonstrated that antagonistic rhizosphere and endosphere bacteria synthesise exoenzymes and secondary metabolites capable of proteolytic (73.3%), lipolytic (76.4%), and

chitinolytic (56.8%) activity, and synthesise IAA and siderophores at 75.3% and 76% rates, respectively (Aranda et al., 2011). *Paenibacillus polymyxa* strain n PIC73 has been shown to inhibit a variety of olive pathogens in vitro and has shown a high potential to be used as a biocontrol agent against *Verticillium dahliae* fungus (Gómez-Lama Cabanás et al., 2018).

Annexure (B)

5. Interactions and defence mechanisms between the pathobiome and the host plants:

Olive trees can be exposed to pathogens by wounds, natural orifices, or insect vectors. Trees use different ways to protect against pathogen infection, such as physical resistance, chemical resistance, and systemic resistance (War et al., 2012). The physical barriers consist of the plant cuticle, which does not allow the entry of pathogens into the plant, and the plant cell wall, which forms a physical barrier against invasion of pathogens. The synthesis of antimicrobial compounds and phytohormones is a chemical defence and can suppress the growth of pathogens and induce systemic responses to the presence of pathogens. Systemic responses involve activation of genes that produce defence-related proteins, including pathogenesis-related proteins (PRs). These proteins can directly inhibit pathogen growth or cause systemic acquired resistance (SAR) (Métraux, 2001) (Table 3).

Annexure (C)

6. Management of the olive tree's pathobiome

6.1 The effectiveness of biocontrol agents

In addition to competing with harmful bacteria for resources and space, beneficial microbes produce antibiotic substances that stop pathogens from growing (Köhl et al., 2019). The bacterium *Pseudomonas fluorescens* is one of the biocontrol agents

for managing olive tree diseases that has received the most research. This bacterium has proven to be effective in the protection of olive trees against numerous pathogens, such as *Verticillium dahliae* against *Fusarium oxysporum* (Gómez-Lama Cabanás et al., 2018). Another bacterium with the potential to be utilised as a biocontrol agent against olive knot disease is *Bacillus subtilis* (Krid et al., 2012). In addition to bacteria, fungi have also been investigated as potential biocontrol agents. *Verticillium dahliae*, *Fusarium oxysporum* (Gómez-Lama Cabanás et al., 2018), and *Phytophthora cinnamomi* were among the olive tree pathogens that could be controlled by the fungus *Trichoderma harzianum* Balla et al., 2021. Although biocontrol agents may be effective against olive tree diseases, many factors, including host genotype, environmental conditions, and the presence of other microorganisms, can affect their efficacy. In addition, additional research is required to develop novel and effective biocontrol agents and maximise their use. The olive tree's microbiome is significantly influenced by management practices like fertilisation, irrigation (Delitte et al., 2021) and organic matter (Köhl et al., 2019).

7. Future directions in the study of the pathobiome of olive trees

The pathobiome of olive trees is still a mystery, and more research is needed to understand this intricate system. The development of molecular and genomic methods has made it easier to study microbial communities and how they interact with the plants they live in. One of the potentially promising areas of study is the pathobiome's role in ecosystem dynamics and interactions between plants and microbes. The development of disease management strategies based on the microbiome is another area of research that

holds promise for success. Even though the olive tree's pathobiome hasn't been fully figured out yet, recent research has shown that some microorganisms can help prevent pathogen infection, improve plant development, and make the plant more resistant to stress.

8. Conclusion

The pathobiome of the olive tree is a dynamic, complex system crucial to the plant's health and productivity. Although much remains to be understood about this system, recent studies have provided valuable insights into the diversity and roles of the microorganisms that constitute the pathobiome. Numerous bacterial, fungal, and viral pathogens pose a threat to olive tree health, and their interactions with the pathobiome can have significant consequences for disease development and plant health. In addition to providing defence against pathogens, the beneficial microorganisms associated with olive trees, including mycorrhizal fungi and other bacteria and fungi, can play an important role in plant growth and stress tolerance. Understanding the interactions among infections, helpful microorganisms, and host defence mechanisms can inform the creation of more effective management strategies.

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Abbreviations

The following abbreviations are used in this manuscript:

VOCs: Volatile organic compounds

OQDS: Olive quick decline syndrome

AMF: Arbuscular mycorrhizal fungi

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Annexure (A)

Table 1: Different pathogens and their responses.

| Sr/no | Pathogen | Type of Pathogen | Response | Ref. |
|-------|----------------------------|--------------------|--|-----------------------------|
| 1. | Fusarium oxysporum | Fungal pathogen | Causes Fusarium wilt disease in olive trees by infecting the roots and interfering with the absorption of water and nutrients. | (Bizos et al., 2020)] |
| 2. | Verticillium dahliae | Fungal pathogen | Causes Verticillium wilt disease in olive trees. It also infects the roots and can cause the tree to grow more slowly, turn its leaves yellow, and eventually die. | (Keykha Saber et al., 2018) |
| 3. | Phytophthora cinnamomi | oomycete pathogen | It infects the roots and interferes with the absorption of water and nutrients, leading to wilt and eventual death of the tree. | (Balla et al., 2021) |
| 4. | Xylella fastidiosa | bacterial pathogen | Leads olive trees to develop OQDS. It infects the tree's xylem tissue and causes leaf scorch, wilting, and eventually death. | (Ravindra et al., 2017) |
| 5. | Meloidogyne spp. | Nematodes pathogen | Root-knot nematodes that infect the roots of olive trees cause gall formation, stunted growth, and reduced yield. | (Ali et al., 2014) |
| 6. | Pratylenchus spp. | Nematode pathogen | Infect the roots of olive trees and cause damage to the root system, leading to reduced growth and yield. | (Downer & Perry, 2019) |
| 7. | Armillaria mellea | Fungal pathogen | Causes Armillaria root rot in olive trees. It infects the roots and causes decay, leading to reduced growth and eventual death of the tree. | (Hu, 2012) |
| 8. | Phymatotrichopsis omnivora | Fungal pathogen | Causes cotton root rot disease in olive trees. It infects the roots and can cause sudden wilting and death of the tree. | (Mbaka, 2013) |
| 9. | Phytophthora nicotianae | Oomycete pathogen | Causes root rot in olive trees, especially in poorly-drained soils. | (Moral et al., 2017) |
| 10. | Pythium spp. | Fungal pathogen | Cause damping-off disease in young olive trees by infecting the roots and causing decay. | (Balla et al., 2021) |
| 11. | Botryosphaeria spp. | Fungal pathogen | Cause canker disease in olive trees. They infect the bark and can cause dieback of branches and eventually the entire tree. | (Mbaka, 2013) |
| 12. | Colletotrichum spp. | Fungal pathogen | Causes anthracnose disease in olive trees. They infect the leaves, twigs, and fruit and can cause spots, lesions, and defoliation. | (Moral et al., 2017) |
| 13. | Sclerotinia sclerotiorum | Fungal pathogen | Causes stem rot in olive trees. It infects the stem and can cause the tree's wilt, collapse, and death. | (Ruano-Rosa et al., 2017) |

| | | | | |
|-----|-------------------------|-----------------|---|-------------------------|
| 14. | Macrophomina phaseolina | Fungal pathogen | Cause charcoal rot in olive trees. It infects the roots and can cause sudden wilting and death of the tree. | (Sergeeva et al., 2005) |
|-----|-------------------------|-----------------|---|-------------------------|

Annexure (B)

Table 2: Beneficial Microorganisms and their responses.

| Sr. No | Beneficial Microorganisms | Type of Microorganism | Response of Microorganisms | Ref. |
|--------|------------------------------------|-----------------------|---|-----------------------------------|
| 1. | Arbuscular mycorrhizal fungi (AMF) | Fungi | Creates symbiotic connections with plant roots and aids in enhancing the absorption of nutrients, especially phosphorus. Increase the plant's resistance to environmental stressors like drought as well. | (Porras-Soriano et al., 2009) |
| 2. | Rhizobium spp. | Bacteria | Forms symbiotic relationships with legume plants and can fix atmospheric nitrogen, providing a source of nitrogen for the plant. | (Fernández-González et al., 2019) |
| 3. | Azospirillum spp. | Bacteria | Fix nitrogen from the atmosphere and create hormones that encourage plant growth to increase plant productivity. | (Bartolini et al., 2023) |
| 4. | Pseudomonas fluorescens | Bacteria | Produces antibiotics and other antimicrobial compounds that can protect the plant against pathogens. It can also enhance nutrient uptake and promote plant growth. | (Prieto et al., 2011) |
| 5. | Bacillus spp. | Bacteria | Produces antibiotics and other antimicrobial compounds that can protect the plant against pathogens. | (Cha et al., 2013) |
| 6. | Streptomyces spp. | Actinomycetes | Produces antibiotics and other antimicrobial compounds that can protect the plant against pathogens. | (Dede et al., 2020) |
| 7. | Trichoderma spp. | Fungi | Produces enzymes and other compounds that can degrade plant pathogens and induce the plant's natural defence mechanisms. | (Reghmit et al., 2021) |
| 8. | Penicillium spp. | Fungi | Produces antibiotics and other antimicrobial compounds that can protect the plant against pathogens. | (Robles et al., 2000) |
| 9. | Aspergillus spp. | Fungi | Aspergillus produces enzymes and other compounds that can degrade plant pathogens and induce the plant's natural defence mechanisms. | (Elias et al., 2016) |
| 10. | Actinomycetes spp. | Actinomycetes | Produces antibiotics and other antimicrobial compounds that can protect the plant against pathogens. | (Dede et al., 2020) |
| 11. | Burkholderia spp. | Bacteria | Produces antibiotics and other antimicrobial compounds. It can shield the plant from diseases. | (Bach et al., 2016) |
| 12. | Serratia spp. | Bacteria | Produces antibiotics and other antimicrobial compounds. It can shield the plant from diseases. | (Muller et al., 2007) |
| 13. | Enterobacter spp. | Bacteria | Fix atmospheric nitrogen. | (Upadhyay et al., 2011) |

Annexure (B)**Table 3:** Key pathogens and associated microbes as biocontrol agents.

| Pathogen (Olive) | Type | Beneficial Microorganism (Biocontrol Agent) | Mechanism | Ref. |
|---------------------------------------|-----------|---|---|-------------------------------|
| Verticillium dahliae | Fungus | Pseudomonas fluorescens PICF7 | Root colonisation induces systemic resistance | (Prieto et al., 2011) |
| Verticillium dahliae | Fungus | Trichoderma asperellum Bt3, T25 | Mycoparasitism overgrows the pathogen | (Carrero Carrón et al., 2016) |
| Verticillium dahliae | Fungus | Streptomyces spp. | Inhibits mycelium & microsclerotia | (Dede et al., 2020) |
| Pythium schmitthenneri | Oomycete | Bacillus amyloliquefaciens SF14 | Antifungal metabolites reduce root rot | (Cha et al., 2013) |
| Pythium schmitthenneri | Oomycete | Alcaligenes faecalis ACBC1 | Pathogen suppression inhibits mycelium | (Legrifi et al., 2022) |
| Pseudomonas savastanoi pv. savastanoi | Bacterium | Bacillus subtilis F1 | Antimicrobial compounds reduce knots | (Delitte et al., 2021) |
| Fusarium spp. | Fungus | Bacillus licheniformis | Inhibits mycelial growth | (Mina et al., 2020) |
| Fusarium spp. | Fungus | Enterobacter spp. | Suppresses root infection | (Muller et al., 2007) |