Microorganisms for Sustainability 40
Series Editor: Naveen Kumar Arora

Udai B. Singh · Pramod K. Sahu ·
Harsh V. Singh · Pawan K. Sharma ·
Sushil K. Sharma *Editors*

Rhizosphere Microbes

Biotic Stress Management

Springer

Microorganisms for Sustainability

Volume 40

Series Editor

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Microorganisms perform diverse roles on our planet most of which are important to make earth a habitable and sustainable ecosystem. Many properties of microorganisms are being utilized as low input biotechnology to solve various problems related to the environment, food security, nutrition, biodegradation, bioremediation, sustainable agriculture, bioenergy and biofuel, bio-based industries including microbial enzymes/ extremozymes, probiotics etc. The book series covers all the wider aspects and unravels the role of microbes towards achieving a sustainable world. It focuses on various microbial technologies related to sustenance of ecosystems and achieving targets of Sustainable Development Goals. Series brings together content on microbe based technologies for replacing harmful chemicals in agriculture, green alternatives to fossil fuels, use of microorganisms for reclamation of wastelands/ stress affected regions, bioremediation of contaminated habitats, biodegradation purposes. Volumes in the series also focus on the use of microbes for various industrial purposes including enzymes, extremophilic microbes and enzymes, effluent treatment, food products.

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Preface

For sustainable food production and food security at global level in the line of Sustainable Development Goals (SDGs), food supplies must keep pace with increasing population that can partially be accomplished by reducing the yield losses caused by the devastating pest and pathogens. Doubling in global food demand by 2050, changing climatic conditions impose a number of challenges towards agricultural sustainability. Today, crop production to fulfil food demands is being enhanced by the increasing application of agrochemical inputs, which act as plant growth regulators, plant nutrients supplier and plant protectors. Apart from increasing cost of production, excessive use of agrochemicals increases the possibilities of residual effects in agricultural commodities, land degradation and deterioration of environmental health. With the increasing world population and global demand for food, there is an urgent need to adopt sustainable approaches to ensure perpetual agricultural production with less or no use of agrochemicals. Besides urbanization, reduction in arable land and land degradation, numerous biotic stresses cause significant crop loss from field to storage. The biotic factors include insect-pest, pathogens, weeds and others including both vertebrates and invertebrates. The average yield losses due to biotic stress factors, i.e. insect-pest and disease, have been reported to be as high as 40% every year at global level (FAO 2015). Management of biotic stresses mainly relies on the use of toxic chemical pesticides and resistant plant varieties. The use of resistant plant varieties is an important approach for conferring agricultural sustainability. However, non-availability of suitable donor parents and the breakdown of resistance have still remained a great concern. Further, negative impact of plant protection agrochemicals on the non-target microflora and fauna, environment, animal and human health has forced researchers to explore alternative measures for management of biotic stresses of important crop plants. Among the more recent strategies, stress tolerance/resistance induced by inducers of microbial origin and/or rhizosphere microorganisms has emerged as a promising approach in crop protection. The multidimensional factors involved in microbial communities present in the ecosystems which can provide the answers to the current agricultural problems. Microbial communities play a significant role in microbe-microbe,

microbe-insect/pest and plant-microbe interactions which have not yet been fully exploited to harness their potential benefits to achieve agricultural sustainability. There are numerous microorganisms comprising fungi, bacteria, actinomycetes and cyanobacteria having mechanisms of plant growth promotion and biological control properties.

The rhizosphere is a micro-environment contrastingly different from non-rhizosphere. Plant rhizosphere is the battlefield for beneficial and harmful organisms. Microorganisms in the rhizosphere co-exist in perfect communities which show division of labour and different functions for microbe-plant interactions. The significance of microbe-plant interactions in the rhizosphere ecosystem is enormous for agricultural sustainability. The positive interactive effect of the beneficial rhizosphere microorganisms on plants is induction of plant growth, conferment of abiotic and biotic stress tolerance and modulation in several pathways of the plants for the proper establishment in all kinds of environments including degraded and contaminated soils. Moreover, interactions among microbes, plants, soil and insects play a crucial role in the rhizosphere ecosystem functioning and modulate the physico-biochemical properties of the rhizosphere soil. Further, the plant secretome influences the rhizospheric microbial communities by recruiting the specific microflora around the root system and interacting with them. However, rhizospheric interactions are quite complex and dynamic. It is rather difficult to elucidate as they take place under different circumstances and at different interfaces such as endosphere, rhizoplane and rhizosphere. In view of the above facts, large-scale exploitation of rhizospheric interactions is crucial for enhancing the agro-ecosystems resilience to biotic stresses by adopting novel microbe-based strategies for maximizing the sustainable food production under changing climatic conditions. Therefore, strategic and applied researches are essential to explore and exploit all root-associated microorganisms for harnessing benefits from all kinds of interactions for biotic stress management in low-input sustainable agriculture under changing climatic conditions. In this context, the book *Rhizosphere Microbes: Biotic Stress Management* edited by Udai B. Singh, Pramod K. Sahu, Harsh V. Singh, Pawan K. Sharma and Sushil K. Sharma is a topical and timely contribution on plant-microbe interactions and offers a great scope for harnessing the beneficial interactions for biotic stress tolerance and agricultural sustainability. The objective of the present book is to furnish a broad-based review on updated critical developments on the management of biotic stresses by using rhizospheric microbes. Chapters which provide a consolidated state-of-the-art work in this area have been incorporated in this book. This much awaited book is aimed to impart a vision for the advancement of science with a special focus on the development of biological control researches worldwide. The book contains critical reviews, mini-reviews, case studies and success stories within the ambit of its title. It covers the complete knowledge on all spheres of stress tolerance, i.e. diverse role of microbes and microbial communities in biotic stress tolerance, diversity, ecology and population dynamics of biocontrol agents, exploring the microbial resources for antimicrobial bioactive compounds, microbe-mediated mitigation of biotic stresses in many crop plants, microbial signalling in the rhizosphere, biofilm formation, plant-microbe

interactions under biotic stresses, role of microorganisms in ecosystems functioning under various biotic stress conditions, development of sustainable techniques/bioformulation, increased agricultural productivity through the application of microbial bio-pesticides, molecular studies using microbial systems, etc. Further, the present book volume *Rhizosphere Microbes: Biotic Stress Management* is very particular to rhizosphere microbe-mediated management of biotic stress with special reference to disease management. This book does not deal with the management of insect-pests, weeds and invertebrates-vertebrates. This book has 16 contributory chapters from well-experienced researchers in plant pathology, microbiology and biotechnology working on different aspects and issues of detection of plant pathogens and characterization of biological control agents for the management of diseases in plants of agricultural importance. This book is unique with complete knowledge about rhizosphere microbe-mediated biotic stresses in major crop plants. Last but not least, this book highlights the role of microbial technologies in sustainable crop protection that may help increase food production for food security to achieve targets of SDGs by the year 2030.

Maunath Bhanjan, India — Udai B. Singh
Maunath Bhanjan, India — Pramod K. Sahu
Maunath Bhanjan, India — Harsh V. Singh
Maunath Bhanjan, India — Pawan K. Sharma
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Pramod K. Sahu has completed his B.Sc. from IGKVV and M.Sc. (Gold-Medalist) and Ph.D. (Agricultural Microbiology) from UAS, Bengaluru, India. He was a DST-Inspire fellow and was selected in various national level exams including ICAR-JRF, ICAR-SRF, ICAR-NET, ARS and GATE. Being a Scientist of Agricultural Microbiology at ICAR-NBAIM, Mau, India, working on plant-endophyte interaction, consortium of bioinoculants, biological control and salinity stress alleviation, he has to his credit more than 48 research/review papers, 1 book, 11 book chapters, 6 training manuals, 13 extension folders, 5 popular articles and 30 abstracts. He has won NAAS Young Scientist Award in Plant Protection 2021, Dr. B. P. Pal Gold Medal award, Dr. G. Rangaswamy Gold Medal, Willium Rigo award, several best posters and best oral presentation awards along with Young Scientist in Agricultural Microbiology-2018 award.

Harsh V. Singh is currently working as a Principal Scientist (Plant Pathology) at ICAR-National Bureau of Agriculturally Important Microorganisms, Kushmaur, Maunath Bhanjan, Uttar Pradesh, India. He is specialized in the areas of plant-microbe interactions, biological control, biotic and abiotic stress management. He has vast research experience in the field of plant pathology with special reference to management strategies of biotic stress with application of microbes-based technologies. He has also experience of biotic stresses of temperate crops of Jammu and Kashmir and grasses and fodder crops. He has worked on microbial role in grassland productivity and nutrient cycling. Studies on microbial diversity and seasonal dynamics in different horti- and silvopastoral systems are his significant contributions towards grassland productivity. He served as a Junior Scientist-cum-Assistant Professor in Plant Pathology (2002–2007) at RARSS, SKUAST-K, Kargil, Programme Coordinator (2007–2009) at KVK, Poonch, SKUAST-J and Senior Scientist (Plant Pathology) at ICAR-IGFRI, Jhansi, from 2009 to 2018. So for he has published more than 40 publications as research articles, 4 books and 6 reviews and 15 book chapters both in national and international journals. He is an editor and reviewer of journals of reputed societies. He has delivered several expert talks and has been an invited speaker for different national and international symposium and conferences.

Pawan K. Sharma did his M.Sc. and Ph.D. in Plant Pathology from DR YSP University of Horticulture & Forestry, Solan, HP. He is working as Principal Scientist at ICAR-NBAIM at Mau, UP, since September 2013. He had been associated with 14 projects as PI or Co-PI sponsored by various agencies. Currently, he is working on biological control of wilt and collar rot diseases through liquid-based formulation of *Trichoderma* and is also in charge of National Agriculturally Important Microbial Culture Collection. He has published 46 research papers in national and international journals, edited 4 books and authored 1 book.

Sushil K. Sharma is currently working as a Principal Scientist (Agricultural Microbiology) at ICAR-National Institute of Biotic Stress Management, Raipur, Chhattisgarh, India, in the area of microbial resource conservation, secondary metabolites and antimicrobial peptides for biotic stress management of crops. So far he had published 110 articles (research, review, book chapter, book, etc.) in both national and international journals/book/conferences. His team has developed (1) a simple novel method for the detection of operation of tryptophan-independent pathway in *Micrococcus aloeverae* DCB-20 and 20 low-cost preservation method of *Chromobacterium violaceum* using natural gums at room temperature. He has been decorated with *Best Scientist* award by PEARL Foundation, Madurai, India, and *Excellence in PGPR Research* by Asian PGPR Society. Recently, he has been nominated as a member of *Biosafety and Biosecurity Cell* (*B2Cell*) of Indian Council of Medical Research (ICMR), New Delhi. He has also delivered talks in PGPR conference organized in Columbia, Uzbekistan and Indonesia.

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Chapter 1
Detection and Identification of Soil-Borne Pathogens: Classical to Recent Updates

Manjunath Hubballi, I. Johnson, V. A. Anjali, T. S. Archana, and S. Nakkeeran

Abstract Soil being biologically complex atmosphere offers shelter to diverse microbes. The survival of microbes in soil is greatly influenced by both edaphic and atmospheric factors. In addition, microbiome dwelling in soil competes with each other for space nutrients and other essential elements. The microbes in soil causing disease in crop plants are called soil-borne pathogens. They mainly encompass actinomycetes, bacteria, fungi, and viruses. These pathogens, though represent a very small portion of total microbial biomass in soil, are responsible for yield losses of varying dimensions in a range of crops. The fact that they reside and cause damage underground remains unnoticed many a times. The presence of a favorable environment for the establishment of host–pathogen relationship and delayed diagnosis of the interaction of soil-borne pathogens contributed to a huge loss in many crops. However, proper detection and diagnosis of the diseases at an early stage can aid in saving the losses caused by these pathogens. There has been an enormous number of methodologies for a diverse group of pathogens. The traditional methods of detecting soil-borne pathogens using direct quantification of pathogens from soil, enumeration of fungal and bacterial pathogens present in soil, and use of selective media for culturing desired pathogen are all laborious and time consuming. Recent advances in science have led to the development of immunological and molecular techniques for the detection of pathogens in soil. These improved methods are not only quick and efficient but are also reliable in detecting particular pathogens.

Keywords Soil · Bacteria · Fungi · Transient visitor · Resident visitors · Inoculum · PCR · LAMP · Immunoassays

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1.1 Introduction

Soil is a biologically active, complex environment developed on the uppermost layer of the earth's crust. It is more porous in nature and has an immense role in the existence of life on earth as it forms a major reservoir of water and nutrients. It is mostly dominated by a multitude of invertebrates, microbial organisms, and a highly complex animal biota. It is said that one gram of soil is composed of innumerable microbes having immense ecological significance. According to the study, approximately 10^8–10^9 bacteria, 10^5–10^8 actinomycetes, 10^1–10^2 nematodes, and 10^3–10^5 invertebrates are present in one gram of soil (Trevors 2010). In addition, soil fertility is greatly contributed by a large number of earthworms present in soil. It is estimated that around 300 earthworms are present in one square meter area. Thus, soil is a microbial biochemical gene library (Dindal 1990). The enormous population of microbes present in soil can be broadly grouped into beneficial, neutral, or harmful to plants. The harmful category of microbes causing harm to plants is considered soil-borne plant pathogens. In other words, microbes residing in soil and causing economic damage to the plants growing in soil are considered soil-borne pathogens. According to Stevens et al. (2003), the term soil-borne pathogens can be defined as pathogens that cause plant diseases via an inoculum that comes to the plant by way of the soil.

1.2 Classification of Soil-Borne Pathogens

Soil is complex in nature, harboring a large number of microbes in it. This has created complexity in its ecology, and hence, it is very essential to establish the role of each microbe in soil as they largely determine the growth and establishment of plants. Based on their ecological role, the soil-borne pathogens are divided into three major categories: transient visitors, resident visitors, and residents (Schuster and Coyne 1974).

1. Transient visitors: The pathogens that in their life cycle spend very less time in soil and commonly don't perpetuate in soil are grouped under this category. These pathogens are more specialized parasites, and the presence of such pathogens is usually associated with a particular host. A prolonged absence of the host in particular soil eliminates these pathogens from the soil owing to their inability to compete with general soil saprobes for existence on nonliving matter. This intimate relation of host and pathogen is conditioned by general soil microflora. These are also called soil invaders, soil transients, root inhabitants, root-specific pathogens, or short-lived exotics. Most bacteria infecting plants fall in this group. The typical examples of this are *Verticillium*, *Rhizoctonia solani*, *Pythium debaryanum*, *Erwinia stewartii*, *E. amylovora*, *E. tracheiphila*, *X. citri*, *X. vesicatoria*, *X. vasculorum*, *E. rubrifaciens*, *X. malvacearum*, *X. juglandis*, *X. vesicatoria*, *X. pruni*, *P. syringae*, *P. pisi*, *P. phaseolicola*, *P. solanacearum*

race 2, *P. tabaci*, *P. glycinea*, *P. lachrymans*, *P. mors-prunorum*, and *P. savastanoi* (Buddenhagen 1965).

2. Resident visitors: These pathogens are typified by a gradual decline of population in soil, and populations of these pathogens largely depend on host or cropping practices followed in soil. The examples of this category would include *Agrobacterium tumefaciens*, *Ralstonia solanacearum*, and *Erwinia carotovora*.
3. Residents: These pathogens are primitive types and are general or unspecialized parasites having a large host range. These pathogens are distributed throughout the soil, and their parasitism appears to be incidental to their saprophytic existence as members of the general soil microflora. Unlike the previous group, these pathogens survive in soil for a longer period, and their relation with plants is ephemeral in nature (Stevens et al. 2003). In general, the competitive saprophytic ability of these pathogens is very high. The species of the genera *Pythium*, *Rhizoctonia*, and *Sclerotium* and bacteria species of *Erwinia* and *Pseudomonas* fall in this group (Veena et al. 2014).

1.3 Significance of Soil-Borne Diseases

The losses incurred due to soil-borne diseases in many agriculture and horticultural crops are largely underestimated because they appear underground. It is estimated that more than 50 different species of fungi, a large number of bacteria, nematodes, and a few viral species and also a few parasitic plants are reported to be soil-borne (Acuf 1988). According to Papavizas (1985), the loss incurred due to soil-borne diseases alone in annual crops is tolling 50% in total. The damage incurred by these diseases is considered the major factor limiting the growth, establishment, and health of plants ultimately influencing negatively on yield both quantitatively and qualitatively (Buchenauer 1998). In a study, the major pathogenic species belonging to *Sclerotinia*, *Pythium*, and *Phytophthora*, *Fusarium*, *Verticillium*, and *Rhizoctonia* inflict yield losses of 50–75% in selected agricultural crops like maize, cotton, wheat, and horticultural crops viz., ornamental crops and fruits (Lewis and Papavizas 1991; Mokhtar and El-Mougy 2014; Baysal-Gurel and Kabir 2018). Furthermore, in the USA, the loss caused by soil-borne diseases was assessed, and it was inferred that around $ 4 billion was lost due to these diseases. Mokhtar and El-Mougy (2014) reported 90% yield losses in about 2000 diseases infecting major crops in the USA (Table 1.1).

1.4 Soil-Borne Pathogens Vs. Foliar Pathogens

The line of difference between soil-borne pathogens and foliar pathogens cannot be always demarcated. The diseases caused by foliar pathogens and soil-borne pathogens differ greatly in the way of spread. The foliar diseases are polycyclic whereas

Table 1.1 Yield loss due to soil-borne diseases in major crops

Crop	Disease	Pathogen	Yield loss (%)	Reference
Rice	Sheath blight	*Rhizoctonia solani*	50	Zhao et al. (2021)
Wheat	Soil-borne wheat mosaic virus disease	*Soil-borne wheat mosaic virus*	10–80	Liu et al. (2020)
Maize	Late wilt	*Magnaporthiopsis maydis*	100	Degani and Dor (2021)
Pigeon pea	Fungal wilt	*Fusarium udam*	50	Kumar et al. (2020)
	Dry root rot	*Rhizoctonia bataticola*	10–100	Vamsikrishna et al. (2021)
	Stem canker	*Macrophomina phaseolina*		
Ground nut	Bacterial wilt	*Ralstonia solanacearum*	20	Yuliar et al. (2015)
	Stem rot	*Sclerotium rolfsii*	25–30	Acharya et al. (2021)
Cotton	Verticillium wilt	*Verticillium dahliae*	10–35	Song et al. (2020)
Tobacco	Bacterial wilt	*Ralstonia solanacearum*	10–30	Yuliar et al. (2015)
Potato	Root knot nematode	*Meloidogyne incognita*	35	Mardhiana et al. (2017)
	Bacterial wilt	*Ralstonia solanacearum*	33–90	Yuliar et al. (2015)
Tomato	Root knot nematode	*Meloidogyne incognita*	24–38	Mukhtar (2018)
	Fusarium wilt	*Fusarium oxysporum*	10–80	Patil et al. (2011)
	Early blight	*Alternaria solani*	79	Dhaval et al. (2021)
	Bacterial wilt	*Ralstonia solanacearum*	90.62	He et al. (2020)
Brinjal	Damping-off	*Pythium* sp.	60	Mahadevakumar and Sridhar (2020)
	Dry root	*Macrophomina phasiolina*	10	Pugalendhi et al. (2019)
Bean	Root knot nematode	*Meloidogyne incognita*	20	Mardhiana et al. (2017)
Cucumber	Root knot nematode	*Meloidogyne incognita*	69.2	Singh and Balodi (2021)
	Fusarium wilt	*Fusarium oxysporum*	70–100	
	Root rot	*Rhizoctonia solani*	5–80	
Banana		*Ralstonia solanacearum*	80–100	

(continued)

Table 1.1 (continued)

Crop	Disease	Pathogen	Yield loss (%)	Reference
	Bacterial wilt			Yuliar et al. (2015)
	Fusarium wilt	*Furaruim* sp.	30	Bubici et al. (2019)
Pomegranate	Root knot nematode	*Meloidogyne incognita*	17.3	Tulika et al. (2019)
	Fungal wilt	*Fusarium oxysporum*	36	Das et al. (2021)
		Ceratocystis fimbriata	30	Shruthi et al. (2019)
Water melon	Root knot nematode	*Meloidogyne inconita*	24–50	García-Mendívil and Sorribas (2021)
Guava	Wilt	*Fusarium oxysporum* f. sp. *psidii*, *Fusarium solani*, *Gliocladium roseum*, *Cephlosporium* sp., *Nalanthamala psidii*, and *Gliocladium roseum*	5–60	Singh et al. (2021)
Wheat, cotton, maize, vegetables, fruit, and ornamentals	–	*Rhizoctonia* spp., *Fusarium* spp., *Verticillium* spp., *Sclerotinia* spp., *Pythium* spp., and *Phytophthora* spp.	50–75	Panth et al. (2020)

soil-borne diseases are monocyclic in nature (Katan 2017). The fluctuations of climatic conditions greatly influence foliar pathogens. For example, a change in temperature and relative humidity will directly influence the growth and development of pathogens and their spread in the case of foliar diseases as the pathogens are directly exposed. On the other hand, such fluctuation is masked in the soil-borne pathogens due to soil mass (Garrett 1970). The research progress in the case of soil-borne diseases is hindered by various factors.

1. The opaque nature of soil prevents in situ examination of pathogens (Cytryn and Minz 2012).
2. Surviving structures of pathogens such as sclerotia, conidia, mycelia, rhizomorph, oospores, and chlamydospores exhibit difference in their resistance to hostile environment and also their survival capacity. These differences contribute to the quantity and quality of inoculum present in soil, thereby influencing pathogenicity.
3. The heterogeneous nature of soil conditioned by a huge microbial population leads to uneven distribution of pathogens in soil, especially in the rhizosphere region (Campbell and Van der Gaag 1993).
4. A large number of microbial species present in soil mask the population of disease-causing organisms in soil.

1.5 Groups of Soil-Borne Pathogens

Streptomyces These are filamentous prokaryotes having the capacity to produce mycelium colonizing the organic matter present in soil. Similar to fungi, these also have an immobile lifestyle, and they also produce spores for dispersal. The species of *Streptomyces* are well known for the production of metabolically active antibiotics, and these compounds improve the fitness in soil. It is interesting to note that only a small proportion of the described *Streptomyces* species are known to be plant pathogens (Table 1.2).

Bacteria These are single-celled microscopic organisms lacking a true nucleus. The structure of bacteria is simple as they do not possess nucleus and membrane-bound organelles. Their genetic information is placed in a loop of DNA. One gram of soil contains approximately 40 million bacterial cells. Among this huge population, a very minute portion of bacteria cause plant diseases, and the important genera reported to be plant pathogenic and reside in soil are *Erwinia*, *Streptomyces*, *Rhizomonas*, *Pseudomonas*, and *Xanthomonas* (https://ausveg.com.au/biosecurity-agrichemical/crop-protection/overview-pests-diseases-disorders/bacterial-diseases/) (Table 1.3).

Fungi These are eukaryotic organisms having well-defined nuclei and membrane-bound organelles. These organisms grow from the tips of hyphae that make up mycelia. They are very successful inhabitants in soil owing to their high adaptive nature in adverse conditions (Sun et al. 2005). According to Gardi and Jeffery (2009), the soil fungi can be grouped into fungi involved in biological control activity, fungi involved in the regulation of ecosystem, and fungi involved in the decomposition organic matter and transformation of compounds. Apart from this, a small group of fungi cause diseases in different crops. The predominant soil-borne pathogenic fungi are *Sclerotium rolfsii*, *Rhizoctonia solani*, *Fusarium* sp., *Pythium*, and *Phytophthora* with diseases (Table 1.4).

Table 1.2 *Streptomyces* spp. associated with different diseases

Streptomyces spp.	Disease name	Reference
S. scabies or *S. scabiei*, *S. acidiscabies*, *S. stelliscabiei*, and *S. turgidiscabies*	Common scab disease	Lerat et al. (2009)
S. aureofaciens and *S. griseus*	Potato superficial scab	Loria et al. (1997)
S. europaeiscabiei, *S. niveiscabiei*, *S. microflavus* or *S. luridiscabiei*, and *S. puniciscabiei*	Common scab disease in Korea	Park (2003)
S. reticuliscabiei	Netted scab of potato	Bouchek-Mechiche et al. (2000)
S. ipomoeae	Soil rot of sweet potato	Zhang et al. (2003)
Streptomyces sp.	Root tumor of cucurbits	Loria et al. (1997)

Table 1.3 Bacterial species associated with various diseases in different crops

Bacterial species	Disease name	Crop	Reference
Xanthomonas campestris pv. *campestris*	Black rot	Brassicas	Ignatov et al. (1998), Vicente and Holub (2013)
Clavibacter michiganensis pv. *michiganensis*	Bacterial canker	Tomato, capsicum, and chilli	Chang et al. (1992), Nandi et al. (2018)
Pseudomonas spp. and *Erwinia* spp.	Bacterial soft rot	Wide range of vegetables, including lettuce, brassicas, cucurbits, tomato, capsicum, potato, sweet potato, carrots, and herbs	Charkowski (2018), Sławiak et al. (2009), Charron et al. (2002)
Xanthomonas campestris	Bacterial leaf spot/bacterial spot	Range of vegetables including lettuce, cucurbits, tomato, and capsicum	Batista et al. (2021)
Ralstonia solanacearum	Bacterial wilt	Potato, tomato, capsicum, and eggplant	Sharma et al. (2021)
Pseudomonas syringae	Bacterial leaf spot/bacterial spot/bacterial blight	Beet, spring onions, leeks, rocket, and coriander	Fonseca-Guerra et al. (2021)

Table 1.4 Different diseases caused by fungal pathogens

Pathogen	Diseases	Reference
Cylindrocladium, *Pythium*, *Phytophthora*, and *Rhizoctonia*	Root rot	https://www.thespruce.com/what-are-soilborne-diseases-1402990
Phytophthora, *Rhizoctonia*, *Sclerotinia*, and *Sclerotium*	Stem, collar, and crown rots	https://www.thespruce.com/what-are-soilborne-diseases-1402990
Fusarium oxysporum and *Verticillium* spp.	Wilt	https://www.thespruce.com/what-are-soilborne-diseases-1402990
Pythium, *Phytophthora*, *Rhizoctonia*, and *Sclerotium rolfsii*	Damping-off	https://www.thespruce.com/what-are-soilborne-diseases-1402990
Ganoderma sp.	Stem rots and root rots	

Viruses These are obligate parasites that require living hosts for their multiplication and survival. They usually require vectors insects, nematodes, or fungi for transmission and spread. However, these vectors contribute to local movement within the field or adjacent fields. The long-distance movement of soil-borne viruses is due to the movement of infected planting materials and shifting of soils. Soil-borne viruses typically infect plant roots or other underground parts, causing significant losses in different crops (Roberts and Alison 2014) (Table 1.5).

Nematodes These are unsegmented worms with round bodies and pointed ends, otherwise called roundworms. The wide adaptability of these worms has made them as most abundant animals on earth. These occur as both free and parasites in nature

Table 1.5 Soil-borne viruses and their vectors

Virus name	Vectors	References
Barley mild mosaic virus	*Polymyxa*	Kanyuka et al. (2003)
Cherry rasp leaf virus	*Xiphinema*	Griffin and Epstein (1964)
Strawberry latent ringspot virus	*Xiphinema*	Griffin and Epstein (1964)
Arabis mosaic virus	*Xiphinema* and *Longidorus*	Griffin and Epstein (1964)
Freesia sneak virus	*Olpidium*	Sekimoto et al. (2011)
Cucumber soil-borne virus	*Abiotic transfer*	Kakani et al. (2003)
Melon necrotic spot virus	*Olpidium*	Sekimoto et al. (2011)
Carnation ringspot virus	*Olpidium*	Sekimoto et al. (2011)
Cucumber necrosis virus	*Olpidium*	Sekimoto et al. (2011)
Chinese wheat mosaic virus	*Polymyxa*	Kanyuka et al. (2003)
Peanut clump virus	*Polymyxa*	Kanyuka et al. (2003)
Beet soil-borne virus	*Polymyxa*	Kanyuka et al. (2003)
Beet virus Q	*Spongospora*	Falloon et al. (1996)
Potato mop-top virus		Santala et al. (2010)
Pea early-browning virus	*Paratrichodorus* and *Trichodorus*	Karanastasi et al. (1999)
Beet necrotic yellow vein virus	*Polymyxa*	Kanyuka et al. (2003)
Tobacco rattle virus		Karanastasi et al. (1999)
Lettuce big-vein virus	*Olpidium*	Lot et al. (2002)
Watercress yellow spot virus	*Spongospora*	Falloon et al. (1996)

(https://www.britannica.com/animal/nematode). A very minute portion of nematodes has been identified to be pathogenic to different crops. The major genera of nematode infecting plants include *Meloidogyne*, *Globodera*, *Heterodera*, *Pratylenchus*, *Ditylenchus*, *Rotylenchulus*, *Xiphinema*, *Aphelenchoides*, and *Bursaphelenchus* (https://ohioline.osu.edu/factsheet/plpath-gen-8). About 90% of nematodes reside in the top 15 cm of soil. The plant parasitic nematodes are reported to consume 10% of global agricultural production, tolling to 125 billion loss every year (Chitwood 2003). In addition to acting as pathogens, they also act as vectors of plant viruses (Table 1.6).

1.6 Detection Methods for Major Soil-Borne Pathogens

Soil-borne diseases represent a major share of the reported 80,000 plant diseases across the globe. Most of the diseases are fatal to crops reflecting huge yield loss as indicated in the tables earlier. Furthermore, they cause 10–20% more diseases compared to airborne and seed-borne pathogens. As they reside in soil, they cause their initial damage to crops, which is underestimated many times. Thus, the early detection of these microorganisms in the soil could help farmers to optimize their

Table 1.6 Nematodes acting as vectors of different virus

Nematode vector	Virus
Xiphinema diversicaudatum	Arabis mosaic virus
X. index and *X. italiae*	Grapevine fanleaf virus
X. americanum and *X. rivesi*	Peach rosette mosaic virus
X. americanum, *X. californicum*, *X. intermedium*, *X. rivesi*, and *X. tarjanense*	Tobacco ringspot virus
X. americanum, *X. californicum*, and *X. rivesi*	Cherry rasp leaf virus
X. diversicaudatum	Strawberry latent ringspot virus
Longidorus apulus and *L. fasciatus*	Artichoke Italian latent virus
L. elongatus	Beet ringspot virus
L. martini	Mulberry ringspot virus
L. elongatus, and *L. macrosoma*	Raspberry ringspot virus
L. attenuates and *L. elongatus*	Tomato black ring virus

crop yield by suppressing pathogens and avoiding disease development. The detection of major pathogens is discussed hereunder.

1.7 Detection of Soil-Borne Pathogens

1.7.1 Traditional Methods

1.7.1.1 Direct Quantification

The method of estimating soil-borne fungi mainly depends on the direct counting of resting structures of pathogens, and it is more precisely applicable to fungi producing sclerotial bodies such as *Sclerotium rolfsii* and *Rhizoctonia solani*. In this approach, the number of sclerotial bodies present in a known quantity of soil is estimated by sieving soil through a sieve of 250 mesh. The viable count of sclerotial bodies can be estimated after moistening the 50 g of soil with 12.5 ml of 1% methanol (Rodriguez-Kabana et al. 1980).

1.7.1.2 Enumeration of Pathogens

Soil is a complex environment, and the presence of microbial pathogens is influenced by various biotic and abiotic factors. In order to assess the load of particular pathogens, enumeration of pathogenic propagules (cells and spores) from soil is one of the basic and primeval methods for detection and quantification of soil-borne plant pathogens. Conventional enumeration techniques prerequire sample preparation where the bacterial cells/fungal spores from the soil sample

matrix are dispersed in a suitable diluent (Foght and Aislabie 2005). Sterile distilled water, phosphate-buffered saline, potassium phosphate, or mineral salts medium devoid of carbon source are the most commonly used diluents (Atlas 1995). After the cells/spores are congruously dispersed in these diluents, serial dilutions are performed and the individual cells/spores are then enumerated by microscopic visualization or cultivation methods. The dilution factor employed for the detection varies with the technique used (Foght and Aislabie 2005). The two major enumeration techniques used for the detection and quantification of pathogens are the direct or microscopic visualization method and the culture-based enumeration method.

1.7.1.2.1 Enumeration of Bacteria

Direct or Microscopic Visualization of Bacteria

This technique enables to count the total number of cells present in the sample by staining with a fluorescent dye and subsequently visualizing the cells through epifluorescence microscopy. The most common fluorescent dyes used are acridine orange and 4,6-diamino-2-phenylindole (DAPI) (Bölter et al. 2002). One of the shortcomings of this method is that enumeration takes into account both dead and live cells. However, the recent development of certain new dyes such as 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) (Créach et al. 2003) and propidium iodide + thiadazole orange (Foght and Aislabie 2005) has resulted in detecting metabolically active cells, thus discriminating live and dead cells. Autofluorescence of soil matrix components and occlusion of bacterial cells by soil particles can interfere with detection techniques, thus reducing its efficacy. Recently confocal laser scanning microscopy has been employed to improve the detection and visualization of cells over conventional microscopy.

Culture or Cultivation-Based Enumeration of Bacteria

The viable cells present in the soil suspension can be detected and enumerated using this technique, but it is limited by the fact that only culturable bacterial populations can be detected by this method. As compared to highly sophisticated molecular techniques, this method is relatively simple, inexpensive, and easier to interpret. Culture-based enumeration techniques are of two types: the most probable number method (MPN) and plate count method.

Most Probable Number Technique (MPN)

This method involves the addition of serially diluted soil suspensions to a liquid medium, which is then incubated under required conditions to yield a series of cultures that is scored in accordance with a predetermined criterion (Alef and Nannipieri 1995; Atlas 1995). The cell population can be identified by employing various methods such as turbidimetry or screening the production of certain

metabolites. The data obtained is finally evaluated by statistical tools to infer the MPN of viable cells in the undiluted sample (Eaton et al. 1995; Alef and Nannipieri 1995; Koch 1994). This method can be used to detect and enumerate certain selective bacterial pathogens by providing suitable selective cultivation media. Although it gives only a statistical estimate of bacterial cells in the given suspension, it is more suitable for particulate samples and can detect pathogens that do not grow well in a solid medium.

Plate Count Technique

This is a relatively rapid and inexpensive technique that enables the detection of viable bacterial pathogens present in a soil sample by enumerating colonies formed over a solid growth media inoculated with sample dilutions. This method is based on the speculation that each bacterial colony on the growth media has originated from a single cell or endospore, thus referring to them as colony forming unit (cfu). Although the method is biased as it only allows the detection and counting of culturable cells, it yields well-separated colonies of bacterial pathogens, which can be subsequently purified and characterized (Foght and Aislabie 2005).

1.7.1.2.2 Enumeration of Fungal Pathogens

Enumeration of fungal pathogenic propagules from the soil can be done by using a common technique known as serial dilution. Serial dilution is a step-by-step dilution technique, where the soil dilution factor remains constant with a geometric progression. Tenfold serial dilutions result in 1M, 0.1M, 0.01M, 0.001M, and subsequent concentrations and are plated on specific media to count the number of viable pathogens (Aneja 2005). Mitsuboshi et al. (2016) enumerated *Fusarium* sp. present in soil by plate count technique.

This count gives the colony forming units and not the count of individual microbes. However, these counts are considered very accurate for estimating the number of microbes in original samples. Drawbacks of this test are time- and space-consuming and require specialized equipment that must be prepared correctly. The other important drawback with the enumeration is that only viable pathogenic structures can be assessed (Wetzel 2001).

1.7.1.3 Use of Selective Media

Isolation of pathogen residing in soil in pure form is an important step in the diagnosis of disease. There has been huge amount of literature on the use of specific media that supports the growth of desired organisms. The media supporting the growth of desired organisms by preventing undesired microbes in it through inhibitory chemicals are referred to as selective media. These types of media generally contain an inhibitory chemical that will selectively inhibit all microbes except the

desired group of microbes. The classical example of selective media for fungal pathogens is peptone-pentachloro nitro benzene (PCNB) medium (Papavizas 1967). PCNB was earlier used to prevent the contamination of zygomycetes in cultures. However, due to its hazardous nature and carcinogenic ability, it was banned from usage. Boknam Jung et al. (2013) developed a selective media for the isolation of *Fusarium graminearum.* Furthermore, acidified weak potato-dextrose agar (AWPDA) along with thiabendazole was developed as the selective media for the isolation of *Alternaria* species from samples of soil (Hong and Pryor 2004).

Different pathogenic phytobacteria utilize different metabolic pathways, and this nutritional diversity can be used in the development of selective agar media (Schaad 1987). Kado and Heskett (1970) developed five selective plating media for the detection of pathogenic bacteria in the genera *Xanthomonas*, *Pseudomonas*, *Erwinia*, *Corynebacterium*, and *Agrobacterium*. A major constraint faced in the development of selective media for plant pathogenic bacteria is that most of them have a very narrow nutritional demand. However, it is a relatively easy and rapid method once the growth media specific to a particular pathogen is standardized (Table 1.7).

1.7.1.4 Indicator Plants

The use of indicator plants or bio-indicators can aid in determining whether or not a field is contaminated with a bacterial pathogen. The detection of the pathogen is based on the symptoms observed and the time taken for symptom development. Tomatoes and potatoes are the most common indicator plants used for the detection of *Ralstonia solanacearum* race 3. More particularly, potato seedlings bearing small tubers can serve as a rapid diagnostic tool for the detection of *R. solanacearum* race 3 (Graham and Lloyd 1978). Also Paret et al. (2009) evaluated three different varieties of ginger and found that tissue-cultured edible ginger was most suitable for the detection of *R. solanacearum* race 4. Similarly in fungi, the presence of *Ganoderma* in coconut gardens was detected through the use of pigeon pea as indicator plants (Snehalatharani et al. 2016). The use of indicator plants for detection is time consuming, labor intensive, and not widely preferred.

1.7.1.5 Baiting or Trapping Techniques

Bait is any substance that is preferred by an organism for its growth, and in the presence of such substance, the growth of the organism is enhanced. The small piece of plant parts/substance is placed near soil for a known period of time so as to allow the desired organisms to grow into the bait. The baiting material will be afterward placed into selective culture media. The material used for the growth of the pathogen is called the bait and the method is referred to as baiting. In this method, the parasitic nature of the pathogen will be exploited to separate the pathogen from a diverse

Table 1.7 Selective media for isolation and enumeration of fungi and bacteria

Media	Pathogen	References
Fungi		
PARP (pimaricin, ampicillin, rifampicin, pentachloronitrobenzene) medium	*Pythium* spp.	Tojo (2017)
3P medium and PV medium	*Phytophthora*	Eckert and Tsao (1962)
DCPA (dichloran-chloramphenicol peptone agar) medium	*F. oxysporum*	Bragulat et al. (2004)
NS medium (Nash and Snyder medium)	*F. oxysporum*	Bragulat et al. (2004)
PDID medium (potato dextrose iprodione dichloran agar)	*F. oxysporum*	Bragulat et al. (2004)
CZID (Czapek Dox iprodione dichloran agar) medium	*F. oxysporum*	Bragulat et al. (2004)
PSAA (potato sucrose acidified agar) medium	*Sclerotinia Sclerotiarum*	Steadman et al. (1994)
Neon agar medium	*Sclerotinia sclerotiorum*	Peres et al. (2002)
TB-CEN medium	*Thielaviopsis basicola*	Specht and Griffin (1985)
Bacteria		
Kritzman's selective medium	*Xanthomonas campestris* pv. *campestris*	Kritzman and Ben-Yephet (1990)
MMG medium (maltose, methyl green, and antibiotics)	*Xanthomonas campestris* pv. *vitians*	Toussaint et al. (2001)
Modified Miller–Schroth medium	Pectolytic *Erwinia*	Pierce and McCain (1992)
MSCFF	*Curtobacterium flaccumfacciens* pv. *flaccumfaciens*	Maringoni et al. (2006)
Crystal violet pectate (CVP)	Pectolytic *Erwinia*	Cuppels and Kelman (1974)
Tetrazolium medium	*Ralstonia solanacearum*	Kelman (1954)
XAS medium	*Xanthomonas albilineans*	Davis et al. (1994)
SMSA	*Ralstonia solanacearum*	Elphinstone et al. (1996)

organism present in soil. In the earlier studies, many soil-borne pathogens were isolated and purified using this simple method. The common examples of baits are dead insects, boiled seeds, pollen grains, and nails (Shew and Meyer 1992). *Thielaviopsis basicola* is a soil-borne pathogen that is reported to be a pathogen on 200 plant species that produce two kinds of spores known as cylindrical endoconidia and as aleuriospores. This pathogen was proved to be isolated by carrot disc in soil (Yarwood 1946). Sharadraj and Chandra Mohanan (2016) identified leaves of the badam tree as baiting agents for the isolation of *Phytophthora palmivora*. Anandaraj and Sarma (1990) reported that *Albizia falcafaria* (L.) leaflets