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Microbiota and Biofertilizers

A Sustainable Continuum for Plant and Soil Health

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Dedicated to our parents and teachers.

Foreword

Undoubtedly, the agricultural industry feeds an enormous population globally. Numerous techniques and technologies along with modern strategies have been employed in order to increase the agricultural yield. The faith of agriculturists to rely on synthetic substances has enhanced agricultural yield, but this has been achieved at the cost of the environment. Synthetic substances have a history of deteriorating the quality of different environs significantly and these substances not only degrade the ecosystem but have led to human health hazards. There are numerous traditional methods available to control the overuse of these substances. However, microbiota as biofertilizers is a viable option to deal with grave issues that rose due to application of synthetic chemicals and may act as a solution against the discriminate use of synthetic substances. Furthermore, exploring these sustainable approaches can eliminate the accumulation of persistent contaminants from environs in a versatile and eco-friendly manner.

The book contains coherent topics, relevant to the trustworthiness of microbiota as biofertilizer and for the safeguard of the environment. The book is an assembly of 15 chapters covering different regions of the world. Chapter 1 entitled ‘Chemical fertilizers, formulation, and their influence on soil health’ by a group of authors from Pakistan presents information on intensive land use with continuous and injudicious application of high doses of inorganic fertilizers. The authors stress the fact that these chemical substances intensively affect soil properties, and keeping in view the grave concerns on using synthetic substances, an understanding of chemical fertilizer formulations and their effect on soil health is necessary to overcome high synthetic fertilizer applications. The scientists from northern part of India provide a valuable picture about 'Organic agriculture: principles, current status, and significance' in Chap. 2. The authors have skillfully drawn a picture regarding the impacts of population growth on agricultural industry and highlighted the major challenges to exploring organic agriculture globally. In Chap. 3 on the 'Responses of soil properties to organic amendments' by scientists from India, the authors present information pertaining to the synthetic fertilizers and chemicals which persistently pose a threat not only to human lives but create ecological imbalance too. Otherwise, they put forth an argument that organic amendments not only improve the soil organization by enhancing aggregate stability, hydrophobicity, soil porosity and soil permeability, but also help in reducing bulk density. However, they lay emphasis on the fact that application of organic amendments should follow references in order to get desirable results. In Chap. 4 a team of workers from India while addressing a trending topic in their work entitled 'Vermicomposting: Sustainable tool for agricultural environs' have added information on compost, a quality product containing ingredients beneficial for plants that prevents environmental degradation. Chapter 5 'Application and viability of macrophytes as green manure' is written by a group of researchers from the USA and Mexico. The authors investigated that the increase in the human population is an important challenge to food security in the world and it is important to develop agro-industrial and biotechnological strategies that are eco-friendly and to avoid the use of chemical fertilizers. Therefore, they urge using themacrophytes as green manure because of their eco-friendly nature. The scientists again from India present their work on 'Role of microorganisms as biofertilizers' in Chap. 6, wherein authors demonstrate that microbes promote growth, productivity and physiological properties of plants either directly or indirectly. Furthermore, authors highlight that bio-fertilizers increase the growth as well as development of plants by building up the accessibility of plants to mineral nutrients, biological nitrogen fixation, solubilizing phosphorus and growth hormones. A group of authors from India have expanded the information on the title 'Nano-agriculture: A novel approach in agriculture' in Chap. 7 and established the fact that nanotechnology has a great potential to enhance the quality of life explored in various regions of agro-industry and the food system. Again the researchers from India have shared their thoughts and information on the title 'Biofertilizers: sustainable approach for growing population needs' in Chap. 8. The authors enumerate that biofertilizers offer an economically and environmentally attractive route to increase the supply of nutrients. Furthermore, they suggest that information acquired from the literature evaluated will assist in comprehending the physiological foundation of biofertilizer for viable farming in order to reduce the issues of utilizing chemical fertilizers. Chapter 9, titled 'Role of recombinant DNA technology in biofertilizer production' written by a scientist from India highlights important features with respect to biofertilizer production by using the tools of molecular biotechnology, like recombinant DNA technology, which can improve the metabolic pathways and production of important plant growth promoting factors like phytohormones and enzyme activity. Another group of authors from India prepared Chap. 10, titled 'Root-associated ectomycorrhizal mycobionts as forest biofertilizers: Standardized molecular methods for characterization of ectomycorrhizal wood wide web'. This chapter highlights the role of root-associated ectomycorrhizal fungi as biofertilizers in forest ecosystems and efficient molecular methods specially optimized for characterization of ectomycorrhizal fungi associated with conifers. Scientists from Pakistan have pooled up the information on the 'Plant Growth-Promoting Rhizobacteria (PGPR) as biofertilizers and biopesticides' in Chap. 11. The authors report that plant growth-promoting rhizobacteria (PGPR) play an important role in sustainable agriculture through the improvement of plant growth via different processes like biological nitrogen fixation, phosphate solubilization, siderophore production and phytohormone synthesis. Furthermore, PGPR can work as biocontrol agents providing protection to the plants, enhancing the plant growth through the synthesis of antibiotics. Chapter 12 titled 'Halotolerant microorganism reclamation industry for salt-dominant soils' has been prepared by researchers from India. The authors provide a detailed picture on bacterial domain halophiles, which are considered moderately tolerant and a good choice for reclamation of salt affected soils. The chapter also highlights the role of *Bacillus* species to maintain the friendly behaviour of plant root zone changes under stressed environs and accelerate plant development. Chapter 13 entitled 'Allelopathic bacteria as an alternate weedicide: progress and future standpoints' has been critically overviewed by workers from Pakistan. The chapter deals with grave effects of chemical substances on different environs utilized in agricultural industries. Otherwise, it focuses on allelopathic bacteria as an alternative and more effective weed control approach which not only eradicates the weed problem but also enhances the growth of the crops. A group of scientists from India have presented a valuable description under the title '*Azotobacter* as biofertilizer for sustainable soil and plant health under saline environmental conditions' in Chap. 14. The authors report that application of *Azotobacter* as biofertilizer has had a positive impact on the germination of seeds, growth and increased proliferation of root and shoot length, and yield of different crops in isolation and in consonance with other bacterial biomass under saline conditions. It has also proven beneficial with other phosphate solubilizing microbes for improving the quality of compost. Chapter 15 deals with the topic 'Role of microbiota in composting' as the closing chapter, presented by researchers from India, and highlights the importance of composting and the role of microbiota in it. The authors mention that composting helps to reduce the waste dumped in landfills, recycles humus and nutrients, protects and improves the microbial diversity of the cultivated soils and thus reduces the overall contamination in soil environs.

The order of chapters included and information established in this book cover the highly sensitive issues related to sustainable agriculture practices, and many crucial aspects of scientific valuation are addressed in this volume. The book can act as a repository of knowledge on the subject and can act as a source of attraction to the scientific community. The editors must be highly praised and appreciated for their creditable hard work in bringing forth this book.

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Preface

In the current era, an increasing population has put tremendous pressure on agricultural productivity to fulfil the demands of the huge population. Numerous agricultural activities and techniques have been developed to raise yearly crop production globally. No doubt, agriculturists have succeeded in the scenario to enhance yearly crop productivity at the cost of environmental degradation by applying synthetic persistent substances, *viz.,* synthetic fertilizers, pesticides, and herbicides since chemical fertilizers are nearly as destructive as productive, like monocultures and consequences associated with the elimination of key biota, nutrient pollution, as evidenced by an algae bloom, eutrophication, water quality issues, lower oxygen levels and danger to fish stocks. Therefore, the scientific approach to maintain sustainable fertility in soil and plants is to switch over to biofertilizers.

Biofertilizers are compounds of organic matter that are applied to crops for growth and health. Their constituent microorganisms interact with the soil, root and plant seed in an eco-friendly manner, promoting the growth of micro-flora that enhance soil fertility. They are known to play several vital roles in soil fertility, crop productivity and production in agriculture. The application of biofertilizers results in increased mineral and water uptake, root development, vegetative growth and nitrogen fixation. They liberate growth-promoting substances plus vitamins and help in maintaining soil fertility. They act as antagonists and play a pivotal role in neutralizing the soil-borne plant pathogens and thus, help in the bio-control of diseases. The application of biofertilizers in lieu of synthetic fertilizers could be a promising technique to raise agricultural productivity without degrading the environmental quality.

The book highlights grave consequences and enumerates plenty of examples on the degradation of different environs due to synthetic substances. Interestingly, various eco-friendly agricultural practices, which have been proven a vital asset and an alternative production system that principally disallows the usage of synthetic substances in farm fields, have been discussed in a coherent manner. Besides, modern approaches, especially recombinant DNA technology, for food production that aim to promote and maintain edaphic factors, human health and ecological balance have been elaborated logically. The book also deals with the role of earthworms and microbes as a part of eco-friendly farming, which helps in conversion of organic waste and macrophytes into quality products and prevents environmental degradation. It also contains a detailed description of the application of nano-agricultural intrusion in crop cultivation to boost agricultural quality and productivity. Also, it focuses on the utilization of advantageous microbial organisms as biofertilizers to aid in the maintenance of food security and elevating crop produce.

In general, the book is a depot of advanced research about the role of microbiota and biofertilizers to prolong plant and soil health. This book addresses diverse challenges and the possible future action of useful biota in the development of a sustainable agricultural system. It shall act as a valuable reference to the latest advances in research in the concerned area. The content of the book is diverse and shall pay keen attention to the needs of students, researchers and scientists the world over.

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About the Book

Population explosion has put an immense load on the agricultural industry to keep up with the increasing basic requirements. Copious farming strategies have been developed to increase the yield globally. Undoubtedly, agriculturists have succeeded in this scenario, but at the cost of environmental degradation as a consequence of using an enormous quantity of persistent substances. Since, these substances are nearly as vicious as fruitful, like monocultures and consequences associated with elimination of key biota, nutrient pollution, eutrophication, water quality issues, lower oxygen levels and danger to fish stocks. Therefore, in this backdrop the present book on *Microbiota and Biofertilizers – A Sustainable Continuum for Plant and Soil Health* has been formed.

The book provides a perceptive picture on impediments raised due to the overuse of synthetic substances on quality environs. Besides, the impact of degrading soil environs on the flora and fauna is also discussed in detail. This book aims to promote a comprehensive account on microbiota, applied to crops for growth and health. Otherwise, modern approaches, especially recombinant DNA technology, for food production that aim to promote and maintain ecological balance have been elaborated logically. This book also deals with the role of earthworms and microbes as a part of eco-friendly farming, which helps in converting of organic waste and macrophytes into quality products and preventing environmental degradation. It also contains detailed description on the application of nano-agricultural intrusion in crop cultivation to boost agricultural quality and productivity. In addition, it focuses on the utilization of advantageous microbial organisms as biofertilizers to aid in the maintenance of food security and elevating crop produce. In general, the book shall act as a valuable reference to the latest advances in research in the concerned area. The content of the book is diverse and shall pay keen attention to the needs of students, researchers and scientists the world over.

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About the Editors

Dr. Khalid Rehman Hakeem (PhD) is Professor at King Abdulaziz University, Jeddah, Saudi Arabia. After completing his doctorate (Botany; specialization in Plant Eco-physiology and Molecular Biology) from Jamia Hamdard, New Delhi, India, in 2011, he worked as a lecturer at the University of Kashmir, Srinagar, for a short period. Later, he joined Universiti Putra Malaysia, Selangor, Malaysia, and worked there as Postdoctorate Fellow in 2012 and Fellow Researcher (Associate Professor) from 2013 to 2016. Dr. Hakeem has more than 10 years of teaching and research experience in plant eco-physiology, biotechnology and molecular biology, medicinal plant research, plant-microbe-soil interactions as well as in environmental studies. He is the recipient of several fellowships at both national and international levels; also, he has served as the visiting scientist at Jinan University, Guangzhou, China. Currently, he is involved with a number of international research projects with different government organizations. So far, Dr. Hakeem has authored and edited more than 50 books with international publishers, including Springer Nature, Academic Press (Elsevier) and CRC Press. He also has to his credit more than 120 research publications in peer-reviewed international journals and 60 book chapters in edited volumes with international publishers. At present, Dr. Hakeem serves as an editorial board member and reviewer of several high-impact international scientific journals from Elsevier, Springer Nature, Taylor and Francis, Cambridge, and John Wiley Publishers. He is included in the advisory board of Cambridge

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Dr. Mohammad Aneesul Mehmood (PhD) has specialization in Limnology and Environmental Toxicology. He completed his doctorate from the Division of Environmental Science, SKUAST- K, with a meritorious certificate from the university. He received Dr. Mumtaz Ahmad Khan Gold Medal and Shri Bhushan Memorial Gold Medal for being a topper during his master's programme. He has qualified various national competitive tests in the discipline of Environmental Science. He was also awarded with INSPIRE Merit Fellowship (JRF & SRF) by the

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Dr. Rouf Ahmad Bhat (PhD) is working in Cluster University Srinagar (J&K), India, in the capacity of Assistant Professor specializing in Limnology, Toxicology, Phytochemistry and Phytoremediation. Dr. Bhat has been teaching graduate and postgraduate students of Environmental Sciences for the past 3 years. He is author of more than 50 research papers and 25 book chapters, and has published more than 15 books with international publishers. He has presented and participated in numerous State, National and International conferences, seminars, workshops and symposiums. Dr. Bhat has worked as an associate Environmental Expert in World Bank funded Flood Recovery Project and also Environmental Support Staff in Asian Development Bank (ADB) funded development projects. He has received many awards, appreciations and recognitions for his services to the science of water testing, air and noise analysis. He has served as editorial board member and reviewer for reputed international journals. Dr. Bhat is still writing and experimenting with diverse capacities of plants for use in aquatic pollution.

Chapter 1
Chemical Fertilizers, Formulation, and Their Influence on Soil Health

Shazia Iqbal, Umair Riaz, Ghulam Murtaza, Moazzam Jamil, Maqshoof Ahmed, Azhar Hussain, and Zafar Abbas

Abstract The world population continues to increase at an alarming rate. To meet the increasing demand for food, intensive cultivation using more cropland areas and increased use of fertilizers had been practiced. According to the FAO, chemical fertilizers are the solitarily most important contributor to the rise in the world's agricultural production. Fertilizers comprising of nitrogen, phosphorus, and potassium are regarded as the drivers of modern agriculture. Their worldwide use had been increased since the inception of the so-called green revolution. Chemical fertilizers recently provided 192 million tons as input to the agricultural soils in which 109 million tons was nitrogen, 45 million tons was phosphorus (expressed as P_2O_5), and 38 million tons was potassium (expressed as K_2O). Fertilizer use increased by about 30% per hectare from 2002 to 2017, which was about 95 tons per hectare. By nutrient, the increase was about 24% for nitrogen, 25% for P_2O_5, and 53% for K_2O. Low fertilizer use efficiencies in most of the soils are another factor adding in more use of chemical fertilizers. Intensive land use with continuous and injudicious use of higher doses of inorganic fertilizers significantly influences soil health and crop growth. Soil health is collectively defined by physical (texture, bulk density, infiltration rate, hydraulic conductivity, porosity, etc.), chemical (essential nutrients, cation exchange capacity, electrical conductivity, etc.), and biological (microbial community including bacteria, fungi, algae, archaea, protozoa, earthworm, etc.)

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properties. Chemical fertilizers affect soil properties both positively and negatively. Keeping in view these points, an understanding of chemical fertilizer formulations and their effect on soil health is necessary to overcome low fertilizer use efficiencies and more fertilizer use.

Keywords Soil · Chemical · Fertilizers · Agriculture · Green Revolution

1.1 Introduction

Soil is a complex matrix in which a lot of processes are taking place at the same time. Soil functions and reactions are imperative in understanding the behavior of nutrients in the soil. These processes affect the availability of nutrients to plants. Plants need about 18 nutrients like nitrogen, phosphorus, and potassium essential for their growth and life cycle completion (Table 1.1). When the crop is harvested, these nutrients do not reimburse to the soil, and levels of these nutrient decrease with time. This decrease in nutrient level affects the crop both qualitatively and quantitatively. Henceforth, these nutrients need to be remunerated either by returning soil extracted nutrients to soil return, by the natural decomposition (decay of plants and organism bodies) process, or by directly applying these nutrients as fertilizers (Zaman et al. 2019). From ancient times, organic fertilizers were used to supply nutrients to crop, but this practice decreased since the 1980s because of more availability and affordability of chemical fertilizers (Gong et al. 2009).

Fertilizer application has to turn out to be a crucial practice to contemporary agriculture to feed the increasing population. The application of fertilizers, chemical fertilizers, has become a consecration on humanity. The use of chemical fertilizers helps in overcoming hunger and death in many areas of the world (Zaman et al. 2019). Chemical fertilizers are amendments comprising of nutrients needed for plant growth. These nutrients are classified as primary nutrients, secondary nutrients,

Table 1.1 Essential nutrients for plant growth

Primary or macronutrients	Secondary or macronutrients	Micronutrients	Non-mineral elements (from air and water)
Nitrogen	Calcium	Boron	Oxygen
Phosphorus	Magnesium	Chlorine	Carbon
Potassium	Sulfur	Copper	Hydrogen
		Iron	
		Manganese	
		Molybdenum	
		Zinc	
		Nickle	
		Cobalt	

Source: Savoy (2009)

micronutrients, and non-mineral elements. Primary and secondary nutrients are mutually termed as macronutrients. Macronutrients are required by plants in large quantities, while micronutrients are required in smaller quantities. Apart from main constituent elements, oxygen and hydrogen, and carbon, are uptaken by plants in gaseous form and obtained from water and air. The fertilizer industry is usually involved in the manufacture of primary plant nutrients, e.g., nitrogen, phosphorus, and potassium, appropriate for soil application (Cheremisinoff 1995; Scherer 2000). Agriculture is provided with essential nutrients through the use of these fertilizers though it is also facing many severe issues like declining productivity, low fertilizer use efficiencies, the disproportion in between addition and removal of nutrients from the soil, and low soil organic carbon (Bhatt et al. 2019; Riaz et al. 2020). Chemical fertilizers not only upsurge the crop production by supplying more nutrients in the soil for plant uptake, but it also affects the soil physical, chemical, and biological properties both positively and negatively (Zaman et al. 2019). These all soil properties maintain soil health and improve crop growth.

Soil quality is measured by all soil properties (Shukla et al. 2006). Soil physical properties, such as texture, compaction, infiltration rate, seepage, hydraulic conductivity, soil porosity, bulk density, and soil chemical properties; and nutrients status, cation exchange capacity, electrical conductivity, pH, and soil microbial community change with long term and intensive application of chemical fertilizers. This chapter addresses the effect of chemical fertilizers on all soil processes and properties.

1.2 Background

In the medieval era, Babylonians, Egyptians, early Germans, and Romans are known for using manure and minerals for increasing their farm productivity. In ancient times, wood ash was used for field reclamation (Scherer 2000) in the Andes, for at least 1500 years guano is known and used, and in nineteenth century, it was taken in vast quantities from Chile and Peru to the United States and Europe. Johann Friedrich Mayer (1719–1798) firstly uses gypsum in agriculture. Louis Augustin Guillaume Bosc used gypsum to intimates its septic quality. But this opinion is overthrown by the experiments of Humphry Davy. French agronomist Charles Philibert de Lasteyrie (1759–1849) used gypsum for nutrient improvement (Armstrong and Buel).

The foundation for the modern fertilizer industry was laid by Liebig, starting in 1840 (Russel and Williams 1977). Chemist Justus von Liebig (1803–1873) significantly contributed to understanding plant nutrition. His persuasive work first criticized the vitalist theory of humus, by arguing ammonia importance and then endorsing the importance of inorganic minerals for plant nutrition (Chisholm and Hugh 1911). In England, he produced first fertilizer by chemical processes that were superphosphate, made early in the nineteenth century by treating phosphate of lime in bones with sulfuric acid. It was cheaper than the guano that was used at the time, but it failed because of its low absorbance by crops (Russel and Williams 1977).

John Bennet Lawes, an English entrepreneur, in 1842 patented a manure formed by treating sulfuric acid with phosphates and therefore was the first to generate and initiate the artificial manure industry (Chisholm and Hugh 1911). By 1853 in the United Kingdom, there were 14 manufacturers, and several were in other countries. By 1870, their number increased to 80 in the United Kingdom (Hignett 1985). Triple superphosphate did not turn out to be an imperative fertilizer material until the 1950s, although triple superphosphate comprised of higher phosphate content than rock phosphate from which it was made. Production of concentrated phosphate, also known as triple superphosphate, is linked to phosphoric acid production. In the 1870s in Germany, the first known commercial production of triple superphosphate was made using iron and aluminum content with low-grade phosphate rock. Other plants were built soon in America and Europe, but they were small. Products of these small units were used in sugar clarification rather than for fertilizer. In 1927, Erling Johnson produced nitrophosphate, by an automated method known as Odda process after his Odda Smelteverk of Norway. In the 1930s, nitrophosphate fertilizer production was started in Europe, and many units of nitrophosphate production were built in other continents. Many units were large and produced in 1500 or more tons of product per day. Phosphate rock is finely ground, and raw form was used as a direct application in China, Russia, and the United States to a substantial extent and in other countries to some extent. The practice declined later in the United States. Ammonium phosphate did not become a general fertilizer until the 1960s. It proved an effective fertilizer, and, in several countries, small quantities had been produced from time to time. Now, ammonium phosphates are the main form of phosphate fertilizer used in the world (Hignett 1985).

The first synthetic nitrogen fertilizer was calcium nitrate, made in 1903 from nitric acid through Birkeland-Eyde process by a Norwegian scientist and an industrialist, Kristian Birkeland, along with his business partner, Sam Eyde, on the basis of electric arc method used by Henry Cavendish in 1784 (Ihde 1984). In Notodden and Rjukan in Norway, a factory based on this process was built along with hydroelectric power facilities (Leigh 2004). The process was inefficient in terms of energy usage and substituted by the Haber process (Williams and Derry 1982). Wilhelm Ostwald in 1902 developed an Ostwald process for the production of nitric acid. This process was similar to the Haber process that provides ammonia. The availability of synthetic ammonia started after 1913, but the physical quality of this fertilizer was not good. The availability of synthetic ammonia after 1913 led to many new nitrogen fertilizers, but the physical quality was poor. Tennessee Valley Authority was formed in 1933 to upsurge the manufacture and use efficiency of fertilizer. Tennessee Valley Authority produced more than 75% of the fertilizer in the United States (Russel and Williams 1977). The potassium fertilizer industry started in 1861, firstly in Germany. During World War I, in North America the potassium industry started, and due to the development of deposits of New Mexico in 1931 and the Saskatchewan in 1958, it was flourished. Today's potassium fertilizers are not the product of chemical processes and are the results of physical processes (Russel and Williams 1977). After the green revolution, more production units were built all over the world, but the United Kingdom, North America, and many other

European countries have monopolized the fertilizer industry. First names no longer exist except brand names. Major industry holder is a Russian company Uralkali ranked by Forbes in 2008.

1.3 Chemical Fertilizers and Their Formulations

Chemical fertilizers are industrially manufactured and available in many formulations in markets. Fast-acting and low-cost, commercially available chemical fertilizers, are in solids (granular, crystalline, powder, and pills), slow-release spikes and pellets, liquids, and tablets (Table 1.2). Fertilizers mostly contain major nutrients (nitrogen, phosphorus, and potassium) in a specific ratio. Chemical fertilizers comprising micronutrients and secondary macronutrients are also available.

1.4 Chemical Fertilizer Effect on Soil Parameters

1.4.1 Soil Chemical Parameters

Sustainable agriculture is an important global issue (Lin et al. 2019). Fertilizers are applied to soil for improving soil quality by providing nutrients for plant growth. Plants entail nutrients for completing their life cycle (Sharma and Chetani 2017). To supply plants with these nutrients, chemical fertilizers are added to the soil. Application of chemical fertilizers, like nitrogen, phosphorus, and potassium, are considered as the most beneficial way to provide nutrients to plants. Different chemical fertilizers usually enhanced the soil nutrient available and increased nitrogen, phosphorus, and potassium available in soil (Azizi et al. 2016). The application of these fertilizers not only improves nutrients but also affects soil health either positively or negatively. Soil health is collectively described by physical, chemical, and biological aspects of soil.

Nitrogen fertilizers contain nitrogen as ammonium, nitrate, and urea. Plants can uptake nitrogen in either as ammonium (NH_4^+) or nitrate (NO_3^-) (Fig. 1.1). At the point when a charged particle is taken up by plant roots, the plant ordinarily discharges a particle with similar charges to keep up a balanced pH. Nitrate is the main form of nitrogen that plants uptakes in crop production conditions ideally. Ammonium-based nitrogen fertilizers are converted into nitrate bacteria under aerobic conditions and release hydrogen ions (H^+). This H^+ reacts with hydroxide ion (OH^-), released during nitrate uptake by plants. The overall effect on soil pH is close to neutral. Nitrate- nitrogen-based fertilizers are directly plants take up nitrogen in nitrate forms. Urea nitrogen rapidly hydrolyzes to ammonia. Thus it shares similar characteristics as ammonia-based nitrogen fertilizers. Overall, an H^+ ion is released by plants and reduces pH in the rhizosphere when ammonium ion is

Table 1.2 Different chemical fertilizers and their formulations

Fertilizer	Matrix	Formula	Fertilizer	Formulation	Formula
Urea	Solid (prill or granules	$Co(NH_2)_2$	Magnesium sulfate (Epsom salts)	Solid (crystalline)	$MgSO_4.7H_2O$
Anhydrous Ammonia	Liquid	NH_3	Potassium magnesium-sulfate	Solid (crystalline)	Langbeinite: $K_2Mg_2(SO_4)_3$
Ammonium nitrate	Solid (prills, granules, crystalline)	NH_4NO_3	Ammonium sulfate	Solid (crystalline or granules)	$(NH_4)_2SO_4$
Urea ammonium nitrate solution	Liquid		Borax	Solid (crystalline)	$Na_2B_4O_7$. $10H_2O$
Ammonium sulfate	Solid (crystalline or granules)	$(NH_4)_2SO_4$	Boric acid	Solid (crystalline)	H_3BO_3
Monoammonium phosphate	Solid (granules)	$NH_4H_2PO_4$	Solubor	Solid (powder)	$Na_2B_4O_7$. $5H_2O$ $Na_2B_{10}O_{16}.10H_2O$
Diammonium phosphate	Solid (granules)	$(NH_4)_2HPO_4$	Calcium sulfate (gypsum)	Solid (powder)	$CaS0_4.2H_2O$
Nitrophosphate	Solid (granules)	NO_6P^{-2}	Calcium nitrate	Solid (crystalline)	$Ca(NO_3)_2 \cdot 4H_2O$
Ammonium polyphosphate	Liquid	$[NH_4PO_3]_n(OH)_2$	Calcium ammonium nitrate	Solid (Prills and granules)	$5Ca(NO_3)_2 \bullet NH_4NO_3 \bullet 10H_2O$
Single superphosphate	Solid (granules)	$Ca(H_2PO_4)_2$	Calcium cyanamide	Solid (granules)	$CaCN_2$
Triple superphosphate	Solid (granules)	$Ca(H_2PO_4)$	Bone meal	Powder	
Phosphoric acid	Liquid	H_3PO_4	Iron sulfate	Solid (crystalline)	$FeSO_4{\cdot}xH_2O$
Potassium chloride	Solid (granules)	KCl	Iron chelates	(soluble powder)	
Potassium sulfate	Solid (granules)	K_2SO_4	Manganese oxy-sulfate	Solid (crystalline)	$MnSO_4{\cdot}H_2O$
Potassium nitrate	Solid (granules)	KNO_3	Manganese chelates	(soluble powder)	
Magnesium oxy-sulfate	Solid (granules, crystalline)	$MgSO_4(H_2O)_x$	Zinc oxy-sulfate	Solid (crystalline)	$ZnSO_4$
Dolomitic limestone	Solid (crystalline)	$CaMg(CO_3)_2$	Zinc chelates	(soluble powder)	
Calcium chloride	Solid (crystalline)	$CaCl_2$			

Source: Gowariker et al. (2008), Savoy (2009), Kant and Kafkafi (2013)

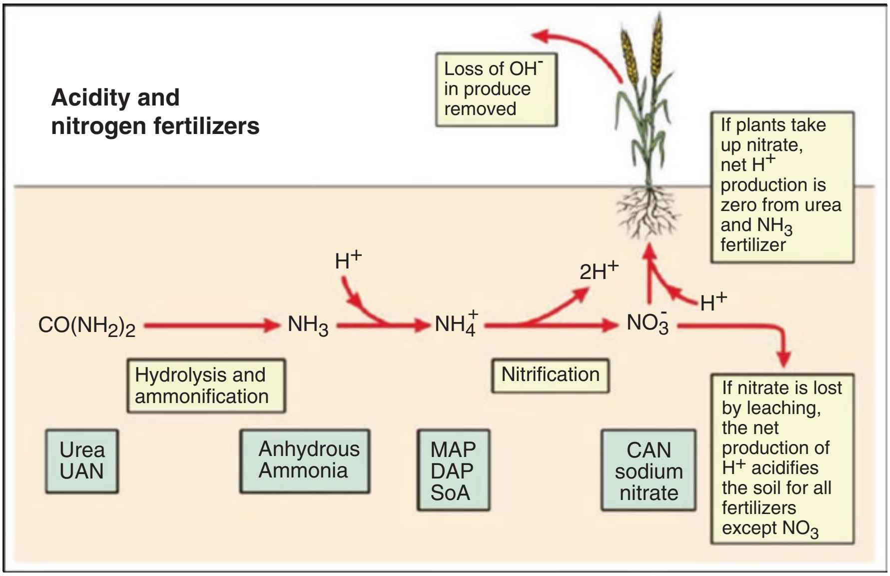

Fig. 1.1 Soil acidity and nitrogen fertilizers. (Purbasha et al. 2017). *MAP* monoammonium phosphate, *DAP* diammonium phosphate, *SoA* sulfate of ammonia, *CAN* calcium ammonium nitrate, sodium nitrate

taken up (Belay et al. 2002). An OH^- is released when a nitrate ion is taken up and increases soil pH. However, ammonium-based nitrogen fertilizers are over-applied to reimburse for the nitrate leached. Therefore soil pH is decreased over time. This may occur because of H^+ accumulation through the nitrification process (Guan 2016).

Phosphorus fertilizer affects soil pH when added to the soil (Iqbal et al. 2020). The decrease in pH by application of phosphorus fertilizers is minor comparative to nitrogen because phosphorus is used in lower amounts. pH changes mainly by the gain or release of H^+ ions by the phosphate (Fig. 1.2). Monoammonium phosphate and single and triple superphosphate added phosphorus to the soil as $H_2PO_4^-$ ion. This situation can decrease the pH in soil with pH > 7.2 but did not affect soil pH in already low pH soil. Most acidifying phosphorus fertilizer is phosphoric acid. When phosphoric acid is applied in soil, H^+ ions will always be released and acidify the soil. One H^+ ion is released at soil pH < 6.2, and two H^+ ions are released at soil pH > 8.2. Phosphorus from diammonium phosphate is added as HPO_4^{2-} and makes acidic soils with pH < 7.2 more alkaline but does not affect the soil with a pH >7.2. The ammonium polyphosphate hydrolysis process is pH neutral, where phosphorus is present as a molecule of $P_2O_7^{4-}$ that changes to HPO_4^{2-}. Hence any decrease in pH due to adding phosphorus is regarded as alike as in diammonium phosphate. Single superphosphate and triple superphosphate are sometimes stated to decrease soil pH due to acidic reaction (Table 1.1). In soils with pH values <7.7, the reaction described in Table 1.3 neutralizes the low pH of soil, so no net acidification actually occurs there (Purbasha et al. 2017).

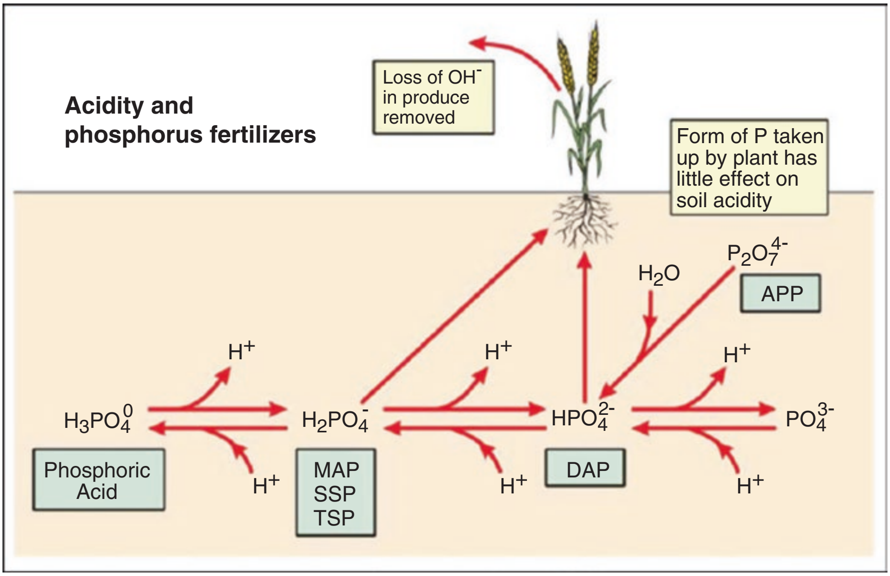

Fig. 1.2 Soil acidity and P fertilizers. *MAP* monoammonium phosphate, *DAP* diammonium phosphate, *SSP* single superphosphate, *TSP* triple superphosphate, *APP* ammonium polyphosphate (Purbasha et al. 2017)

Table 1.3 Chemical fertilizers reaction affecting soil pH

Phosphorus	Acidifying reaction	$Ca(H_2PO_4)_2 + 2H_2O \rightarrow CaHPO_4 + H^+ + H_2PO_4^-$
	Neutralizing reaction	$CaHPO_4 + H_2O \rightarrow Ca_2+ + H_2PO_4^- + OH^-$
Nitrogen	Ammonia oxidation	$NH_3 + O_2 \rightarrow NO_2^- + 3H^+ + 2e^-$
	Nitrite oxidation	$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ +2e^-$

In soils with high pH (>7.2), some acidity is produced by H^+ ion dissociation from the $H_2PO_4^-$ molecule. Phosphorus uptake by crop has a minute effect on pH of soil because a small amount of phosphorus is uptaken.

Sulfur addition affects soil pH by the release of H^+ ion. H^+ ion is released when elemental sulfur (S^0) or thiosulfate ($S_2O_3^{2-}$), in ammonium thiosulfate is applied to soil (Fig. 1.3). With the addition of one molecule of S^0, two H^+ ions will be produced. Production of two H^+ ions is balanced by the production of OH^- or H^+ uptake (same as the release of OH^- ions). Where produce is removed, a net decrease in soil pH will occur if elemental sulfur or thiosulfate ($S_2O_3^{2-}$) are used. However, small amounts of sulfur are added and reserved by plants compared to nitrogen. Potassium fertilizer has no influence on pH (Purbasha et al. 2017).

Chemical fertilizers affect the soil quality by changing cation exchange capacity (CEC) in acidic soils. It is the ability of soil to hold exchangeable cations on adsorption sites. Cation exchange capacity indicates the fertility of the soil, and it is decreased by chemical fertilizer application (Titilola 2006). Low pH induces a

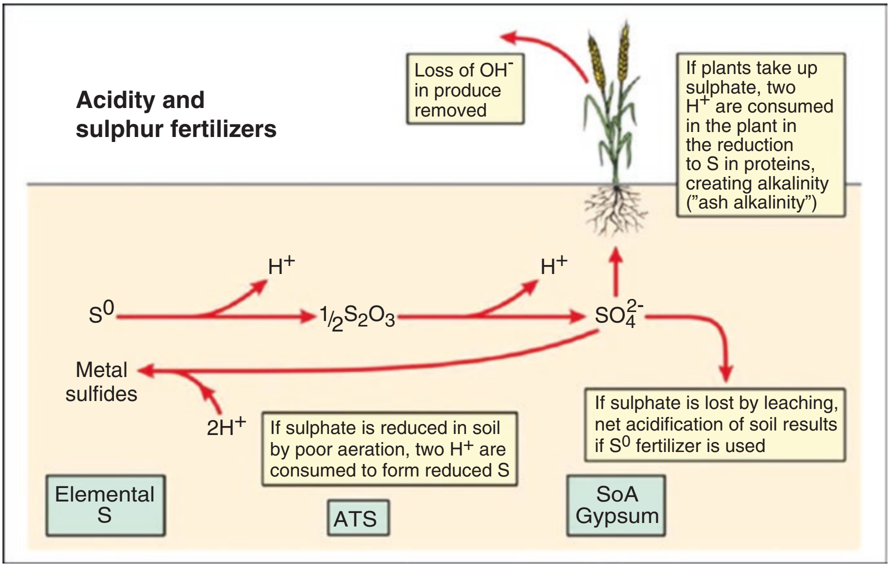

Fig. 1.3 Soil acidity and S fertilizers. S^0 = elemental S, *ATS* ammonium thiosulfate, *SoA* sulfate of ammonia. For each molecule of S^0 added to soil, two H^+ ions will be generated, and these can be balanced through plant uptake by either uptake of H^+ (same as excretion of OH^- ions) or the generation of OH^- (effectively organic anions) within the plant to form alkaline plant material ("ash alkalinity"). Where produce is removed (which is often the case in agricultural systems), net acidification of soil will occur if S^0 or ATS are used Add Source: (Purbasha et al. 2017)

reduction in CEC because of acidifying nitrogen fertilizer application and by nitrate leaching (McKenzie et al. 2004). High CEC soils often have high exchangeable calcium, reducing the effect of increased pH by hydrolysis of urea to NH_3 volatilization (Jones et al. 2013). Cations linked with soils CEC are exchangeable calcium, magnesium, sodium, and potassium. Usually, large quantities of negative charge in the soil make it more fertile as they can hold more cations. In acid soils, these cations are substituted by aluminum, manganese, and H^+ ion and produce much higher CEC values (McKenzie et al. 2004). In a soil slightly alkaline soil, CEC increased with nitrogen phosphorus and potassium fertilizer application because of the production of more aromatic nature compounds of organic matter (Brar et al. 2015)

Soil organic carbon (SOC) and total nitrogen decreased in soil with low pH (<7) due to chemical fertilizer application (Titilola 2006). Soil organic carbon sequestration contributes to the improvement of soil fertility (Reddy et al. 2017). Total nitrogen reduced more under chemical fertilizer application because of fast nutrients availability from these sources. Decreased SOC and total nitrogen result from stimulated organic matter decomposition in soil and crop residue because of applied fertilizer. This process leads to higher nitrogen mineralization and ultimately, higher crop nitrogen uptake or loss through leaching (Titilola 2006). However, in long-term experimentation in above seven pH soil, an increase in SOC is also reported when NPK fertilizers were applied. Excessive use of these fertilizers increases the

Table 1.4 Acidification potential of different fertilizers in the soil

Acidity level	Fertilizer name	Fertilizer composition
Neutral	Potassium nitrate	13% nitrogen
	Calcium nitrate	15.5% nitrogen
Moderate	Anhydrous ammonia	82% nitrogen
	Urea	46% nitrogen
	Ammonium nitrate	34% nitrogen
	Urea ammonium nitrate	32% and 28% nitrogen
Moderately high	Diammonium phosphate	18% nitrogen, 46% P_2O_5
High	Ammonium sulfate	21% nitrogen, 24% sulfur
	Monoammonium phosphate	11% nitrogen, 52% P_2O_5

Source: (Ag Professionals 2013)

SOC, while low dose will not affect SOC as much as affected by a full dose of nitrogen phosphorus and potassium (Brar et al. 2015). A gradual increase in chemical fertilizer application will increase organic carbon gradually (Azizi et al. 2016). Carbon-to-nitrogen ratio (C/N) affected nonsignificantly by nitrogen phosphorus and potassium fertilizer application, and a slight increase in C/N ratios of soil with time occurred (Dong et al. 2012). This increase occurred because of SOC and total nitrogen accumulation gradually with time (Darilek et al. 2009).

Electrical conductivity is the measure of electrical current passing through a solution. Electrical conductivity increased when chemical fertilizers are added to the soil. A large number of salts and nutrients are added in soil along with the application of inorganic fertilizer addition (Table 1.4). An increase in salts increases salinity that ultimately results in increased electrical conductivity (Azizi et al. 2016).

1.4.2 Physical Parameters

Soil physical properties are affected by exogenously applied chemical fertilizers. Application of nitrogen fertilizer is the utmost essential tactics for improving soil physical-chemical parameters (Azizi et al. 2016).

Application of chemical fertilizers increased cumulative infiltration and infiltration rate with time. Nitrogen fertilizer application improved SOC concentration that results in better soil physical properties especially infiltration rate (Bhattacharyya et al. 2007). A positive correlation exists between SOC and infiltration rate (Brar et al. 2015; Rasool et al. 2007). Phosphate fertilizer with organic matter application improves hydraulic conductivity and infiltration rate. The pores size distribution, pore continuity, and soil aggregate stability affect the value of infiltration rate (Laddha and Totawat 1998). Phosphorus addition improves soil structure and soil aggregation by increased rooting density, more release of exudates, and improved concentration of SOC (Brar et al. 2015). Phosphorus addition improves pore size and biochannels and ultimately accelerates the water flow (Haris and Megharaj

2001). Chemical fertilizer had increasing or sometimes no effect on the bulk densities of soil upon application (Malik et al. 2014). For the reduction in bulk density, more soil organic carbon is required that results in higher root biomass (Bhatt et al. 2019). Soil aggregation is closely related to soil bulk density. As chemical fertilizers don't have a noticeable effect on soil aggregation and flocculation, no significant results observed on the bulk density of soil (Kumar et al. 2011). In contradiction, a marginal reduction is also reported in bulk density at nitrogen phosphorus and potassium application, maybe because of improved production of biomass with the consequential upsurge in organic matter content of the soil (Bhatt et al. 2019).

Other soil physical properties like soil aggregation, flocculation, infiltration rate, porosity, water holding capacity hydraulic conductivity, etc. are affected by phosphorus fertilizer application. Phosphorus addition increases rooting density, more release of exudates that increases the concentration of SOC and the associated improvement in soil structure and soil aggregation. Better soil aggregation improves soil pore continuity and porosity (Brar et al. 2015). Improvement in soil aggregation happens because of precipitation of phosphate hydroxides and carbonates (Bronick and Lal 2005).

1.4.3 Biological Parameters

Soil biological properties are connected with microbial activity in the soil. These organisms include bacteria, fungi, earthworms, nematodes, protozoa, and different arthropods. Soil organisms perform many functions like break down of organic matter, nutrient cycling, and making them available for plant uptake, reduce nutrient leaching loss as nutrients are stored in soil organisms' bodies, and maintain the structure of the soil. Earthworms are significant in soil bioturbation. Bacteria play a dynamic role in the nitrogen cycle (FAO, USA).

The microbial community comprises of living microorganisms smaller than 5–10 μm^3. Microorganisms produce gums and mucilages and help in aggregate formation (Watts et al. 2005) and play a vital role in nutrient cycling that sustains the productivity of soil (Vineela et al. 2008). Microbial communities produce and use different pools of carbon and release nutrients that are plants available biologically (Calbrix et al. 2007). Enzyme activities participate in soil physical, chemical, and biological processes and effect agronomic management on productivity of soil (Mikhailouskaya and Bogdevitch 2009).

Chemical fertilizers (nitrogen phosphorus and potassium) affect microbial diversity in two ways. The effect of chemical fertilizer depends on the type, nature, and composition of the microbial community (Rousk et al. 2010). The acidophilic microbial community will be decreased when chemical fertilizers will release OH^- and increase the pH. An increase in pH will affect microbial communities adversely. While high pH will be favorable for those microbes, which are high pH loving. Chemical fertilizers that cause acidity will hinder the growth of high pH loving bacteria, while the acidophilic microbial community will flourish (Lin et al. 2019).

A fungal community grows better usually in the acid environment, while the bacterial community is decreased, so the progressions carried out by the bacteria will be affected negatively. Nitrification rates reduced in very acidic soil, because of lower activity of nitrifying bacteria (Cheng et al. 2013). Bacterium phyla, *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, and *Proteobacteria*, are abundantly identified bacteria (Zhao et al. 2012; Li et al. 2016). Acidophilic bacteria, *Acidobacterium*, *Acidicaldus*, and *Acidothermus*, increased in an acidic environment, created by chemical fertilizer in the soil. *Acidibacter* was found to be exceedingly related to soil pH of soil. Long-term chemical fertilizers application decreased the soil pH, as well as activated the heavy metal ions in the soil. This results in the deterioration of physicochemical properties and crop quality (Lin et al. 2019). Phosphorus fertilizer hinders the growth of mycorrhizal fungi, but the extent of hindrance is dependent on fungal species and soil available P level (Seymour 2002).

Chemical fertilizer effects on soil microbes are usually short term because soil pH changes only for a short time, and soil buffer capacity helps to attain original pH of soil. Short-term effects of anhydrous ammonia and urea application on soil were observed in a project in New South Wales. Total microbial activity reduced by the application of ammonia and urea for 5 weeks and returned to normal after that. The microbial activity starts to recover after that; however, it was more or less for some organisms. Large increase in nitrifying bacteria population occurs in the soil after 5 weeks of application. Protozoa and their population reduced in the number of about 80% and did not return to normal numbers after 5 weeks (Angus et al. 1999).

Chemical fertilizer application at different crop stages also affects the behavior of microbial communities. Flowering stage showed more microbial community compared to other stages of soil sampling. Enzyme activity was also different at differing stages. All three-chemical fertilizer, nitrogen, phosphorus, and potassium, increased invertase activity at all sampling stages. Potassium has more effect on enzyme activity as nitrogen and phosphorus increasing effect were on two of three sampling stages. Nitrogen at flowering increased urease activity compared to other fertilizers (Li et al. 2012).

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Chapter 2
Organic Agriculture: Principles, Current Status, and Significance

Peer Saffeullah, Neelofer Nabi, Saima Liaqat, Naser Aziz Anjum, Tariq Omar Siddiqi, and Shahid Umar

Abstract Agriculture is facing the pressure to grow high in order to feed burgeoning world population. Intensive agricultural activities have impacted soil fertility and decreased crop productivity and quality. Organic agriculture has been proposed as a holistic and alternative production system that principally disallows the usage of synthetic fertilizers, pesticides, livestock feed additives, and growth hormones. Combining science, tradition, and innovation, organic agriculture nurtures ecosystems and soil health and helps in accomplishing the global food and ecosystem security. This paper (i) introduces organic agricultural system by highlighting major concepts and principles and also its current status and (ii) overviews the role of organic agriculture in sustainably improving crop productivity/yield, and quality parameters, and in environmental and human health. In addition, major challenges to organic agriculture and aspects so far unexplored in the current context have also been highlighted. Outcomes of the discussion may provoke future research in the current direction.

Keywords Crop quality · Environmental sustainability · Human health · Organic agriculture · Sustainable agriculture

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2.1 Introduction

2.1.1 Concept and Principles

A matter of contentious since its advent, organic agriculture has been debated as an ineffective approach to ensure food security (Connor and Mínguez 2012; Pickett 2013). The extent of organically managed farmlands, numbers of organic farms, and global market size for organically grown foods have increased steadily (Willer and Lernoud 2017). The sales of organic foods and beverages have enormously been increased by almost fivefold between 1999 and 2013 to US$ 72 billion and are expected to double by 2018 (Willer and Lernoud 2017). Focused mainly on harmonizing multiple sustainability goals, organic farming has been recognized as an innovative production system, and its importance is expected to increase in accomplishing the global food and ecosystem security (De Schutter 2010).

Organic agriculture is a holistic and alternative farming system that evades or largely excludes the usage of synthetic fertilizers, pesticides, livestock feed additives, growth hormones, and more recently the use of genetically modified organisms (GMO's) (Lairon 2010; Goh 2011). It enriches biodiversity and promotes biological activity of soil (USDA-NOSB 1995; Gold 2007). Organic agriculture nurtures health of soils, ecosystems, and humans. Additionally, organic agriculture also puts together science, tradition, and innovation to promote joint environment and boost fair relationships and a decent quality of life for all (IFOAM 2005). To accomplish any particular function within a system, organic systems utilize agronomic and mechanical methods rather than usage of synthetic resources (FAO 1999). Organic agriculture is based on the management practices embracing preservation, restoration, maintenance, or enhancement of ecological harmony; relies on the principles of sustainability; and hence helps in attaining objectives of environmental, economic, and social sustainability (Keatinge et al. 2001; Haas et al. 2010; Pamela 2010). In organic agriculture, the soil fertility is protected in long run through sustaining levels of organic matter and returning all the wastes to it chiefly as compost additions and animal or green manures. The effectual recycling of organic wastes including plant residues, animal wastes, and weeds is ensured to curtail the gap among N, P, and K supplementation and exclusion from the soil (Chhonkar 2002). Organic farming fosters soil microbial activity, cautious mechanical involvement, use of better crop varieties, and water and soil conservation practices. Herein, crop rotation, intercropping, and mulching can enhance soil nutrients; and the use of appropriate cropping techniques, biological control, and natural pesticides can help in pest control (Gomiero et al. 2011; Yadav et al. 2013). A brief summary of historical aspects related with the organic agriculture is given in Table 2.1.

Table 2.1 Summary of historical aspects related with the organic agriculture

Date/ year/ period	Details	Reference/ remark
1924	Rudolf Steiner, Kobierzyce (Poland), in his agricultural course expressed disquiet at the new directions in commercial agriculture and sparked the evolution of organic agriculture in Europe. Steiner's course of 1924 eventually led to the publication of his widely read book *Bio-Dynamics Farming and Gardening* which simultaneously appeared in English, German, Dutch, French, and Italian editions	Lockeretz (2007), Paull (2011)
1930s, 1940s	The foundation of organic agriculture was established in its own right in Britain by Lady Eve Balfour and Sir Albert Howard, who is often regarded as father of organic agriculture	Lockeretz (2007)
1940s	Masanobu Fukuoka, a microbiologist working in soil science and plant pathology, quit his job as research scientist and returned to his family farm and devoted the next 30 years to devise a radical no-till organic system for growing crops, now known as "Fukuoka Farming." The introduction of organic agriculture in Japan was influenced by work of Masanobu Fukuoka. The development of organic agriculture in Switzerland (called ecological agriculture there) is associated with the writings of Hans Mueller	Lockeretz (2007), Pearson et al. (2011)
1940	The term organic agriculture was introduced by Lord Walter Northbourne, a British agriculturalist in his book *Look to the Land*	Paull (2010)
1943	Lady Eve Balfour published her book entitled *The Living Soil* in which she established a direct influence of farming practices over plant, animal, human, and environmental health	Gomiero et al. (2011)
1943	Sir Albert Howard, a British agronomist based in India, upon return to the UK tried to develop a scientific-based system for preserving soil and crop health. In his milestone book *An Agricultural Testament* Howard formulated one of the fundamental concepts of organic agriculture *The Law of Return*. The law of return elucidates the importance of recycling of all organic waste materials including sewage sludge back to farmland to maintain the soil fertility and soil organic content	Gomiero et al. (2011), Howard (1943), Conford (2001)
1945	J. I. Rodale expanded the ideas of Albert Howard in his book *Pay Dirt* and familiarized the concept of organic agriculture in the USA. He also introduced the techniques like crop rotation and mulching in an article published in Fact Digest.	
1946	The book entitled *The Living Soil* exerted a significant influence on public opinion and led to the foundation of "the Soil Association" in the UK	
1972	International Federation of Organic Agriculture Movement (IFOAM), Versailles (France) merged all streams of agriculture (including biodynamic, organic, biological, and ecological) that eschewed the use of synthetic fertilizers and pesticides. The vision of IFOAM was worldwide adoption of organic agriculture	IFOAM (2011)

(continued)

Table 2.1 (continued)

Date/ year/ period	Details	Reference/ remark
1977	Eve Balfour, one of the founders of IFOAM, claimed that "the criteria for a sustainable agriculture can be summed up in one word "permanence", which means adopting techniques that maintain soil fertility indefinitely; that utilize only renewable resources, to avoid those that contaminate the environment; and that foster biological activity throughout the cycles of all the involved food chains." She performed the famous "Haughley experiment" to compare the conventional and organic agricultural systems	Balfour (1977)

Table 2.2 The number and share of countries performing organic agriculture

Region	No. of countries performing organic agriculture	Countries per region	Share of countries performing organic agriculture
Europe	47	47	100%
Africa	39	56	70%
Asia	37	47	79%
Latin America and Caribbean	33	46	72%
Oceania	13	26	50%
North America	3	5	60%
World	**172**	227	**76%**

2.2 Current Status

Today, almost 160 countries of the world practice organic agriculture (Willer et al. 2009). Some new countries continue to join the community of organic producers taking the count to 181 (IFOAM 2019) (Table 2.2). A steady progress in the expansion of organically cultivated land in the world has been noticed during last decade. About 43 million hectares of land were under organic agriculture worldwide in the year 2014 (Willer and Lernoud 2017). 500,000 more hectares of land were under organic management in 2014 than for 2013. In total 81.2 million hectares of land are organic (Fig. 2.1). Globally there are nearly 2.3 million organic producers (Willer and Lernoud 2017). Currently 0.99% of the cultivated land is organic in the world. The region of Oceania covers major extent of organic land traversing 17.3 million hectares, constituting about 40% of worlds organic agricultural land. Europe at second position cuts across 11.6 million hectares of organic land contributing about 27% of it. Latin America constitutes 15% (6.8 million hectares), Asia (8%, 3.6 million hectares), and North America shares 3.7 million hectares contributing only 7%. Africa lags behind contributing an area of 1.3 million hectares sharing about 3% of total organic agricultural land (Figs. 2.2, 2.3, 2.4, and 2.5).

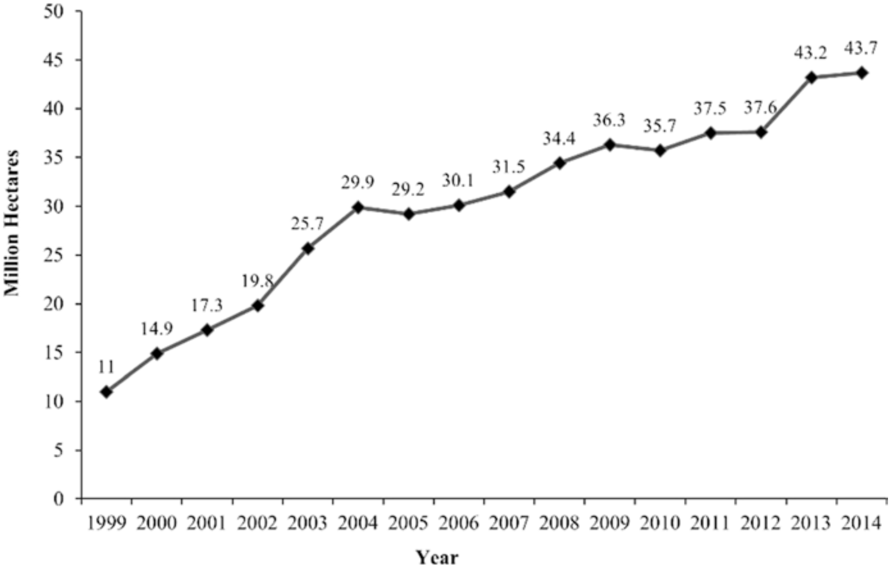

Fig. 2.1 Global trends of organic agricultural land

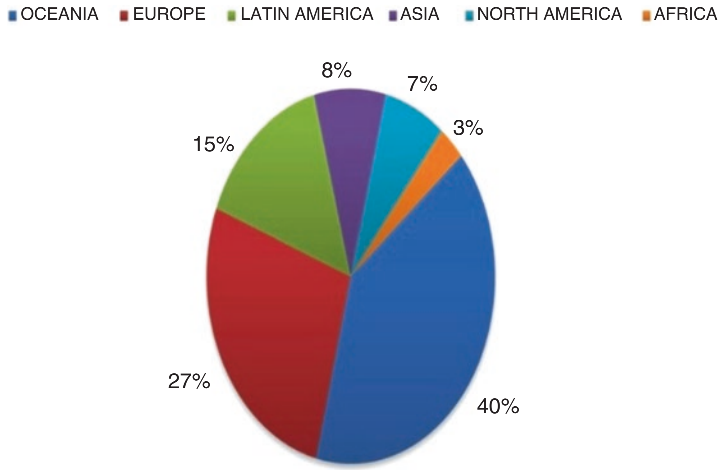

Fig. 2.2 Bar graph showing percentage of area under organic cultivation in seven continents of the world

2.3 Organic Agriculture: A Sustainable Agriculture

The world community is facing the challenges of feeding the burgeoning 9 billion people, and also the limits of resources have become more apparent. In this scenario, sustainable agriculture is gaining increased consideration. Sustainable

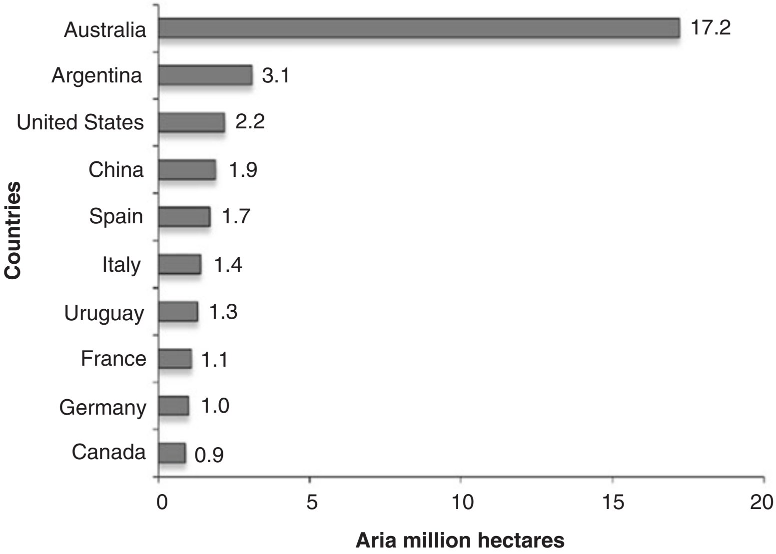

Fig. 2.3 Top ten countries with largest areas of organic agricultural land

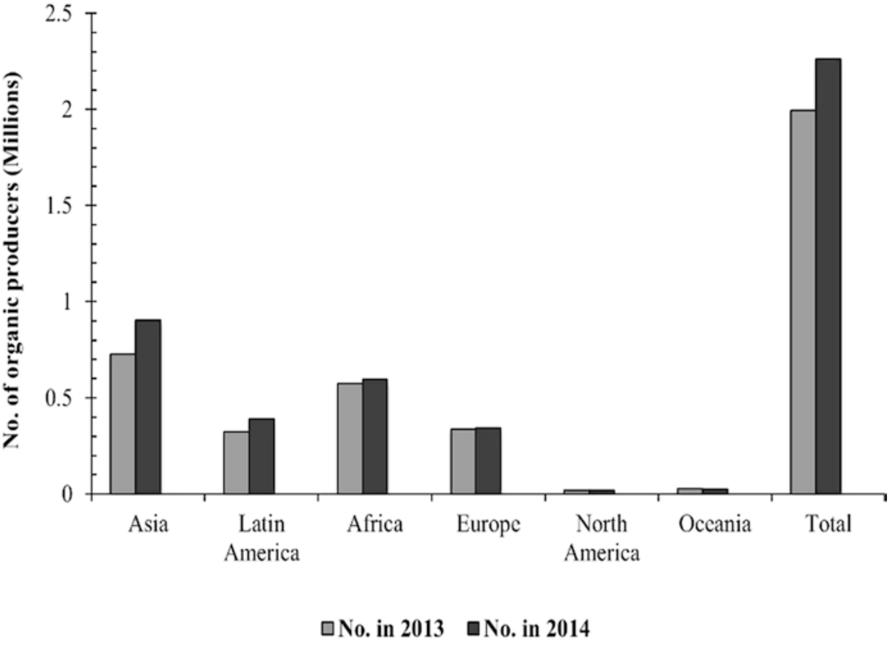

Fig. 2.4 Global increase in no. of organic producers

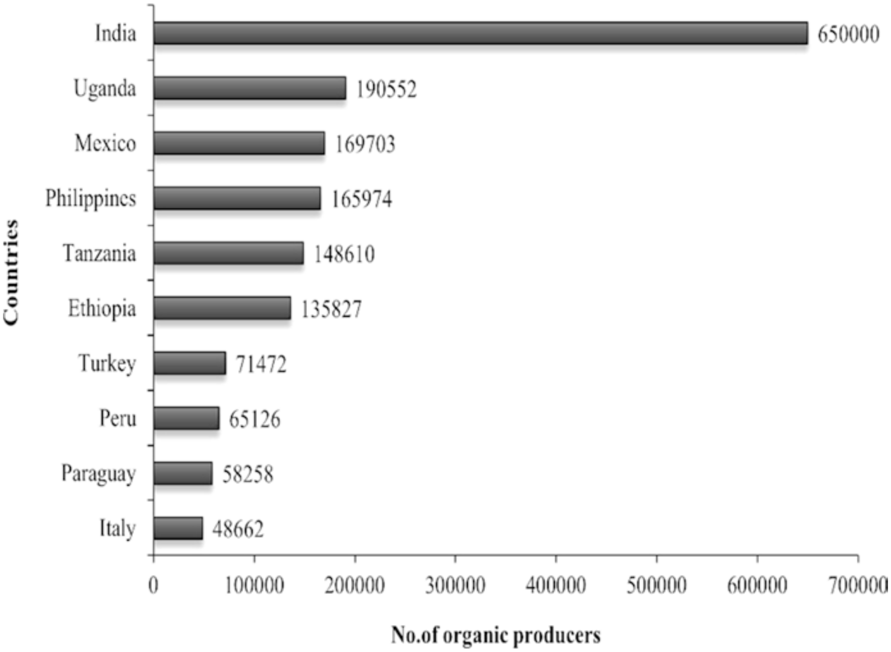

Fig. 2.5 Top ten countries with highest no. of organic producers

agriculture is an effective resource management system which fulfills changing human needs without compromising on the quality of environment and depleting natural resources (FAO 2008). Thus, sustainable agriculture emphases on such a production system which can sustain the food needs of all without draining the treasured resources. Sustainable agriculture is often referred to as a system central for attaining the goal of sustainable development.

In fact, organic farming is looked upon as unique alternative approach established to fulfill the goals of sustainable agriculture. Various practices employed in organic farming like mulching, intercropping, and integration of cattle and plants are not new to agriculture systems but have been used traditionally. However, organic farming is centered over several legislations and certifications, which exclude the use of nearly all synthetic or chemical inputs, and the core issue of this system is soil health. Moreover, exclusion of synthetic fertilizers in traditional agriculture was primarily due to unavailability of synthetic fertilizers, whereas organic farmers voluntarily restrict the use of readily available chemical fertilizers. Modern agricultural practices have been well documented to impart negative impacts on the human health as well as the farm. The practice of irrational and excessive usage of chemical fertilizers and pesticides has influenced people to search for alternatives. Organic farming has become one of the extensively used production system, which is assumed as a suitable substitute to evade the harmful effects of conventional agriculture.

Although the road toward sustainability stands unclear, organic farming has the prospective to attain it (Rigby and Cáceres 2001). Soil quality, one of the major issues of sustainability (Nannipieri 1994), can be addressed by organic agricultural practices (van Diepeningen et al. 2006). There has been a rapid diminution in soil organic matter due to intensive synthetic inputs and intensive cultivation, besides disrupting soil physical properties. Howbeit, to enforce the sustainable management practices, there is a great need to understand the nexus between cycling of nutrient and the aspects leading to their decay in soil. Nutrient cycling involves various physicochemical and biochemical reactions, which are catalyzed by enzymes secreted by soil microbiota (Kiss et al. 1975) and plant roots. As a result, any variation in soil microbial populations will modify the activity of soil enzymes. Fertility of soil is determined by mineralization of some essential elements like C, N, P, and S which are dependent upon microbial populations (Frankenberger and Dick 1983). Hence soil fertility and microbial activity are closely linked. Due to introduction of cover crops and application of manures, inputs of C, P, K, Mg, and Ca were found to be higher in organic systems (Clark et al. 1999). However, the levels of NPK in some organically managed soils were lower than synthetic cultivation systems (Mäder et al. 2002). Although, in some organic cultivation systems, higher amounts of total C, N, and P were found (Cavero et al. 1997; Clark et al. 1999; Poudel et al. 2002), while Mäder et al. (2002) stated minor dissimilarities for chemical characteristics of soil for instance: organic C and P. In contrast, the pH was found to be slightly alkaline in the organic soils (Clark et al. 1999; Mäder et al. 2002).

2.4 Organic Nutrition and Crop Productivity/Yield and Quality Parameters

Global food security is one of the challenging problems in the debate on performance of organic agriculture in increasing or maintaining crop yield. The crop yield or productivity of organic system and its potential contribution to fulfill the world's ever-increasing food demands are key questions (Padel and Lampkin 1994). Organic agricultural systems generally require additional land than conventional systems and hence put pressure on land resources. This, in turn, will lead to a decrease in area of natural ecosystems; however the magnitude of biodiversity on and around the agricultural systems will increase.

Statements on the practicability of achieving food security with organic systems are generally established by comparing organic and conventional yields. Researchers are highly divided on the issue of feasibility of organic farming to sustain the global population (Reganold and Wachter 2016). Organic agriculture has the potential to meet the food demands of burgeoning population at global scale, but not regionally (De Vries et al. 1997). Lotter (2003) compared a lot of reviews on organic versus conventional yield and argued that if intake of meat is brought down, large-scale transition to organic agriculture is possible without bringing any nourishment deficiencies. Organic agriculture can "contribute significantly" to bolstering the present and future populace, and it might even be conceivable to shrink the agricultural land base (Badgley et al. 2007). These arguments were intensely questioned by Cassman (2007), Connor (2008), Emsley (2001), and Goulding et al. (2009), as the authors contended that the data utilized by Badgley et al. (2007) and the presumptions made on nutrient accessibility in organic systems, especially N, were excessively optimistic.

Some researchers found a positive impact on crop yield by using organic fertilizers in long-term experiments (like Bi et al. 2009), whereas others (like Reganold and Wachter 2016) refuted this argument. However, the performance of organic agriculture in terms of yield trends is highly reliant on soil type and cropping methods, and the application of organic fertilizers gradually enhances soil physical properties and its organic content which might lead to a positive yield trend (Zhang et al. 2009). Surprisingly, most of the comparison studies have concentrated on increased yield rather than on a holistic natural resource management for food security. Moreover, the potential crop yields are specified as the maximum yield obtained from a particular plant under well-defined agroclimatic conditions, whereas ignoring the losses caused by pests and diseases as well as water or nutrient constrains (Lobell et al. 2009). In organic agriculture, these yield-limiting factors largely hamper the yield; so these factors need to be coped suitably so as to lessen the yield gaps concerning organic and conventional agriculture.

Soil fertilization is an essential feature which ultimately determines the plant yield and harvest quality (Koutroubas et al. 2016). Soil fertility can be managed efficiently by utilizing better fertilization practices. But, the influence of diverse fertilizers on soil fertility and crop performances is highly variable, dependent on type, composition, rate, and time of fertilization. Several beneficial impacts of organically grown plants are attributed to lack of pesticide residues (Reganold and Wachter 2016). However, the proponents of organic agriculture proclaim nutritional superiority of organic foods as compared to conventionally grown plants, due to proper soil management and fertilizer practices.

During last few years, organic food industry has witnessed a phenomenal progress in all food sectors. In the USA, a 40-fold increase was witnessed in the organic food demand from 1986 to 1996 and valued over $4.2 billion at the end of 1999 annually and estimated to grow over 24% annually (Fisher 1999). In the UK, the market of organic food is projected to be valued above US$567 million (Reavell 1999) and constitutes 3–4% of total food sales (Wright 2000). Though, in some European nations, organic markets are substantially larger than that in the UK. For instance, in 1997, Germany had the leading market share estimated over US$1.92 billion (Reavell 1999).

A number of whys and wherefores have been placed behind the substantial rise in demand of organic foods, although the relative preferences may vary from person to person or country to country. Regularly surveys report the content of pesticide residues in conventionally grown foods to be the main decisive factor for rise in purchasing of organic produce. In some countries the concerns for environment are also an impetus for purchasing organic products. In the USA, consumers preferred organic products since they believed that parameters like safety, freshness, general health benefits, nutritional quality, environmental influence, flavor, and general product were crucial when they purchased organic foods (Jolly et al. 1989). Some organic consumers referred to environmental and health motives for buying organic foods (Wandel and Bugge 1997). Some studies also point out that the vegetables grown under conventional agriculture exhibit higher tissue nitrate levels as compared to vegetables grown in organic management (Woëse et al. 1997).

2.5 Organic Agriculture and Environmental Sustainability

Presently, we are facing one of the emergent challenges of the twenty-first century: meeting society's rising food demands and in chorus decreasing agriculture's ecological damage. It would be imperative to highlight issues originating from the current agricultural system. Varied agrochemicals being used in the agricultural activities are adversely affecting soil, water, food, and atmospheric environment as well. The use of chemical-based fertilizers, pesticide, and insecticide have contributed in polluting soil and water resources and exacerbated nitrate pollution; have led to the accumulation of several heavy metals in soils and eutrophication of water; brought stratospheric changes; and also have badly impacted farmer's health (Bender et al. 2016; Feng and Zhu 2017; Galloway et al. 2003; Gorski et al. 2019; Ngatia et al. 2019; Tomich et al. 2016).

The use of synthetic external inputs like fertilizers and pesticides during green revolution has no doubt brought about enormous increases in productivity but consequently leads to prodigious environmental pressures. Organic agriculture attempts to tackle this problem by restraining the synthetic chemicals and integrating several environmentally sustainable practices. The organic system endeavors at a miniscule interference of the natural equilibrium. It also strives for providing superior food by prohibiting chemicals unsafe for humans.

There is currently substantial interest in organic farming as a system to provide environmental benefits. It is considered as a systemic approach to agronomic production that is striving for an inclusive environmental sustainability including social and economic aspects. A basic principle in organic agriculture is to abate environmental impacts while maintaining an economically feasible level of production. Though, a complex relation exists between the environment and the agricultural system. Organic production system has been suggested as a possible way to lessen agriculture's environmental constrains (Ponisio et al. 2015). It is often endorsed for having lesser environmental impacts as compared to high-input conventional farms because it substitutes synthetic agrochemicals with natural inputs like compost or through ecosystem services like pest control (Azadi et al. 2011).

2.5.1 *Organic Nutrition and Soil Fertility*

An ultimate motive of organic farming is to preserve soil fertility. Soil fertility may be described as the capability of soil to yield an adequate crop with least usage of resources like fertilizers. Soil quality, which is interrelated to fertility, is the potential of the soil to perform inside the confines of ecosystem by promoting biotic activity and conserving the environment of living organisms (Doran et al. 1996). A fertile soil offers vital nutrients for crop growth, upholds various biotic communities, and shows a distinctive soil structure.

The stock of organic matter acts as a backbone in maintaining soil fertility. Any changes in this stock triggered by new farming methods may be evident more than hundred years later. Retaining same agricultural system over longer periods will create equilibrium amid accumulation and decomposition processes with a constant stock of organic matter.

Organically managed farms include the practice of catch crops, the reusing of plant residues, and apply organic manures as opposed to chemical fertilizers. Stolze and Lampkin (2009) claimed that organic agriculture accomplishes well than conventional agriculture in terms of maintaining soil organic matter. Zhang et al. (2018) stated that organic fertilizers promoted aggregation and had high C levels. Li et al. (2017) also reported that application of organic manures leads to higher C levels in rhizosphere. Application of organic fertilizers and exploitation of perennial crops are presumed to increase organic matter levels in the soil, even though more studies are required to establish this.

2.5.2 *Organic Fertilizers and Soil Biological Properties*

Organic systems intend to maintain a remarkable intensity of soil biological activity, so as to improve soil quality and thus create a harmonious metabolic interaction between the plant roots and rhizosphere (Stolze and Lampkin 2009). Soil harbors a large diversity of bacteria and fungi (Hawksworth 1991; Brussard 1997). A wide variety of organisms is also essential to assure the sustainability of the biome. These key organisms (bacteria or fungi) are sometimes selected as environmental indicators in order to depict the soil health.

The role of microbes in preserving the soil fertility is critical in organic farms. Beneath the soil, the microbial activity leads to decay of organic matter. The species of mesofauna act upon organic matter and enhance the availability of N to plants (Setälä and Huhta 1990). Earthworms, a key species, are regarded as farmer's friend, and boost the fertility of soil. The application of organic fertilizers clearly increases the population of earthworms in the rhizosphere. For instance, the number of earthworms increased (20 times) as a result of conversion of conventional farm to organic unit (Christensen and Mather 1997). Axelsen and Elmholt (1998) assessed that the population of earthworms rises >2.88% after organic conversion; however it depends on the nature of fertilizer. Li et al. (2017) stated that organic amendments strongly enhance composition of bacterial communities. The presence of micro-arthropods is likely to be greater in organic than conventional production systems (Krogh 1994).

Various decisive factors like type of soil, kind of fertilizer, and agronomic conditions influence the populations of microorganisms in the soil. Hence it becomes ambiguous to interpret that whether these effects are due to organic farming or due to other factors.

2.5.3 Organic Agriculture and Climate Change and Global Warming

Greenhouse effect is a natural phenomenon, but latest developments linked to climate change are worrying largely attributable to anthropogenic activities. Climate change has by now started affecting livelihood of people as well as ecosystems and poses a serious challenge globally in general and for affect-prone countries in particular. Rapid industrialization and urbanization lead to an upsurge in greenhouse gases (GHGs) – predominantly carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_20). Higher emissions of GHGs have led to modern global warming, which consequently altered global temperatures and rainfall patterns. Climate change has affected agriculture both positively and negatively. The effects are, however, reliant on geographical location and plant species. According to IPCC (2007a), food production has been hampered in tropical regions due to temperature fluctuations, and it is projected that, by 2050, food produce within South Asia will reduce by about 30%.

On the other, agriculture is regarded as one of main contributors of GHG emissions. The agriculture adds over 20% of GHG emissions worldwide (FAO 2008). Agricultural practices like use of livestock manure, N fertilizers, result in nitrous oxide and methane emissions, which persist in atmosphere for longer time than CO_2. Agricultural intensification has resulted in heavy consumption of resources like fossil fuels, contributing considerably to GHG production. The overuse of synthetic fertilizers added substantial volume of GHG releases. During last 35 years, 6.9-, 3.5-, and 1.7-fold increase in N and P fertilizers, and irrigated land, respectively, has doubled the pace of GHG emissions (FAO 2008).

Organic farming is perceived as a suitable agricultural system that possibly will accomplish the purposes of mitigating climate change. It allows ecosystems to adapt suitably to climate change besides having prospect to lessen the GHGs emissions from agricultural systems. Organic agriculture can thus comparatively accomplish the carbon sink notion of the Kyoto Protocol (FAO 2011). The improvement in crop land management, reducing and improving N fertilization, and use of alternative resources instead of fossil fuels are some of the recommendation to cut emissions of GHG from agricultural systems (IPCC 2007a, b). Studies reveal that fossil fuels usage in organic farming is about 50% as compared to conventional systems.

In conventional agriculture, nearly 75% of CO_2 discharges are due to N applications, fuels, and feedstuff, whereas in organic systems, almost 70% of CO_2 releases were because of usage of fuel and machinery (FAO 2008).

Very little evidence is available on the subject of CH_4 emissions in organic agriculture. Organic management possibly does not influence the CH_4 emission by ruminants; however, further investigations need to be done to establish the impact of organic agriculture on CH_4 emissions.

2.5.4 Organic Agriculture and Nitrate Leaching and Carbon Sequestration

The main goal of organic production is to have minimum impacts on environment. Organic farming has been found to decrease nitrate leaching and increase carbon sequestration. These benefits are achieved by practices like crop rotations, cropping of legumes, low external inputs of nutrients, and exclusion of mineral fertilizers and synthetic chemicals for crop protection. The exclusion of pesticides in organic farming provides protection to natural resources such as ground and surface water against harmful chemicals. Furthermore, organic farms intend to curtail fertilizer losses for two basic causes. First, fertilizers are valuable resources and lead to the maintenance of agricultural system. Secondly, a foremost objective in organic practices is to work in closed nutrient cycles to minimize losses to hydrosphere and atmosphere.

Of all the elements, N poses more difficulties with leaching. This is caused by the movement of nitrate in hydrosphere and also the discharge of NH_3, N_2O, and N_2 from the lithosphere to atmosphere. The application of low N inputs, low stock densities, and routine of catch crops in different seasons in organic systems leads to reduction in leaching. Nevertheless, improper management of organic systems may also lead to ground and surface water pollution. It may be caused by poor tillage of land and destruction of root systems of crop cover.

On the other, the carbon sequestration of soil is a vital aspect in agriculture and might compensate higher amounts of CH_4 and N_2O emissions caused by agricultural practices (UNFCCC 2008). The application of organic fertilizers is an effective strategy of enhancing carbon sequestration in soil (Lal 2004). Organic fertilizers enhance carbon sequestration of soil mostly in following three ways: First, it straightaway increases organic matter of soil; second, it raises root and root exudate input by inducing crop growth; and third, it proliferates growth of roots by enhancing physical conditions of soil (Gong et al. 2009).

2.6 Organic Farming and Human Health Implications

Globally a quick upsurge in demand and sale of organic food have been seen over past few years (Barański et al. 2017). Demand is largely compelled by customer perceptions that organic farming promotes environmental sustainability, and biodiversity, and improves food quality and safety. Consumers favor organic products largely by reason of health apprehensions, to evade contamination from chemicals, and for the perception of higher nutritional quality (Hughner et al. 2007). Although there are growing scientific reports for benefits of organic farming in improving biodiversity and environmental sustainability (Mondelaers et al. 2009; Tuck et al. 2014), however, there are still substantial scientific disagreements over whether or not and to what magnitude does organic products result in higher nutritional value and safety (Brandt et al. 2011; Palupi et al. 2012; Smith-Spangler et al. 2012). Some of the existing research for nutritional variances and possible health benefits of organic products are discussed below (Table 2.3):

- Organic produce has higher antioxidant potential (18–69% higher); intake of polyphenolics and antioxidants has been related to a reduced threat of certain

Table 2.3 Effect of organic management on nutritional quality of various crops

Study(year)/references consulted	Crop/s tested	Design of study/ management	Parameters evaluated	Content increased or decreased
Alföldi et al. (1996)	Barley	Organic	Ca, Cu, Zn	Increased
Rembialkowska (2007)	All	Organic	Fe, Ca	Increase
Kolbe et al. 1995	Potato	Organic	Vit. C	Increase
Caris-Veyrat et al. (2004)	Tomato	Organic	Vit. C	Increase
Leclerc et al. (1991)	Celeriac	Organic	Vit. C	Increase
Weibel et al. (2000)	Apple	Organic	Vit. C	No difference
Gutierrez et al. (1999)	Olive	Organic	Vit. E	Increase
Woëse et al. (1997)	27 crops	Organic	β-Carotene	No difference
Lucarini et al. (1999)	Apple	Organic	Phenols and polyphenols	Increased
Carbonaro et al. (2002)	Peach	Organic	Phenols and polyphenols	Increased
Mitchell et al. (2007)	Tomato	Organic	Phenols and polyphenols	Increased
Pérez-López et al. (2007)	Pepper	Organic	Phenols and polyphenols	Increased
Levite et al. (2000)	Grapes	Organic	Resveratrol	Increased
Rossi et al. (2008)	Tomato	Organic	Salicylic acid	Increased
Hoefkens et al. (2009)	Carrot Lettuce Potato	Organic	Nitrate	Decreased
Hoefkens et al. (2009)	Spinach	Organic	Nitrate	Increased

ailments like neurodegenerative and cardiovascular diseases [discussed by Barański et al. (2014)].
- Brandt et al. (2011) found increasing amounts of antioxidants in plants grown under organic farms.
- Smith-Spangler et al. (2012) found higher levels of phenolics in organically cultivated plants.
- Crops grown in conventional system retain higher amounts of Cd and possess four times more pesticide residues; there are all-purpose recommendations to minimize the ingestion of pesticides and Cd to evade their deleterious health impacts [discussed by Barański et al. (2014)].
- Plants cultivated under conventional farming also contain higher levels of protein, nitrogen, nitrate, and nitrite; increased consumptions of these compounds have been associated with both positive and harmful health effects [discussed by Barański et al. (2014)].
- Omega-3 fatty acid content has been found to be higher in organic meat, milk, and dairy products [discussed by Średnicka-Tober et al. (2016a,b)].

Keeping in view these results, it is tempting to come to a conclusion that organically managed crops may result in higher intake of a variety of nutritional components like antioxidants, certain vitamins, and omega-3 fatty acids, but lesser ingestion of adverse pesticides, heavy metals (like Cd), and saturated fatty acids (Reganold and Wachter 2016).

A lesser number of human cohort studies have recognized relations between organic food intake and human health. Most of these reports were mother-and-child dyad cohort studies and found positive relation among organic vegetable and/or dairy intake and menaces of preeclampsia in mothers (Torjusen et al. 2014), eczema in infants (Kummeling et al. 2008), and/or hypospadias in babies (Brantsaeter et al. 2016; Christensen et al. 2013). Besides, another UK cohort study examined incidence of cancer in mid-aged women but found no strong relation concerning consumption of organic products and lesser occurrence of non-Hodgkin's lymphoma, though the observation period was only 7 years (Bradbury et al. 2014). Thus, more human-based cohort studies are needed to ascertain the claims of nutritional superiority and health welfares of organic products.

2.7 Conclusions and Prospects

Opponents and proponents of organic agriculture often appear to pronounce diverse actualities. Despite the fact, both sides have scientific evidences to support their arguments, though neither side is completely right, there is some ambiguity in several dimensions. Organic farming has some promising benefits; it improves soil fertility, carbon sequestration, and biodiversity; helps attain environmental and economic sustainability; and arguably enhances nutritional quality and performance of crops. Yet, the main impediment for organic agriculture is reduction in crop yield (at least during initial years), nutrient gaps, and lack of proper pest management system. In some countries, lack of labor guidelines also restricts the expansion of organic farming. In the course of few years, some conventional farms have also introduced specific practices of organic farming, for instance, conservation tillage, cover crops, or composting. More expansion of organic farming and integrating effective organic management practices into conventional agriculture may be alternative ways to attain sustainable agriculture.

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Chapter 3
Responses of Soil Properties to Organic Amendments

Shazia Ramzan, Ifra Ashraf, Tahir Ali, Tabasum Rasool, Pervez Ahmad, Mushtaq A. Wani, Rohitashw Kumar, and Abdul Rouf

Abstract The escalating population of the world is forcing the nations to rely on the synthetic fertilizers and chemicals which persistently pose threat not only to the human lives but create ecological imbalance. The rescue to this alarming condition is switching to the human-friendly mode of production system alias organic farming. Organic farming is a holistic approach to food production which aims to promote and maintain edaphic factors, human health, and ecological balance. This chapter presents a brief and comprehensive review of the impact of organic amendments on soil physical, chemical, and biological properties. The organic amendments enhance the soil structure by enhancing aggregate stability, aggregate hydrophobicity, soil porosity, and soil permeability and reducing bulk density. The effect of the organic waste surcharge on the chemical properties can be recognized in terms of the increased organic carbon and macronutrient concentration in soil and decreased toxic concentration of the heavy metals. Organic farming helps in the improvement of soil biological health by proliferating the microbial mass and activ-

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ity. However, the application of organic amendments should follow recommendations in order to get desirable results.

Keywords Organic farming · Soil health · Soil properties

3.1 Introduction

Howard pioneered the work of the organic movement in India who articulated and hypothesized most of the views which were later accepted by those people who became active in this movement (Howard 1940). Organic farming can be referred to as a production system aiming at the sustenance of social, environmental, and economic parameters (Stockdale et al. 2001; Lampkin 2003). Codex Alimentarius Commission has defined organic farming as a holistic food production management system, which promotes and enhances agroecosystem health, including biodiversity, biological cycles, and soil biological activity (FAO/WHO 2013). The use of pesticides, growth regulators, synthetic fertilizers, and livestock feed additives has been altogether eliminated by the production system referred to as organic farming. The fundamental characteristics encompass nurturing soil biological activity, maintaining soil organic matter content to safeguard long-lasting fertility of soils, careful mechanical interference, and enhancing the use of leguminous crops for nitrogen self-sufficiency. The organic farming also takes care of recycling organic resources including weeds, livestock wastes, and crop residues and controlling diseases and pest banking primarily on organic manuring, crop rotation, natural predators, and resistant varieties (Chhonkar 2002).

In order to create the balance between the NPK addition and its depletion from the soil, soil fertility is maintained on a priority basis by returning all the agricultural wastes by composting (Chhonkar 2002). Organic matter performs a vital and diversified role affecting physical, chemical, and biological properties of soil. Physically, soil structure and other cognate properties are highly affected by organic matter. Soil organic matter in the chemical realm affects the cation exchange capacity and the capability for buffering changes in soil pH. Biologically, organic matter supplies nutrients and energy for microbial biomass and higher plants (Walsh and McDonnell 2012). A soil, despite being chemically and biologically fertile, if cannot substantially support crop development will not satiate its agronomic potential. Soil productivity is consequently governed by an amalgamation of influence of organic matter on chemical, physical, and biological soil properties (Doran and Parkin 1994).

The organic management of soil has been cited to contribute to the institution of an improved soil structure for crop production (Reganold 1995). The improvement in the soil properties has been associated with organic farming practices owing to a number of deliberations including the enhancement of soil organic matter, amplified earthworm population, soil fertility, biodiversity, etc. (Papadopoulos et al. 2006).

The organically managed soils have been found to have greater potential for soil structural improvement than that of conventionally managed soils (Shepherd and Harrison 2002; Pulleman et al. 2005).

Currently, the expanding population pressure has coerced many countries to expend synthetic fertilizers and artificial chemicals to meet the increasing demand of the food. The prolonged and over usage of chemicals has, however, resulted in human and soil health hazards along with environmental pollution (Barar 2015). Long-term field experiments have confided with the negative implications of the uninterrupted use of synthetic fertilizers and chemicals on soil vigor (Yadav 2003). Intensification in the exploitation of chemical pesticides, herbicides, and fertilizers during the last few years has given rise to other detrimental effects like groundwater contamination with nitrates, food contamination, stratospheric changes, eutrophication, etc. The sustainable use of high agricultural inputs for a very long time is not possible unless the inputs are appropriately assessed in relation to both their quantity and quality (Sofia et al. 2006).

All these negative constraints have forced now farmers in the developed countries to transform their prevalent farming system into an organic farming system. The demand for organic food is driven by consumers who are health-conscious and ready to pay high prices and have environmental concerns. Because of these hidden benefits, conventional growers are turning to organic farming. In Western countries as well as India, the government aims to invigorate the organic divide by means of subsidizations, consumer enlightenment, education, research support, and marketing (Yadav et al. 2013).

3.2 Effect of Organic Farming on Soil Physical Properties

Aggregate stability is a basic factor governing the fertility of the soil in physical facets which can be increased by proper management of organic matter content in soil resulting in maintenance of an apt soil structure (Diacono and Montemurro 2010).The inclusion of optimal organic wastes in soil aids in improving the soil physical properties. The organic matter is assumed to stabilize soil structure employing two dissimilar mechanisms: by fostering aggregate cohesion because of the binding capacity of organic polymers or fine roots or fungi (Chenu et al. 1994; Puget et al. 2000; Abiven et al. 2009) and by augmenting aggregate hydrophobicity, thereby slowing the wetting rates and hence degree of slaking (Sullivan 1990; Sander et al. 2004). The former reason has strong support by researchers than the latter one (Chenu et al. 2000). More specifically, the increase in soil structural stability can be attributed to the proliferation in soil microbial activity, particularly due to the surcharge of composted residues (Van-Camp et al. 2004). The microorganisms synthesize hydrophilic polysaccharides during decomposition which have a tendency to get adsorbed to mineral elements and thereby foster their interparticle cohesion (Chenu 1989).

Alternately, humus-containing products like composts and manures are expected to enhance water-repellant properties of aggregates (Jouany 1991). It has been proved in long-term experiments. Significant improvements in water retention and aggregate stability have been reported in a long-term experiment spanning 16 years involving either crop residue or farmyard manure applications because of increased concentration of humic colloids in soil (Dorado et al. 2003). The structural instability index has been reported to decrease by 2.5 units with respect to control plots in particular (Diacono and Montemurro 2010).

The improvisation in soil physical characteristics due to fortification of organic matter has been reported and can be attributed to a reduction in bulk density and increase in the permeability and aggregate stability (Gopinath et al. 2009; Achiba et al. 2010; Kuncoro et al. 2014). The same trend has been reported even in degraded saline-sodic soil of cold regions (Angin et al. 2013). Likewise, soil organic carbon also invigorates hydraulic conductivity by improving aggregate stability and soil porosity (Eibisch et al. 2015). Organic farming makes the soil aggregates resistant to crushing in most of the cases. Under organically managed soils, the greater crushing strength of aggregates has been reported in most of the cases as compared to conventionally managed soils (Król et al. 2013) which could augment confrontation to carbon sequestration and compaction under the previous. Król et al. (2013) reported the lesser tensile strength for larger aggregates under conventionally managed soils, while the reverse trend was noticed with the organically managed soils.

Nesic et al. (2014) reported better soil aggregate stability in organically managed farms in contrast to the plots managed conventionally over the decades as reflected by the soil structural index of mean weight diameter (MWD), with organic farms having high MWD of 0.95 as compared to conventional farming system with MWD of 0.73.

Yazdanpanah et al. (2016) proved the soil textural properties were improved due to integration of municipal solid waste at 30 Mgha^{-1} as it increased soil organic carbon content which in turn increased the soil porosity and water-stable aggregates. Municipal solid waste, when pooled with mineral fertilizer, increased the soil organic carbon content in soil (Meena et al. 2016). Similar results have been reported by Sabir and Zia-ur-Rehman (2015) and Nest et al. (2016). Sudhakaran et al. (2013) reconnoitered the impact of different management practices including conventional farming, sustainable farming, and organic farming on physical properties of soil and established that organic farming showed enhanced soil physicochemical properties in comparison to other farm management practices as shown in Table 3.1.

From Table 3.1, it is quite evident that organic farming showed better results followed by sustainable farming. Based on the experimental findings, Sudhakaran et al. (2013) assured the nutrient status was improved in organic farming as shown by higher nutrient levels in comparison to other farming systems.

Tejada et al. (2009) studied the impact of three different combinations of compost, i.e., nonleguminous plants, leguminous plants, and the combination of both plants, remains on plant cover and soil physical, chemical, and biological properties. All the compost combinations showed positive effect on soil properties, and percentage of plant cover increased which can be attributed to the addition of the

Table 3.1 Implication of different farming approaches on soil physico-chemical properties

Soil physicochemical properties	Control	Sustainable farming	Conventional farming	Organic farming
Moisture content (%)	11.4	4.2	4.1	8
pH	6	7.51	7.13	7.36
Bulk density (g/cm^3)	1.5	1.4	1.3	1.4
Particle density (g/cm^3)	2.9	2.7	2.9	2.7
Volume of the soil particle (cm^3)	18.8	17.9	17	17.9
EC (mS.cm^{-1})	0.4	0.2	0.15	0.3

Source: Sudhakaran et al. (2013)

humic acids to the soil released from composts added. Sodhi et al. (2009) established that soils can be recuperated and can protract carbon and nitrogen levels in soil with the continuous application of rice straw compost either singly or in blend with inorganic fertilizers. The soil recuperation can be attributed to the higher amount of water-stable aggregates which is the outcome of the continuous addition of the organic matter to the soil, stemming the boosted microbial activity and generation of microbial decomposition products. Molina-Herrera and Romanya (2015) studied the antagonistic and synergistic relationship of organic amendments and soil properties and confronted with the increase in the soil organic matter reserves.

Ma et al. (2016) investigated the influence of organic amendments on total nitrogen, soil organic carbon, aggregate stability, bulk density, field capacity, and available water for plants in a representative Chinese Mollisol. They applied four different treatments including inorganic fertilizer, inorganic fertilizer in combination with the maize straw, biochar amalgamated with inorganic fertilizer, and a control experiment. Their study deciphered that after three uninterrupted years of application, there was a significant reduction in the bulk density in amended plots than in the control plot. Biochar fortified with inorganic fertilizer increased the organic carbon content, mean weight diameter, and relative proportion of soil macroaggregates, field capacity, and plant available water. The enhancement in soil water retention has been attributed to the increases in soil organic carbon and aggregate stability. Similar results have been reported by Masulili et al. (2010), Abel et al. (2013), and Scotti et al. (2016).

Imran (2018) conducted the field experiment for 2 years, i.e., 2016 and 2017, consecutively at the Agriculture Research Institute Mingora Swat, Pakistan, to investigate the effect of organic matter amendments in soil on soybean and maize yield and soil vigor. Results revealed that morphological and phenological and traits of both crops showed positive correlation with the organic matter integration. The soil vigor also enhanced due to organic matter integration as obtained from the analysis of soil before and after the harvest.

However, the application of the organic manures is constrained by some of its negative bearings on soil physical properties like accelerated rain erosion due to increased detachment by raindrops, surface crusting, decreased hydraulic conductivity, termination of water-repellent properties, etc.; hence the recommendations should be followed (Haynes and Naidu 1998). On the basis of the information expounded in this subsection, it can be abridged that long-term and repeated applications of organic amendments can boost soil physical properties and, hence, soil fertility.

3.3 Effect of Organic Farming on Soil Chemical Properties

Electrical conductivity, soil pH, organic carbon, and available potassium and phosphorous are some of the most important indicators of soil fertility (Bogunovic et al. 2017). A substantial number of researches involving long-term fertility experiments indicated the increase in the organic carbon in soil due to organic farming. The increase in organic carbon triggered the increase in the cation exchange capacity because of high negative charge of organic matter. The high cation exchange capacity provides the sites for retainment of nutrients, making them available to plants (Weber et al. 2014).

Clark et al. (1998) transformed the conventionally managed plot into organically managed over the period of 8 years and reported that this transition increased the pH, exchangeable potassium, soluble phosphorus, and organic carbon and maintained relatively stable EC level. The increase in soil organic matter subsequent to the transition to organic farming occurs gradually particularly several years (Werner 1997); nevertheless it has potential to impose vivid effect on long-term productivity (Tiessen et al. 1994). Celik et al. (2004) also reported the increased organic matter content due to addition of organic substances, e.g., animal and crop residues, and compost and manure from organic waste. Nesic et al. (2014) also reported an increased organic matter associated with aggregates in plots managed organically over the period of 3–10 years as compared to the plots managed conventionally over the decades.

Habteselassie et al. (2006) compared the effect of different treatments, i.e., dairy waste compost, liquid dairy waste, and ammonium sulfate on nitrification, available nitrogen, nitrogen pool in soil, and yield of corn over 5 years. They uncovered that the dairy waste compost enhanced the C pool 115% and organic carbon by 54% and 143 in comparison to the treatment of liquid dairy waste treatments and ammonium sulfate, respectively. The trials which received the treated dairy wastes showed a threefold surge in nitrifier activity in comparison to the control trials. On the contrary, nitrogen from liquid dairy waste and ammonium sulfate is almost immediately available for plant uptake, as mineralization of the organic N present in the compost continues throughout the growing season, postharvest, and in the following years.

Montemurro et al. (2006) evaluated the environmental impact and agronomic value of two composts municipal solid waste compost and olive pomace compost on cocksfoot and alfalfa in Southern Italy in a 3-year field experiment. They found that the organic amendments including olive pomace compost and municipal solid waste compost showed a positive effect on the organic matter. After 3 years, the total organic carbon appreciably increased by 43.2% and 24.0% for alfalfa and cocksfoot plots, respectively, in comparison to the control experiment. The municipal-industrial wastes alone or in conjunction with the mineral fertilizer have been reported by many other researchers to kindle plant growth due to enhancement of organic matter on long-term basis (Ouédraogo et al. 2001; Hamdi et al. 2002; Mantovi et al. 2005; Cherif et al. 2009).

The conventional agricultural practices including the extensive use of mineral fertilizers and deep soil tillage can cause progressive depreciation of soil fertility, especially in areas characterized by high summer temperatures and scanty rains (Montemurro et al. 2007). Montemurro et al. (2007), determined the consequences of municipal solid waste compost application, and reduced soil tillage on crop growth parameters and on both mineral nitrogen deficit and soil chemical characteristics. They reported that the lack of a significant difference in sucrose and root yields between deep tillage and reduced tillage. Moreover, the blended organic mineral nitrogen fertilizer application presented crop yield statistically not different from mineral nitrogen fertilizer application. They also showed that the municipal solid waste compost not only augmented the humified organic carbon by 25.4% in comparison to the mineral fertilizer but also did not increase the heavy metal concentration in soil.

Castro et al. (2009) compared different treatments including air-dried sewage sludge, municipal solid waste compost and an inorganic chemical fertilizer and reported that organic amendments increase organic matter and macronutrients in soil. They also did not report the contamination due to heavy metals but reported an increase in microbial population in the organic treatment particularly in the air-dried sewage sludge treatment plot.

Despite the devoid of external supplementation with inorganic inputs, the organically managed farms showed improved soil health and increased plant nutrients (both primary and secondary) availability (Sudhakaran et al. 2013) as shown in Tables 3.2 and 3.3. The primary as well as secondary nutrients superseded in organic farms in comparison to conventional and sustainable farming and control plots.

Domagała-Świątkiewicz et al. (2013) stated that the organically managed farms possessed a higher level of the total organic matter (2.02%) than that of the conventionally managed farms (1.75%). They found the increase in the total soil N and P level in beetroot and celery farms. Although, Ca concentration was reported to be higher in conventional farms than in organic farms. Jan and Amanullah-Noor (2011) confirmed that the manure exhibits the toxicity moderating property for some heavy metals in soil and plants. According to Singh et al. (2007), manure alleviated

Table 3.2 Primary nutrients levels under different farming systems

Primary nutrients (g/kg)	Control	Sustainable	Conventional	Organic
Total N	2.4 ± 0.5	1.8 ± 0.2	2.2 ± 0.36	3.7 ± 1.15
Total K	34.4 ± 6.2	36.0 ± 7.0	22.1 ± 9.66	52.5 ± 9.09
Total P	1.2 ± 0.41	1.7 ± 0.21	1.2 ± 0.41	2.0 ± 0.55

Source: Sudhakaran et al. (2013)

Table 3.3 Secondary nutrients levels under different farming systems

Primary nutrients (g/kg)	Control	Sustainable	Conventional	Organic
Total S	3.7 ± 2.2	6.4 ± 2.62	6.1 ± 2.78	4.2 ± 3.07
Total Na	13.7 ± 0.12	10.6 ± 0.32	4.7 ± 0.6	17.7 ± 0.65
Total Mg	47.1 ± 0.54	36.1 ± 3.47	20.7 ± 2.12	49.2 ± 3.76
Total Ca	33.4 ± 1.01	28.2 ± 1.0	13.6 ± 1.39	48.0 ± 1.42
Total Mg	47.1 ± 0.54	36.1 ± 3.47	20.7 ± 2.12	49.2 ± 3.76

Source: Sudhakaran et al. (2013)

chromium toxicity in spinach. Khurana and Kansal (2014) also reported that farm manure reduced cadmium in a maize field. Mgbeze and Abu (2010) reported an increase in pH in soil from 9.4 to 10.39 as an aftereffect of the addition of farm manure.

Wang et al. (2015) found that organic fertilizers alone or in conjunction with inorganic fertilizers help to minimize the N losses and promote crop production. They also observed that continuous application of organic N or mineral fertilizer on long-term basis considerably roused gross N mineralization rates in soil, accompanied with the boosted soil N and C contents. In a 50-year long-term study, Blanchet et al. (2016) proved the use of organic amendments enhanced soil chemical properties and furnished a considerable amount of phosphorous and potassium. They reported that soil organic carbon content was amplified by 2.45% and 6.4% due to integration of crop residues and farmyard manure application, respectively, as compared to the use of mineral fertilizer alone. On the contrary, there was no significant change in soil carbon stock due to fertilization practices. Similar results for P and k were also obtained by Sabir and Zia-ur-Rehman (2015).

Scotti et al. (2016) studied the impact of municipal solid waste on soil quality and discovered that total nitrogen increased by 40 and 60% in the soil treated with on-farm compost and municipal solid waste, respectively; over the control treatment, exchangeable Na and EC were enhanced under municipal solid waste by 25% and 19%, respectively, in comparison to the control experiment. Available P in soil treated with on-farm compost was 36% more than that in the control field. On the contrary, there was no influence of both the organic amendments on some chemical parameters of soil like pH, CEC, limestone, exchangeable Ca, K, and Mg. Some former studies like Bhattacharyya et al. (2003) revealed that the addition of municipal solid waste compost in wetland rice field increased P, N, and K contents from 6.2 to 7.3 g $kg^{-1}$for P1.7 to 1.76 g kg^{-1} for N and 0.11 to 0.13 g kg^{-1} for K, respectively. Heavy metal (Cu, Zn, and Pb) uptake by straw and grain was minimized in comparison to the control. Similar results have also been reported by Walter et al. (2006), Hargreaves et al. (2008), and Montemurro et al. (2006).

After a long-term (40–50 years) experiment, Nest et al. (2016) showed that manure has more capability to increase soil pH and available P as compared to mineral fertilizer. They reported that extractable P was enhanced 2–4 times with manure application in comparison to a mineral P fertilizer application. Additionally, Sabir and Zia-ur-Rehman (2015) proved the capability of manure to increase buffering capacity and CEC of soil. Contrarily, Fe and Ca showed no response to manure application. On the basis of the information expounded in this subsection, it can be abridged that long-term and repeated applications of organic amendments can boost soil physical properties and, hence, soil fertility.

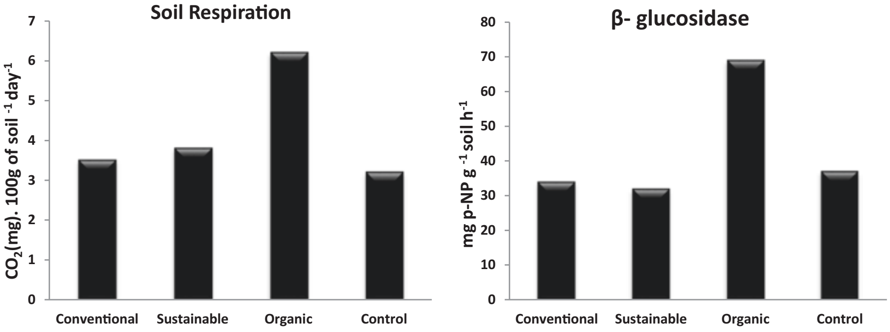
The review cited above clearly uphold the positive influence of the organic amendments on soil chemical properties as attested by an increase in the soil organic C content and macronutrient concentration and availability to the plants and decrease in the toxic concentration of heavy metals.

3.4 Effect of Organic Farming on Soil Biological Properties

Biological properties of soil are very central for safeguarding the fertility of soil because of their role in nutrient cycling. Soil microflora and microfauna play a key role in the mineralization of the soil organic matter, fixing atmospheric nitrogen and reducing nutrient losses by their immobilization in biomass (Blanchet et al. 2016). Additionally, some microbial organisms like mycorrhizal fungi speed up the nutrient uptake by plants (Johansson et al. 2004) or improvise the soil textural properties, e.g., earthworms (Bertrand et al. 2015). In general, microbial population offers a multitude of services aiding in the sustenance of crop (Altieri 1999).

Biochemical and microbiological properties of soil are very sensitive to slight modifications in management practices. This makes it possible to make use of them in order to evaluate the effects of organic amendments of variable sources on soil properties during experimental trials (Diacono and Montemurro 2010). Microbes like fungi, bacteria, microalgae, and actinomycetes perform a fundamental role in cycling of nutrients, decomposition of organic matter, and other chemical transformations in soil (Murphy et al. 2007). The microbes decomposing the organic residues utilize organic carbon for energy, which ultimately gets assimilated into their body tissues, liberated as metabolic wastes, or respired as carbon dioxide. The macronutrients phosphorous, nitrogen, and sulfur present in the organic residues are transformed into inorganic forms. Consequently, they are either immobilized and expended in the microbial tissue synthesis or mineralized and liberated into the mineral nutrient pool of soil (Baldock and Nelson 2000). N is assimilated by microorganisms in a quantity determined by the C/N ratio of the microbial biomass. Precisely, the amount of C required by the microorganisms is 20 times more than that of N. The net mineralization involving the release of inorganic N into soil takes place when the easily decomposable C compounds are present in low concentration and N in greater quantity (Diacono and Montemurro 2010). On the contrary, Corbeels et al. (1999) established that if N is present in lesser amount in the organic residues than that needed by microbes, immobilization of inorganic N from soil will take place in order to accomplish the decomposition process.

Organic farming alters pH, carbon and nutrient accessibility, and other chemical parameters, thereby amending makeup of microbial population in soil (Cookson et al. 2007), nutrient availability, or other chemical parameters. Sudhakaran et al.



Figs. 3.1 and 3.2 Impact of different farming management systems on and soil respiration β-glucosidase activities at $p = 0.05$ and $p = 0.01$, respectively. Source: Sudhakaran et al. (2013)

(2013) envisaged the consequences of distinct farming practices including sustainable, conventional, and organic farming on biological properties of soil and observed improved biological properties in organic farming in comparison to other farming practices as shown by greater soil respiration rates and β-glucosidase in organically managed soils, clearly discernible from Figs. 3.1 and 3.2.

The distinction of direct and indirect effects of an organic amendment on the behavior of microorganisms present in soil is intricate. The microbiological growth and activity can be triggered in the soils supplemented with compost or other raw organic materials, even in association with mineral fertilizer N. However, the direct influence on microorganisms introduced due to compost can be detected (Ros et al. 2006; Kaur et al. 2008). The compost treatments on long-term basis significantly improve the biological properties of soil like microbial biomass C, some enzymatic activities, and basal respiration. This is predominantly manifested in the upper stratums of the soil because of the most degradable added labile fraction of an organic matter (Ros et al. 2006; Tejada et al. 2006 2009). In comparison to the mineral fertilizers, the composts are generally decomposed on slower rates in soil hence continuously releasing nutrients which can survive the microbial population for longer duration (Murphy et al. 2007).

Generally, the quality and quantity of organic amendment to soil are the governing factors which control the copiousness of diverse microbial groups and their role in nutrient cycling. Leon et al. (2006) studied the potential of paper mill residual by-products to subdue the common root rot disease of snap bean relative to soil properties. They reported improved soil quality because of maximized water-stable aggregation and suppression of the disease in the treated plots in comparison to the control plots. Due to creation of optimal conditions in soil, the fungal-dominated microbial community was proliferated, hence able to suppress the disease. This study conforms to the study conducted by Cook (1990) who established that by stimulating antagonist microorganisms, the organic amendments added to the soil can stimulate disease suppression.

Many researchers have reported the positive influence of organic amendments on the biological properties of soil. The municipal solid waste when used in conjunction with the chemical fertilizers proliferates the microbial activities and crop production (Soumare et al. 2003; Castro et al. 2009; Sabir and Zia-ur-Rehman 2015; Wang et al. 2015; Meena et al. 2016). The organic amendments are believed to have positive influence on earthworm community as well because they feed on organic matter offering more organic substrates for earthworm growth (Curry and Schmidt 2007; Eriksen-Hamel et al. 2009; Bertrand et al. 2015).

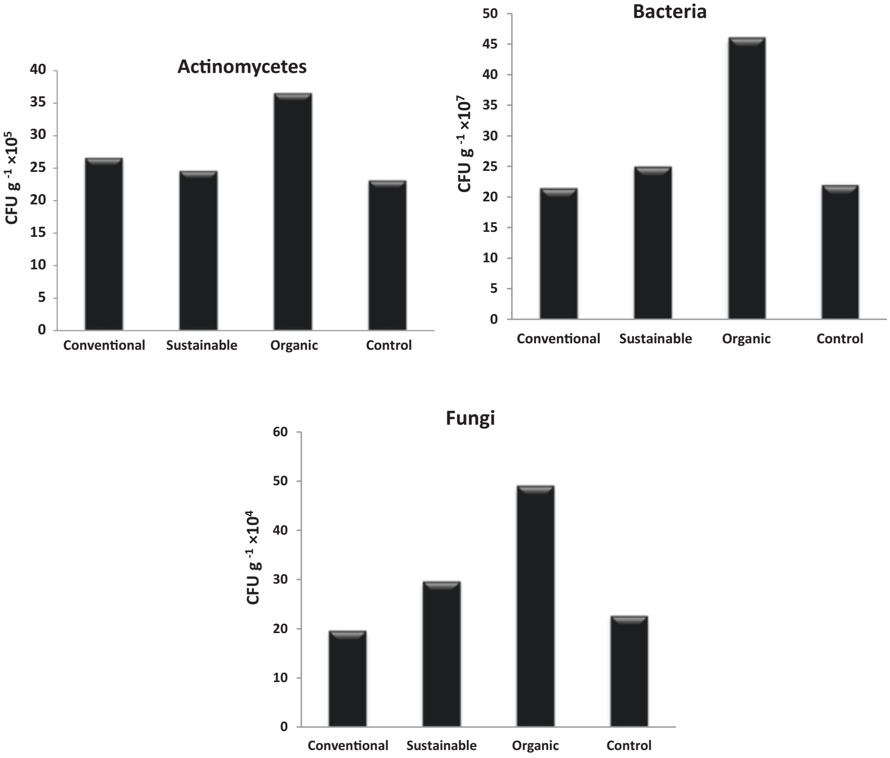
Phospholipid fatty acid analysis undertaken by Elfstrand et al. (2007) showed that in the soils treated with green manure over a long-term study of 47 years, biomass of fungi, bacteria, and total microbial biomass excluding arbuscular mycorrhizal fungi increased as compared to the soil receiving no organic amendments. In a long-term study spanning 50 years, Blanchet et al. (2016) reported an increase in the microbial population due to the incorporation of crop residues and farmyard manure as depicted by increased phospholipid-derived fatty acid contents and microbial biomass. They also reported that microbial biomass was enhanced by both treatments in particular farmyard manure application, leaving the structure of microbial community unaffected. Scotti et al. (2016) assessed the effect of manure and municipal solid waste compost and on soil quality. They asserted that the dormancy of the microorganisms is broken by the organic composts in order to degrade the added exogenic organic matter. They reported an increase in the level of phosphomonoesterase, β-glucosidase, and phosphormonoesterase in lieu of increased availability of nutrients and soil organic carbon, but the effect was pronounced in municipal solid waste compost. However, the increase in urease enzyme was observed in an experimental trial treated with municipal solid waste compost only. Similar results have been confirmed by Spaccini et al. (2009) and Scotti et al. (2015).

Yazdanpanah et al. (2016) reconnoitered the effect of urban municipal solid waste compost and alfalfa residue on hydrological properties of the soil and microbial respiration in the soil. They discovered that the microbial respiration got increased due to addition of organics in comparison to the control plot. The increase in the microbial activity of the order of 4–7 times was reported in a plot amended with organic manure in comparison to control plot (Molina-Herrera and Romanya 2015). Iovieno et al. (2009) observed an increase in the soil respiration, phosphatase and arylsulfatase activities, and fluorescein diacetate hydrolysis in the compost-treated plots after a 3-year long trial. The reviewed results insinuate that exogenic organic matter managements lead to an improvement in soil biological properties due to enhanced microbial biomass and activity.

3.5 Impediments of Organic Farming

Although organic farming has been found to stabilize the structural stability of soil, some researchers have reported some impediments of organic farming in comparison to other farming systems. The soil structure of conventionally tilled plots managed conventionally over the decades was found to be better than that of the organically managed plots because the soil structural indicator (Ks) was found to be higher in organic farms of the order of 5.5 as compared to conventionally tilled farm plots where it was reflected to be 2.6 (Nesic et al. 2014).

In an instance of comparing the various effects of organic and conventional management systems of soil on its ethanol sorptivity, total porosity, tensile strength aggregates, and water and repellency index, Król et al. (2013) elucidated that there was elevated total porosity in soil aggregates managed conventionally as compared to organically managed soil regardless of layer of soil or the aggregate size (Fig. 3.3). Also, it was enumerated that the sorptivity of ethanol (60 mm^3) and infiltration were faster when observed under conventional methodology over organic management irrespective of depth as well as aggregate size (Fig. 3.4 and 3.5). However, in 30–35 mm aggregates, size infiltration and sorptivity of water were found to be greater under organic to that of conventional management. Besides, the repellency index was found to be elevated for aggregate size of about 30–35 mm than 15–20 mm in each management system followed by its conventional management (Figs. 3.3–3.5).



Figs. 3.3, 3.4, and 3.5 Impact of different farming systems on soil actinomycete, bacterial, and fungal population. (Source: Sudhakaran et al. 2013)

3.6 Conclusion

Organic farming provides good quality food without posing any threat to the consumers, soil health, and environment. The positive effect of organic amendments on soil physical, chemical, and biological properties has been addressed in this review article, providing a platform for future research. Application of organic amendments on long-term basis assists in improving the overall soil quality index. The organic farming increases the soil fertility by improving the soil structural and hydrological properties of soil, increasing soil organic matter and other macronutrients. The continuous use of organic amendments helps in triggering the microbial growth, functionality, and activity, hence improving the soil biological fertility. The microbial mass enhancement is accompanied with the suppression of some diseases due to microbial antagonistic relationship with the disease causal agents. The toxicity of some heavy metals like Cu, Zn, and Pb is reduced because of their restricted uptake due to organic amendment. Although organic farming has been found to improve the soil properties, some researchers have reported some impediments of organic farming in comparison to other farming systems.

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Chapter 4
Vermicomposting: Sustainable Tool for Agriculture Environs

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Abstract Vermi-biotechnology is a sustainable technique which helps in recycling of wastes whether in zoo premises or outside the premises. Vermicompost is a quality product which contains necessary ingredients which are very beneficial for plants and helps to prevent environmental degradation at any level. It is a cheaper product which most of the farmers use in agricultural purposes and shows its effect at regular intervals over a long period of time. This is a pilot method, and it should be adopted in other zoological parks for the production of quality vermicompost as in zoo different dung-producing animals are present like hippo, gharial, deer species, elephant, etc. Template flower wastes are often used to form Agarbatti etc. for the successful accomplishment of the vermicomposting process. Waste water of aquatic animals modifies the vermicompost quality, and because of this reason, farmers prefer this eco-friendly product for enhancing the fertility of their productive land.

Keywords Vermicompost · Zoo · Sustainable · Agriculture · Biofertilizer

4.1 Introduction

Earlier the earthworms have been considered as friendly creatures for agriculture and hence called as “farmer’s friend”**,** “engineers of soil”, “nature’s plough”, “intestine of soil” or *soil nutrient-enriching creatures*. Vermiculture is also known as earthworm farming. In this method, earthworms are added to the compost. These worms break the waste and the compost very rich in nutrients. Vermicomposting

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technique also produces economic benefits to farmers by recycling agricultural wastes, and eco-friendly product produced is called vermicompost (Vital et al. 2016), a type of biofertilizer very beneficial for agricultural land. Vermicompost is a biofertilizer which is biodegradable and prevents environmental degradation and is safe for food chains and food webs present in terrestrial and aquatic ecosystems (Joshi et al. 2014). The earthworms feed on wastes generated from zoo, kitchen, and farmyard, and this waste is passed in their gut also called **bio-reactor** (Shankar et al. 2011), where symbiosis relationship occurs between bacteria and mixes gut secretions which contain hormones and other valuable nutrients, very important nourishment for plants. With an understanding of soil health and human sustenance Darwin imminently declared “Worms are energetic than the African Elephant and are more important to the economy than the cow”. He estimated earthworms to produce 10 tons of humus per acre. The excessive use of artificial fertilizers have deletrious impacts on fresh water streams, lakes and ponds. The agricultural runoff are rich in phosphates and nitrates which can cause unwanted growth of algae in these shallow water bodies hence depletes the dissolved oxygen levels, increase number of anaerobic bacteria, below water only respiration occurs, organisms living in this habitat either died due to lack of oxygen or move to other water streams.

This technique is first adapted in Gwalior Zoo in central India to recycle the wastes of wild animals; waste food is collected and brought into vermicomposting plant for biodegradation. The pit method is adapted; mostly exotic species of earthworm is utilized for organic matter degradation. Recently started in Gwalior Zoo, it is very beneficial for zoo premises and visitors; it keeps **Gandhi Zoological Park** attractive for visitors and increases the economy, also protects the zoo animals from diseases and regulates pollution.

4.2 Solid Waste Minimization Using Vermicomposting

The wastes collected from the zoo premises and adjacent area are stored in vermichamber for decomposition at least for 20 days (Manyuchi and Phiri 2013). During this exothermic reaction, it is not safe to put earthworms on waste due to more heat and it is very lethal for the worms. Composting is a biological process in which micro-organisms, mainly fungi and bacteria, decompose degradable organic waste into humus-like substance in the presence of oxygen. Vermi-biotechnology mostly uses exotic earthworms like African night crawler (*Eudrilus eugeniae*), *Eisenia fetida* and *Perionyx excavates* due to their voracious behaviour, and usually their size (14 cm) is more than native species of India. These creatures degrade the waste such as zoo animals, shredded paper, grasses, leaves, cattle, pressmud (Prabhak et al. 2008), etc. in their **gizzard** where these wastes pass and aligned with important nutrient-rich secretions from earthworm’s digestive system, and also symbiotic association occurs between gut and bacteria helps in degradation process of solid wastes.

4.3 Waste Water of Aquatic Animals Is Recycled in Zoo

The excreta of aquatic animals goes in water where they inhabit and contain lot of nutrients when laboratory analysis is done on waste water. To prevent this valuable waste water haggardness, recycling of water occurs only when dilute organic waste is sprayed on vermibed (Li et al. 2008), it helps to increase the quality of vermicompost and increases demand of vermicompost when this product is sold in zoo at cheaper rates (Jjagwe et al. 2019). The dilute organic waste water which is used on vermibed is of hippopotamus, Magar, gharial and turtle in zoo premises. This waste water also increases the population of earthworms in vermibed, and these worms are sold for 500/kg (Yadav and Devi 2009). The waste water of hippopotamus is recycled and analysed which contain valuable nutrients and is poured on vermibed; then quality vermicompost is produced which is used in agricultural purposes and get high yield shown in Fig. 4.1.

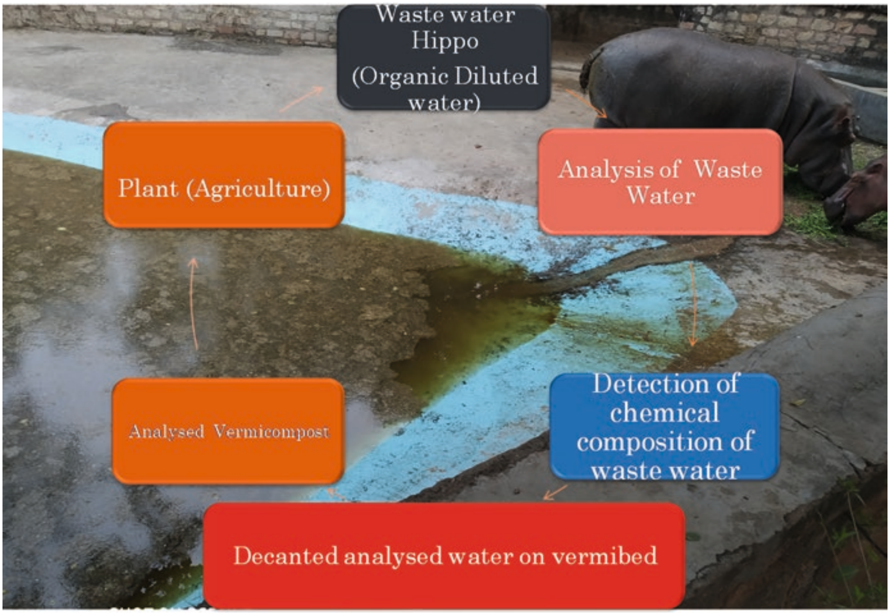

Fig. 4.1 Indicates that quality vermicompost is produced through the above cycle
Waste water of hippo is first analysed and then pour on vermibed; when vermicompost is prepared, it is also analysed by detection of nutrient concentration like nitrogen, phosphorus, potassium, etc. And then sold to formers on cheaper rates and then utilized on crops and vegetables and we get high yield. This agricultural product again used by animals.

4.4 Worms and Their Biological Features

Worms are cosmopolitan in distribution and are anomalous creatures of the animal kingdom. Earthworms are cosmopolitan in distribution, except the Arctic and Antarctic zones. Scientifically they belong to the phylum Annelida, class Clitellata, subclass Oligochaeta, order Haplotaxida. Earthworm's body is metamerically segmented with externally ringlike groove called *annuli* and internally by transverse septa. The segments are called metameric segments and have 100–150 segments; segmentation helps the organism to move. The body of earthworms are divided into preclitellar, clitellar and post-clitellar regions and possess circular ringlike structure called clitellum; it contains albuminous fluid through which fertilization occurs and **"egg cocoon"** and be put into the soil, after this young worms are developed (Shweta et al. 2006). Commonly the respiration in worms occurs through general body surface called **cutaneous respiration** due to their skin richly supplied with blood capillaries, and exchange of gases occurs on the basis of diffusion. The aortic arches function as a heart in earthworms, and different vessels supply blood to their respective body parts like dorsal blood vessel supplies blood to the front body parts and ventral blood blood vessels supply blood to back body parts, and closed type of circulatory system is present, and organ system organization also possesses worms. Digestive system in earthworms is like a bio-reactor which possesses enzymes and necessary secretions from glands of gizzard and intestine and also contains bacteria which shows positive relationship with the worms gut and hence degraded the wastes (Dominguez et al. 2001).

4.5 Temperature, P_H in Vermiprocessing Technique

Several factors have been identified to influence growth and fecundity of earthworms and degree of bio-conversion of waste stuffs (vermicomposting). These factors include the nature and composition of food and availability of necessary nutrients and physical parameters like temperature, light, moisture content and bulk density of the medium and biological parameters like diversity and density of microorganisms, population density of earthworms, etc. (Ansari and Hanief 2015). If the waste medium is suitable in terms of presence of balanced ratio of nutrients (C/N), its bulk density (porosity), moisture content (40–60%) and aeration, the activities of earthworms are able to maintain environmental conditioning and stimulating growth and fecundity of earthworms.

The optimum temperature range for vermicomposting bins ranges from 0° to 35° and favourable composting temperature in between 25° and 30 °C. If temperature is increased above 30 °C, the earthworms cannot survive; to drop this temperature, waste water is sprayed on the vermibed, to bring temperature below 30 °C. Earthworms have good buffering capacity, to increase or decrease $_PH$ value to maintain near neutral and even in too high/too low ambient temperatures; the temperature in the vermibed can be maintained to fairly at optimal range.

4.6 Vermicomposting for Agriculture

Earthworms are called natural conditioners of the soil, which regulates soil fertility. Worm casting produced by worms maintains soil in good condition and plant production and morphology in normal standard. It keeps food chains, food webs and ecological pyramids in proper operation. The worms degrade the complex waste materials into simpler forms which further becomes useful materials for soils (Arancon et al. 2004). Containing a rich nutrient base, worm castings boast an abundance of beneficial minerals, nutrients and microorganisms important for plant growth and disease suppression and necessary source of humus (Piya et al. 2018; Bikle and Montgomery 2015). Calciferous glands of the earthworm excrete calcium carbonate in worm castings, essential for the development of firm cell walls and for the absorption of nitrogen.

4.7 Soil Health vs Organic Fertilizers

Due to modernization man depends on artificial fertilizers like “herbicides”, “pesticides”, “insecticides”, “weedicides” and many other toxic substances which cause environmental imbalances. Aquatic biota and soil biota get disturbed especially our intestines of soil called earthworms. These profusion creatures are very sensitive to these chemicals; these creatures show negative response organic chemicals (Bhat et al. 2016). Some beneficial bacteria living in the soil–symbiotic association with root nodules are also exposed to such kind of threats. Vermicomposting technique is very beneficial for agriculture and organisms present in soil. Worms also produce an important liquid substance called wormwash, which is a good material for soil health and is also a good insect repellent (Samadiya 2017).

4.8 Temperature, P_H in Vermiprocessing Technique

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4.9 Endurable Use of Vermicomposting in Zoo

Gwalior zoo is one of the oldest (established in 1902) and most eminent zoos in central India; Gwalior occupies a strategic location in the Grid region of India. The zoo houses nearly 500 animals including 31 bird, 11 reptile and 21 mammal species as well and is one of a cream tourist terminus in Gwalior. Nearly 100 staff members (including management staff) have been deployed to look after of the animals. The waste is converted into vermicompost with the help of vermicomposting technique and sold to the public at affordable prices (Dhimal et al. 2013). Dung of herbivores and carnivores animals, droppings of birds are good source of raw materials for vermicomposting. On an average, 200 kg of dung is generated every day in the zoo (MCG 2015). Substantial amounts of slain leaves, grasses and other green substances from the garden section are also used. African night crawler (*Eudrilus eugeniae*) is used in zoo to convert mixed waste into agricultural valuable purpose; Figure 4.2 shows the worm on vermibed consume organic waste.

4.10 Use of Earthworms in Aquaculture and Poultry Farming

Earthworms contain high volumes of protein (60–70%), amino acids, niacin, lysine, methionine, phenylalanine and vitamin B12. These qualities are desirable for feedstock within the aquaculture and poultry farming industry (Prayogi 2011; Ahmadi and Karimi Torshizi 2014). In the Gwalior Zoo, earthworms are largely bred for bait for retail within the "fishing community"; however the application of vermicomposting for the production of feed for livestock creates opportunities that empower a sustainable food system (Pucher et al. 2014). The growth of enterprise in aquaponics, where systems are using fish waste to fertilize plants, provides a suitable avenue for the introduction of a vermicomposting system to compliment the sustainability of the process. Earthworm meal provides balanced food for brooding chicks, fishes, zoo birds, etc.

Fig. 4.2 *Eudrilus eugeniae* commonly called African night crawler survives in temperate regions of temperature in between 25 °C and 30 °C and consumes more waste as compared to native species of India

4.11 Formation of Vermiwash and Its Uses

Vermiwash is a liquid portion released during vermicomposting process, which if applied to the agricultural fields enhance the nutrient quality of soil environs and will be the boon for plant health. The collected water contains secretions of earthworms which possess different kinds of valuable macronutrients, micronutrients, vitamins, hormones and enzymes (Zarei et al. 2018). Vermiwash also has insect repellent activity and is also an important feed for juvenile carps. It is applied on ornamental plants and vegetations which get rid pest and other harmful insects on their leaves. Besides vermicompost, we also get secretions of worms called vermiwash shown in Fig. 4.3. These commercial products are important like Agarbati in daily life.

4.12 Soil Health vs Organic Fertilizers

Due to modernization man depends on artificial fertilizers like "herbicides", "pesticides", "insecticides", "weedicides" and many other toxic substances which cause environmental imbalances. Aquatic biota and soil biota get disturbed especially the intestines of soil called earthworms. These profusion creatures are very sensitive to

Fig. 4.3 Vermicompost on polythene bags and vermiwash on bottle and in small pockets contains Agarbati. Vermiwash has mosquito's repellent activity and Agarbati prepared from temple flower waste

these chemicals; these creatures show negative response organic chemicals (Bhat et al. 2016). Some beneficial bacteria living in the soil–symbiotic association with root nodules are also exposed to such kind of threats.

4.13 Vermicomposting for Agriculture

Earthworms are called natural conditioners of the soil, which regulates soil fertility. Worm casting produced by worms maintains soil in good condition and plant production and morphology in normal standard. It keeps food chains, food webs and ecological pyramids in proper operation. Containing a rich nutrient base, worm castings boast an abundance of beneficial minerals, nutrients and microorganisms important for plant growth and disease suppression and necessary source of humus (Piya et al. 2018; Bikle and Montgomery 2015). Calciferous glands of the earthworm excrete calcium carbonate in worm castings, essential for the development of firm cell walls and for the absorption of nitrogen.

4.14 Starving Worms Are Future Biodegrading Agents

Earthworms can eat daily half their body weight; they will be able to eat approximately ½ a pound of scrapes per day assuming ideal bin conditions (Table 4.1). Worms have capability to degrade complex organic wastes and enriches it with essential nutrients for plants and soil health (Aira et al. 2008). Starving worms are future indicators which help to control pollution in developing nations where livestock populations is much more enough (Sandhu et al. 2018).

4.15 Summary/Conclusion and Future Prospects

Worms are negative phototropic species, which play an important role in ecological balance and produce biofertilizers, and are eco-friendly with microbiota of soil. Wormwash is a useful product prepared from earthworms and is good conditioner to the soil and and insect repellent activity. The farmer's productivity is also dependent on these profusion creatures that help in turning and loosening the soil (Agrawal 2009). The uprising economical and ecosystem cost of agricultural chemicals, coupled with the ever-rising cost of landfill, calls for a realigning of management (Bhattacharya and Chattopadhyay 2002). The process of using surface dwelling species of earthworms to finely and ecologically break down organic waste, producing a superior organic fertilizer as a by-product, referred to as vermicomposting, is successfully providing sustainable answers in food production and organic waste management in the current changing environment. The integration of vermicomposting in agriculture and mainstream waste management presents economical, environmental and social benefits for the zoo and the surrounding areas of zoo, building resilience in response to the effects of natural resource depletion. Our future goal is to protect the diversity of worms and use less organic fertilizers which reduce the population of these worms. The excessive use of synthetic chemicals are lethal to non-target organisms and pose long term threats to soil biota. The pesticides, weedicides and insecticides used in agriculture, horticulture and vegetable growing zones are very harmful to these soft bodied animals. Due to this vermitechnology, the life of faithful worms must be protected, and their reproductive behaviour is improved by using environmentally useful products like vermiwash, vermicompost and some works also called vermicompost as **black gold**. Our whole

Table 4.1 Food consume by starving worms

Quantity of worms	Weight	Food	% of food eat by worms
500 worms	½ lb. of worms	¼ lb. of food	25%
1000 worms	1 lb. of worms	½ lb. of food	50%
2000 worms	2 lbs. of worms	1 lb. of food	50%
3000 worms	4 lbs. of worms	2 lb. of food	50%
Total 6500	7.50 lbs	3.75	

surrounding goes lit bit in sustainable development and open sources for unemployed youth, and their skill is used to protect this environment from artificial hazards.

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Chapter 5
Application and Viability of Macrophytes as Green Manure

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Abstract The increase in the human population is an important challenge to the food security in the world. Each year there are fewer areas in the planet destined for agriculture, in addition to soil erosion, and the threat of highly destructive phytopathogenic microorganisms. Therefore, it is very important to develop agro-industrial and biotechnological strategies friendly with the environment, to avoid the use of chemical fertilizers that affect and alter the ecosystems ecology, composition and diversity, as well as human health. An alternative is the use of macrophytes as green manure because of the biological importance of these plants. Macrophytes are aquatic plants with floating or submerged growth and respond to a wide variety of environmental conditions. This review summarizes the information obtained, by scientific sources about the factors affecting the distribution of macrophytes and brief description of their general aspects, the biotechnological applications of macrophytes (applications as organic biofertilizers or green manure), and a case study about macrophytes as phytoremediators. The information here described will be useful for to design strategies in agriculture on the use of organic fertilizers.

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Keywords Macrophytes · Green manure · Biofertilizers

5.1 Introduction

In 1980, United States Department of Agriculture (USDA) defined the organic agriculture as “A production system which avoids or largely excludes the use of synthetically compounded fertilizers, pesticides, growth regulators and livestock feed additives. To the maximum extent feasible, organic farming systems rely on crop rotations, crop residues, animal manures, legumes, green manures, off-farm organic wastes, and aspects of biologic pest control to maintain soil productivity and tilth, to supply plant nutrients and to control insects, weeds and other pests.” In the last two decades, due to social, cultural, economic, and mainly environmental factors, organic agricultural practices, and their production have increased in the world, mainly in countries in Europe, North America, and Oceania (Demiryürek et al. 2008; Gómez-Cruz et al. 2010; Azadi et al. 2011).

Based on these ideas, the production of organic biofertilizers friendly to the environment, made by composting, vermicomposting, or fermentation of agricultural plant wastes, animal excrement, and other organic wastes, has also increased considerably (Sooknah and Wilkie 2004; Mees et al. 2009; Martínez-Nieto et al. 2011; Najar and Khan 2013; Kobayashi et al. 2015; Song 2020; Yatoo et al. 2020). An important source of organic matter used for the production of biofertilizers is macrophyte or hydrophytes (aquatic plant biomass). Similarly as all living organisms, these plants have an ecological importance in freshwater ecosystems. However, many of them are considered aquatic weeds. For example, *Eichhornia crassipes*, also named water hyacinth, which due to its rapid development, rapid colonization of water bodies, and cosmopolitan distribution, is considered an aquatic pest that affects the diversity of flora and fauna in freshwater ecosystems (Lowe et al. 2000; Villamagna and Murphy 2010; Ndimele et al. 2011; Patel 2012). Interestingly, macrophytes, such as *Pistia stratiotes*, *Typha latifolia*, *Typha angustifolia*, *E. crassipes*, etc., also have been described as important phytoremediators, for their capacity to remove heavy metals dragged into water bodies and stored in aquatic sediments (Carrión et al. 2012; Patel 2012; Kouki et al. 2016; Song 2020; Wong-Arguelles et al. 2020). The principal advantage to use macrophytes as a raw material for the production of biofertilizers and as phytoremediators is that they do not compete for the use of land with plants of agricultural importance and do not require fertilization (Moeller et al. 2018). Moreover, its use in the processes described contributes to its control, helping to conserve the diversity of flora and fauna in lakes, lagoons, wetlands, rivers, etc.

Considering population growth and the recent increase in the production and consumption of organic agricultural products, as well as the recent advances in the use and applications of macrophytes. In this work, we will focus on discussing general aspects of macrophytes, their use as phytoremediators, and as aquatic plant biomass for the production of green manure used directly in the organic agriculture and horticulture, or in soil amendments.

5.2 Macrophytes: Brief Description of Their General Aspects

Macrophytes also called hydrophytes are very important photosynthetic organisms of the freshwater ecosystems, due to constitute the base of trophic chains for detritivorous, herbivorous, invertebrates, fish, birds, even microbes as fungi, and bacteria that inhabit these aquatic ecosystems. Interestingly, macrophytes can function as "holobionts," because they provide a niche or habitat for several organisms, e.g., zooplankton, periphyton, bacteria, fungi, amphibians, invertebrates, reptiles, fish, and waterfowl (Peters and Lodge 2009). Although, they are defined as aquatic vascular plants with specialized cells "tracheids" that transport water and nutrients and have true roots (Bowden et al. 2017), macrophytes also includes several macroalgae, mosses, ferns, and liverworts (Chambers et al. 2008; Rejmánková 2011). These organisms grow best in water with low current velocities, high light availability, and in mixture of sand and muck. They can be established by seeds, although the propagules from neighboring macrophytes, involving vegetative and clonal reproduction, are their main mechanism for dispersion and growth (Wetzel 2001). Although they are the dominant element of most freshwater wetlands, and it has been described their influence the structure, composition, and function of these aquatic ecosystems because they compete successfully for nutrients, light, space, etc. (Suren and Riis 2010; Vieira et al. 2012); there are several environmental factors that affect or influence their distribution (Table 5.1).

Table 5.1 Factors affecting the distribution of macrophytes

Factors	Example
Abiotic	Light
	Water temperature
	Water quality
	Changes and nutrient enrichment
	Sediment composition
	Hydrological variations (fluctuations in water levels)
	Exposure to wind and waves
Biotic	Competitive interactions among plants
	Herbivory by invertebrates and vertebrates
	Phytopathogens
Others	Latitudinal and altitudinal pattern
	Kind of soil
	Land cover and land use

Information described by Dar et al. (2014)

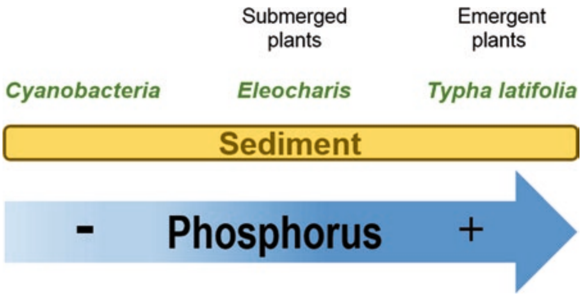

Fig. 5.1 Scheme exemplifying the distribution of macrophytes according to the concentration of phosphorus. (Image modified from Rejmánková 2011)

Although macrophytes can be classified as floating on, submerged, or growing up through the water surface; traditionally, they are classified in four categories: (*i*) emergent plants, plants erect and standing above the water surface and produce aerial reproductive organs; (*ii*) floating-leaved plants, submerged plants that produce floating leaves with floating or aerial reproductive organs; (*iii*) submerged plants, submerged plants with floating, aerial, or submerged reproductive organs; and (*iv*) free-floating plants, plants not attached to the substrate with floating or aerial reproductive organs (Bowden et al. 2017). Also, it has been described that nutrient enrichment, for example, phosphorus concentration, influences the composition and distribution of macrophytes in their ecosystems (Fig. 5.1) (Rejmánková 2011).

On the other hand, some macrophytes are considered a pest or weed, because due to its wide distribution, rapid growth, and colonization of wetlands, rivers, lakes, lagoons, reservoirs, waterfalls, etc. This can affect native flora and fauna, the structures used to irrigate crops, the hydroelectric plants, limit boat traffic and fishing in several countries, e.g., Bolivia, Brazil, Ecuador, Mexico, United States, Australia, parts of Europe, etc. (Instituto Mexicano de Tecnología del Agua 1997; Caffrey et al. 2006; Villamagna and Murphy 2010). Among the most invasive aquatic plants, we can mention *Echinochloa polystachya*, *E. azurea*, *Pistia stratiotes*, *Salvinia* sp., *Hydrocotyle* spp., *Limnocharis flava*, *Lemna* spp., *Potamogeton pectinatus*, *Hydrilla verticillata*, and *Eichhornia crassipes* (Lowe et al. 2000). The latter commonly named "water hyacinth," a native plant from South America and the most studied macrophyte due to its severe invasive effects on aquatic ecosystems and its cosmopolitan distribution (Lowe et al. 2000; Villamagna and Murphy 2010; Ndimele et al. 2011).

5.3 Biotechnological Applications of Macrophytes

In several countries, different strategies have been employed to remove several thousand tons of aquatic weeds from water bodies every year, for example, mechanical control, habitat manipulation, and biological and chemical methods (Caffrey and Monahan 2006; Najar and Khan 2013). Knowing that macrophytes are a rich source of biomass (aquatic plant biomass), recently it has been described their use in biotechnological agro-industrial processes, for example, sewage purification, electricity generation, bioethanol and biogas production, feed for livestock, human food and medicines production, building materials, organic contaminants removal, heavy metal remediation, and organic fertilizer (Engelhardt and Ritchie 2001; Egertson et al. 2004; Bornette and Puijalon 2011; Patel 2012). In this paper we will focus on discussing about their use as phytoremediators and in the organic biofertilizers production or soil amendments.

5.3.1 Macrophytes as Organic Biofertilizers or Green Manure

At present, the use of chemical fertilizers in agriculture has been degrading soil fertility, making it acidic and affected for plant cultivation (Rashid et al. 2016). The intensive use of these fertilizers has caused damages to the environment and consequently to health, for example, soil erosion, salinization, desertification, water and soil pollution, pesticide poisoning, falling of the water table, and depletion of biodiversity (Ramya et al. 2015). This has motivated the search for new alternatives that not only help to combat the problem of pollution but also allow enriching the soil with macro- and micronutrients to increase crop production (Aldás-Jarrín et al. 2016). Currently, organic agriculture has emerged with high priority from the point of view of growth, health, development of sustainability, and concern about environmental pollution (Mishra et al. 2013). One of the alternatives is organic fertilizers or biofertilizers, which are ecological and profitable and improve soil quality without degrading the ecosystem (Baweja et al. 2019). The use of macrophyte-based fertilizers represents an innovative solution that addresses the challenges of sustainable agriculture to ensure optimal nutrient absorption and crop yield. According to the Food and Agriculture Organization of the United Nations (FAO), the term macrophyte includes floating, submerged, emerging aquatic plants, and filamentous algae (Hasan and Chakrabarti 2009).

Taking into account the advantages that macrophytes offer: (*i*) they do not require fertilization, and (*ii*) they do not need land for growth, therefore not compete with plants of agricultural importance (Moeller et al. 2018); in recent years the use of macrophytes has been tested for the production of fertilizers or materials useful in plant nutrition or soil fertilization. For example, vermicomposting of freshwater weeds an ecobiotechnological process that converts the aquatic plants biomass into compounds that can be applied to the horticulture and agriculture (Sinha 2009; Najar and Khan 2013). Moreover, the composting, an aerobic biological process for degradation and transformation of freshwater weeds, by microbial communities, into organic material, phosphorus, nitrogen, and other elements used for plant nutrition (Amir et al. 2008).

Macrophytes comprise different aquatic plants, and therefore the biomass production and constituents vary among them, even it has even been described that the percentage of constituents varies during the different development stages of plants,

Table 5.2 Percentage of constituents in macrophytes

Macrophytes	Celulose	Hemicellulose	Lignin	Crude protein	Ash	References
Eichhornia crassipes	25	35	10		20	Bhattacharya and Kumar (2010)
	21.1	25.9	12	12.4	16.3	Mishima et al. (2006)
	18.2	48.7	3.5	13.3		Nigam (2002)
	17.8	43.4	7.8	11.9	20.2	Patel et al. (1993)
	21.5	33.9	6.01	14.7	11.1	Wolverton and McDonald (1981)
Hydrocotyle bowlesioides	15.7	15.1	7.25	23.4	17.4	
Lemna minor	10	21.7	2.72	37	12.5	
Pistia stratiotes	20.4	16.5	7	16.2	23.2	Mishima et al. (2006)
Potamogeton malaianus	21.2		11.6	35		Kobayashi et al. (2015)
Potamogeton perfoliatus	20		16.5	29.8		
Ceratophyllum demersum	18.5	8.2	18.6	31.5		
Potamogeton dentatus	19.5		15.5	26.6		
H. verticillata	17.8	6.6	12.9	26.1		
Egeria densa	20.2		5	29.4		
Potamogeton inbaensis	21		8.3	28		
Myriophyllum aquaticum	20		5.9	28.6		

or between its different organs, e.g., leaves, stem, root, etc. (Mishima et al. 2006). But interestingly, all macrophytes contain high percentages of cellulose, hemicellulose, lignin, and biodegradable protein (Table 5.2); main compounds used as substrates in the production of biofertilizers and biogas. One of the macrophytes most used for the biofertilizers production is *E. crassipes*; this wild aquatic plants contains approximately 90% of water and 15–20% of solid materials and produces between 11 and 55 Kg/m^2 or between 0.62 and 2.87 Kg/m^2 of wet or dry biomass, respectively (Ndimele et al. 2011). The disadvantage of the aquatic plants in the biogas and biofertilizers production is the sediment attached to the plants, and it's the high water content, which affects the fermentation process by the reduction of the active fermenter volume (Moeller et al. 2018).

Interestingly, it has been described that for better degradation of the substrates as lignin and cellulose present in macrophytes as *Typha latifolia*, *E. crassipes*, *P. stratiotes*, etc., a mixture of microorganisms including bacteria, actinomycetes, and fungi, showing a maximum amylolytic, cellulolytic, and proteolytic activities (Tiquia et al. 2002; Singh and Sharma 2003; Martínez-Nieto 2004; Martínez-Nieto et al. 2011), resulted in better decomposition of organic waste into beneficial metabolites, nutrients, and trace elements used in the plant nutrition or biogas production

(Zehnsdorf et al. 2017). For example, Moeller et al. (2018) analyzed the characteristics of 18 different aquatic macrophytes used as substrate in anaerobic digestion. Interestingly, they found that most of these plants (74%) showed a carbon/nitrogen (C/N) ratio between 10 and 20. Moreover, the methane production was similar to those produced from agricultural residues such as maize and grass. Also, it has been described that water hyacinth, possibly the macrophyte with the highest biomass production (Sooknah and Wilkie 2004), produces a higher percentage of nitrogen and potassium after the composting process, compared to other substrates as animal excrement, earth, and cellulosic gut (Mees et al. 2009).

These data make evident the nutritional advantages and the ecological importance of using macrophyte plants, mainly those considered pest or weed, in the organic biofertilizers production. It not only helps the management of macrophyte plants but also the recovery of nutrients and use in agriculture and horticulture (Moeller et al. 2018).

5.3.2 Macrophyte Applications as Biofertilizers or Green Manure in Agriculture and Horticulture

According to the Food and Agriculture Organization of the United Nations (FAO), the term macrophyte includes floating, submerged, emerging aquatic plants, and filamentous algae (Hasan and Chakrabarti 2009). In the case of floating, submerged, and emerging aquatic plants, there are few scientific reports that mention the use of these macrophytes as organic fertilizers or biofertilizers, although it is important to mention that the published reports demonstrate the effectiveness of using these macrophytes as organic fertilizers because they accumulate high concentrations of nutrients present in the water.

On the other hand, for macroalgae there is a wide variety of scientific articles that demonstrate its effectiveness in its use as biofertilizers. This is because algae have been used since ancient times directly or as a compost to improve crop productivity. Currently, seaweed liquid fertilizer is used as a nutrient-rich organic fertilizer that promotes faster seed generation, increases crop yields, and stimulates the pathogen resistance of many crops. Therefore, liquid fertilizers based on algae extracts are successfully used as fertilizers in horticulture and agriculture (Nabti et al. 2017).

A literature search was performed to analyze studies carried about macrophyte used as biofertilizers or green manure and their applications. The information was searched by consulting the following electronic sources: ScienceDirect, Scopus, Web of Science, SpringerLink, SciELO, PubMed, and Google Scholar. Scientific reports were searched from the databases using the following keywords: macrophyte, biofertilizers, and green manure. The publications considered in this review dated from 1997 to 2019. The information is summarized in Table 5.3.

Table 5.3 Macrophytes used as biofertilizers or green manure

No.	Species name	Plant component	Association	Application	References
1	*Azolla filiculoides* Lam.	Whole	*Anabaena azollae* Strass	*Zea mays* L. cultivation	Wagner (1997), Aldás-Jarrín et al. (2016)
2	*Salvinia molesta*	Whole	*Eisenia fetida*	*Abelmoschus esculentus*, *Cucumis sativus*, *Vigna radiata*	Hussain et al. (2018)
3	*Elodea nuttallii*	Whole	–	Soil nutrition with phosphorus	Stabenau et al. (2018)
4	*Phragmites australis*	Whole	–	Soil amendment and nutrition	Mamolos et al. (2011)
5	*Monochoria vaginalis*	Whole	–	General biofertilizer	Prasad et al. (2016)
6	*Utricularia inflexa* Forsk.	Whole	*Cyanophyta anabaena*	Rice cultivation	Wagner et al. (1986)
7	*Eichhornia crassipes*	Whole	*Tithonia diversifolia*	Soil nutrition and *Pleurotus geesteranus* cultivation	Chukwuka and Omotayo (2008)
8	*Stoechospermum marginatum* brown macroalgae	Liquid extracts	–	*Solanum melongena* cultivation	Ramya et al. (2015)
9	*Sargassum* spp. brown macroalgae	Whole	–	Growing of potatoes, onions, garlic, sweet peppers, and other vegetable	Titlyanova et al. (2012)
10	*Ulva* sp. green macroalgae	Whole	–	Cotton cultivation	Karyotis et al. (2006)
11	*Ascophyllum nodosum* brown macroalgae	Liquid extracts	–	Rice and *Lactuca sativa* cultivation	Silva et al. (2019)
12	*Sargassum muticum* brown macroalgae	Liquid extracts	–	Rice and *Lactuca sativa* cultivation	Silva et al. (2019)
13	*Sargassum wightii* brown macroalgae	Liquid extract	–	*Triticum aestivum* var. Pusa Gold	Kumar and Sahoo (2011)
14	*Sargassum johnstonii* brown macroalgae	Extract	–	*Lycopersicon esculentum* cultivation	Kumari et al. (2011)
15	*Laurencia pinnatifida* red macroalgae	Liquid extract	–	Seed germination and growth of *Vigna mungo*	Jebasingh et al. (2015)
16	*Caulerpa scalpelliformis* green macroalgae	Liquid extract	–	Seed germination and growth of *Vigna mungo*	Jebasingh et al. (2015)
17	*Fucus vesiculosus* brown macroalgae	Liquid extract	–	*Solanum lycopersicum* cultivation	Henrique et al. (2016)

(continued)

Table 5.3 (continued)

No.	Species name	Plant component	Association	Application	References
18	*Saccorhiza polyschides* brown macroalgae	Liquid extract	–	*Solanum lycopersicum* cultivation	Henrique et al. (2016)
19	*Sargassum vulgare* brown macroalgae	Liquid extract	–	Application in *Triticum aestivum*	Mohy El-Din (2015)
20	*Laminaria digitata* brown macroalgae	Liquid extract	–	Carbohydrates (improve aeration and soil structure, especially in clay soils and have good moisture retention properties)	Chatterjee et al. (2017)
21	*Saccharina latissima* brown macroalgae	Liquid extract	–		
22	*Ecklonia máxima* brown macroalgae	Liquid extract	–	Used as source of naturally occurring plant growth regulators. Enhance plant growth, freezing, drought, and salt tolerance	
23	*Phymatolithon calcareum* red macroalgae	Liquid extract	–	Soil nutrition with trace elements	Chatterjee et al. (2017)
24	*Lithothamnion corallioides* Red macroalgae	Liquid extract	–	Soil nutrition with trace elements	Chatterjee et al. (2017)
25	*Palisada perforata* brown macroalgae	Cut fragments	–	Application in *Pisum sativum* L.	Duarte et al. (2018)
26	*Gracilaria caudata* brown macroalgae	Cut fragments	–	Application in *Pisum sativum* L.	Duarte et al. (2018)
27	*Ulva fasciata* green algae	Liquid extract	–	Application in *Pisum sativum* L.	Duarte et al. (2018)
28	*Ulva lactuca* green algae	Liquid extract	–	Application in *Pisum sativum* L.	Duarte et al. (2018)

5.3.3 Macrophytes as Phytoremediators

Typha latifolia (Espadaña) is a macrophyte of the family Typhaceae order of the Typhales and subclass Commelinidae. This plant species is considered a cosmopolitan, perennial, rhizomatous plant, 1–4.5 m tall, cylindrical stems, basal, linear leaves, more than 15 mm wide (Ye et al. 2002). Generally, it is capable of producing 2.9 kg/m2 of biomass as it has high growth and reproduction rates. *T. latifolia* is mainly found in flooded soils and on coastal lagoons or natural wetlands (Fig. 5.2). Due to its high biomass production and rapid growth, this plant species is used in the processes of phytoremediation (Ye et al. 2002). For example, one study showed that the plants of T. latifolia removed effectively Cd and Pb from solutions and was able to accumulate these metals in the roots and, to a lesser extent, in the leaves

Fig. 5.2 *Typha latifolia* developed in the natural wetland Ciénega de Tamasopo, San Luis Potosí, México

(Alonso-Castro et al., 2009). Similarly, it was reported that as the concentration of metal increased in the solution, the greater the internalization in *T. latifolia* plants.

Recent studies conducted by our research group showed that adult *T. latifolia* plants have the ability to remove and accumulate 37 mg of Pb/kg body weight, 110 mg Cr/kg, 1651 mg Mn/kg, and 669 mg Fe/kg, when evaluated in situ conditions. In addition, greenhouse-based *T. latifolia* plants had the ability to remove 0.35, 0.81, and 1.24 ppm of Cd when exposed to 0.85, 1.73, and 2.55 ppm of the metal, respectively, and the rowing efficiency increased to 55% by increasing by increasing by increasing concentration of Cd. However, these studies found that by increasing the concentration and time of exposure to metals, the phytotoxic effects on plants increased. In natural conditions, plants have developed various mechanisms to reduce toxicity from exposure to heavy metals. Some, for example, establish symbiotic interactions with microorganisms present in the rhizosphere, including plant-growth-promoting bacteria (BPCV) (Zhuang et al. 2007). These bacteria take their nutrients mainly from root exudates and develop some activities such as solubilization of phosphate, production of siderophores, acetic indole acid (AIA), 1-aminocyclopropane-1-carboxylate (ACC) deaminase that promote plant growth, and survival to the stressful conditions to which they are subjected (Sarabia Meléndez et al. 2011).

5.3.4 Conclusion and Perspectives

Macrophytes are an alternative to be used as green manure or biofertilizers because they accumulate many nutrients from sediments, grow abundantly and are easy to harvest. Special attention should be paid on native macrophytes to propose strategies for agriculture and thus reduce the use of chemical fertilization. In addition, complementary studies should include the evaluation of the macrophytes phytoremediators of elements such as iron, zinc, or manganese to give them alternating use as green manure.

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Chapter 6
Role of Microorganisms as Biofertilizers

Zeenat Mushtaq, Shahla Faizan, and Alisha Hussain

Abstract Biofertilizers are the living microbes that inhabit the root zone or the interior plant parts. These microbes promote growth, productivity, and physiological properties of plant either directly or indirectly and hence, are also said as plant growth-promoting rhizobacteria. Biofertilizers increase the growth as well as development of plant by amassing the accessibility of mineral nutrients, biological nitrogen fixation, solubilizing phosphorus, and production of growth hormones. Moreover, these microbes and their by-products are eco-friendly organic agro-input that increased the sustainability as well as soil health and thus are considered as the best alternative to synthetic fertilizers. They are effective in very less quantity, have faster breakdown process, and are less likely to make resistance by the pathogens and other kinds of pests. The use of biofertilizers in agrarian practices overcomes the use of chemical fertilizers, which have harmful impacts on all kinds of living beings and depreciate soil health.

Keywords Biofertilizers · Mineral nutrients · Rhizosphere · Pathogens

6.1 Introduction

Presently agricultural practices totally depend on chemical fertilizers, pesticides, herbicides, weedicides, etc. that equally cause destructive influence on the nutritive value of agricultural crops, plantation products, and potency of soil as well as of human beings. They are responsible for food contamination, pathogen, or disease resistance and promote the accumulation of toxic compounds in soil. Large numbers of chemical fertilizers have carcinogenic effects, while some contains acid radicals that increased the acidity of soil, thus adversely affecting the soil, plant, and human health. The amassed consciousness of health challenges due to the intake of pitiable quality crops has led to a journey for new and better tools of improving equally the amount and superiority of agricultural products without threatening human health. A reliable substitute to synthetic fertilizers which are the utmost

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threat to the environment and deteriorate the soil fertility and its health are PGPRs which are the microbial inoculants and can be used as biofertilizers, bio-pesticides, bio-herbicides, and biocontrol agents. These microbes are innocuous and effective in less quantity, have more targeted activity and faster breakdown process, and induce the protection mechanism to plants (Alori et al. 2017a; Babalola 2010).

Biofertilizers are the viable microbes colonizing the rhizosphere or interior plant parts. These microbes are not actually the source of nutrients however, support the plants in accessing the essential nutrients present in the root area of plant. Biofertilizers provide the potential to meet our agricultural requirements, enhance the sustainability, and improve the health of soil. They are active strains of bacterial microorganisms or combination of algae, fungi, and some other microbes, involved directly or indirectly in various actions such as increasing fertility of soil by fixing atmospheric nitrogen, mineralization of elements, and movement of nutrients like phosphorous, sulfur, potassium, zinc, and iron from soil to plant and production of growth hormones, thus improving the crop productivity in an eco-friendly manner. Biofertilizers produce siderophore, protects plants from bio-surfactants and cell wall degrading enzymes etc. (Saraf et al. 2014). Furthermore, biofertilizers are able to proliferate and also have fast decomposition process (Table 6.1).

Biofertilizers can be supplied to fields via roots, seeds, or directly to the soil where they proliferate and translocate the inert nutrients and have proven harmless and valuable technique of enhancing yield (Vejan et al. 2016). Soil microbes used as biofertilizers include free-living nitrogen-fixing bacteria like *Azotobacter*, *Beijerinckia*, *Clostridium*, *Nostoc*, *Klebsiella*, and *Anabaena*; symbiotic bacteria such as *Rhizobia*, *Frankia*, and *Azospirillum*; phosphorus-solubilizing biofertilizers, viz., *Bacillus megaterium* var. *phosphaticum*, *Bacillus subtilis*, *Bacillus circulans*, *and Pseudomonas striata*; and fungi like *Penicillium* sp. and *Aspergillus awamori*, *Glomus species*, *Rhizoctonia*, *Pezizella*, etc. Biofertilizers are cheap and renewable sources of plant nutrients.

6.2 Mode of Action of Microorganisms as Biofertilizers

Interaction of beneficial microbes and plant is an auspicious resolution to increase crop production in place of synthetic fertilizers and has been confirmed as an innocuous method of increasing crop yields (Vejan et al. 2016). Presently different groups of beneficial microbes belonging to bacteria, algae, fungi, and protozoans have been identified and are used as biofertilizers in order to improve the production and sustainability of agriculture (Vessey 2003; Smith and David 2008). Beneficial microbes enhance the growth of plants either directly or indirectly as shown in Fig. 6.1 (Glick 1995).

Table 6.1 List of some plant growth-promoting bacteria as biofertilizers and their function

S. No	PGPR	Function of PGPR	Plant	References
1.	*Azotobacter chroococcum*	Production of ammonia and IAA, HCN synthesis, P solubilization	*Curcuma longa*	Kumar et al. (2014a, b)
2.	*Pseudomonas stutzeri*	N_2 fixation, siderophore production, and IAA	*Capparis spinosa*	El-Sayed et al. (2014)
3.	*Bacillus subtilis*	P solubilization, GA production	Alder	Gutierrez-Manero et al. (2001)
4.	*Azospirillum lipoferum*	N_2 fixation, IAA production	*Haplopappus* sp.	Navarro-Noyaa et al. (2012)
5.	*Rhizobium* sp.	N_2 fixation, cytokinin production	*Oryza sativa*, *Mimosa pudica*	Sev et al. (2016), Sabat et al. 2014
6.	*Enterobacter asburiae*	HCN, ammonia production, P solubilization	Maize	Sandhya et al. (2017)
7.	*Azospirillum strains* (WBPS1 and Z2-7)	Controls rice blast	*Oryza sativa*	Naureen et al. (2009)
8.	*Pseudomonas polymyxa*	Controls fungal disease	Sesame	Ryu et al. (2006)
9.	*Streptomyces marcescens*	Control of blue mold disease	*Tobacco*	Zhang et al. (2002)
10.	*Aeromonas veronii*	Production of IAA	*Oryza sativa*	Mehnaz et al. (2001)
11.	*Bradyrhizobium* sp.	Production of IAA	*Raphanus sativus*	Antoun et al. (1998)
12.	*Pseudomonas fluorescens*	Production of cytokinin	*Soybean, rape*	Garcia de Salamone et al. (2001)
13.	*Variovorax paradoxus*	Production of ACC, ABA, and ethylene	*Pisum sativum*	Belimov et al. (2015)
14.	*Herbaspirillum*	Ethylene and ABA production	*Maize*	Cura et al. (2017)
15.	*Glomus intraradices*	Improvement of relative water content	*Maize*	Naghashzadeh (2014)
17.	*Glomus mosseae*	Enhance seed germination and leghemoglobin content	*Vigna mungo*	Bharti and Kumar (2016)
18.	*Pseudomonas jessenii* M15	Bioremediation of Cu	*Brassica juncea*	Ma et al. (2009)
19.	*Azotobacter chroococcum*	Bioremediation of Pb and Zn	*Brassica juncea*	Wu et al. (2006)
20.	*Brevibacillus* B-1	Bioremediation of Zn	*Trifolium repens*	Vivas et al. (2006)

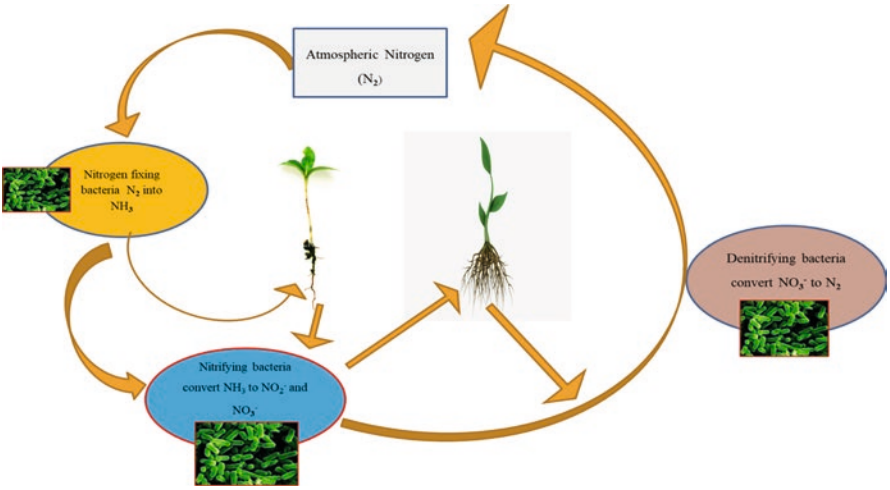

Fig. 6.1 Symbiotic nitrogen fixation by biofertilizers

6.2.1 *Direct Methods*

Direct methods include nitrogen fixation; phosphorus, potassium, and zinc solubilization; siderophore production; production of phytohormones; and enzyme and vitamin production. These actions incite morpho- and physiological changes in plants, thus promoting plant growth.

6.2.1.1 Biological Nitrogen Fixation

Microorganisms play a crucial part in elevation of plant growth by increasing the uptake of minerals from the rhizosphere to other plant parts. Among several other nutrients, nitrogen is an essential nutrient necessary for growth and plant productivity. It is a key component of amino acids, nucleotides, and mineral nutrients. However, it is a main limiting nutrient for growth of plant as being mostly available in an inaccessible form (N_2), which both flora and fauna cannot use which ultimately creates nitrogen deficiency (Pujic and Normand 2009). However, some microorganisms have the capability of fixing inaccessible form of nitrogen into accessible form and ultimately overcome its deficiency. These microbes are called biological nitrogen-fixing bacteria, and the process is called biological nitrogen fixation (BNF) (Fig. 6.2). N_2-fixing microbes fix about 180×10^6 metric tons per year of atmospheric nitrogen by utilizing energy in the form of ATP and convert it into nitrite, nitrate, and ammonia, which plants can easily consume. SNF is a mutualistic relation among plants as well as microorganisms and contributes the maximum part of fixed nitrogen, while the rest portion of the above given estimate is fixed by free-living microbes (Graham 1988). Few examples of SNF bacteria are *Rhizobium*, *Bradyrhizobium*, *Frankia*, and *Azospirillum*, which fix atmospheric nitrogen through symbiotic association with legumes.

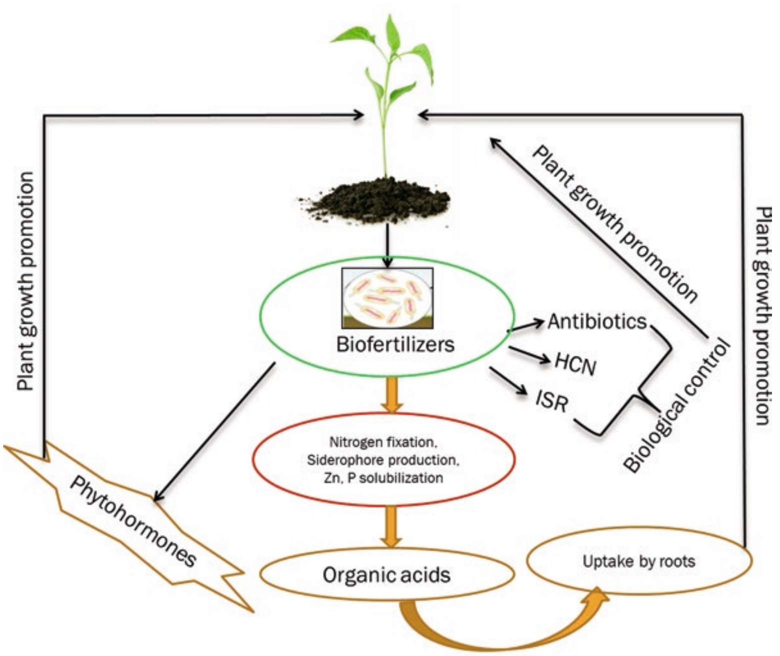

Fig. 6.2 Plant growth promotion and biocontrol properties of biofertilizers

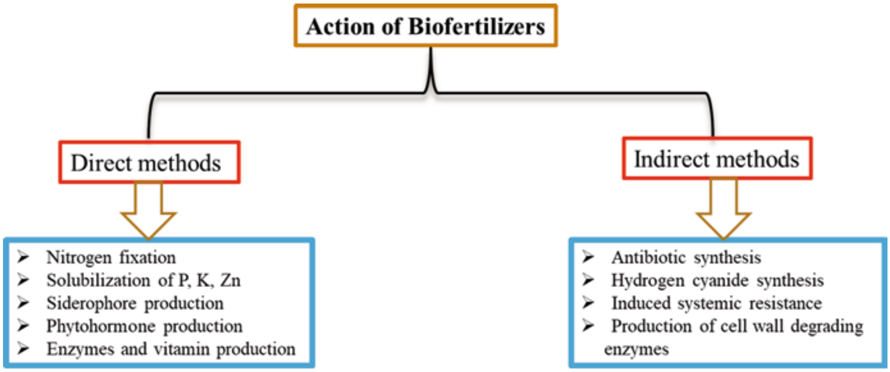

Fig. 6.3 Mechanism of action of biofertilizers in agricultural crop

Rhizobium is a symbiotic nitrogen-fixing bacterium that fixes the N_2 in association with legumes within special structures called nodules present in a root system. These bacteria supply the nitrogen to the plant and increase their photosynthetic activity. *Rhizobium* is a host-specific microorganism and fixes nitrogen only on a specific host. It is a natural system of providing nitrogen to plants as well as to soil, hence a critical component of many terrestrial and aquatic ecosystems across the planet (Fig. 6.3).

Acetobacter is another example of nitrogen-fixing bacteria. It is an endosymbiotic bacterium and fixes nitrogen in underground and in upper parts of plants. It mostly inhabits in sugar beet, millet sugarcane, and coffee. *Acetobacter* fixes the nitrogen under aerobic conditions and has been believed to be capable of producing around half of its nitrogen in a usable form**.**

Azotobacter is an aerobic gram-negative bacterium that fixes nitrogen in free-living conditions. These microbes proliferate in the rhizosphere area of plant and converts atmospheric nitrogen into ammonia. *Azotobacter* also produces phytohormones, such as IAA, GA, cytokinins, etc., and vitamins, thus benefiting plant in several ways (DeLuca et al. 1996). Besides its role as biofertilizer, it has been used as additives in foods such as ice cream, puddings, and in the manufacturing of alginic acid which is applied in medicine as antacid (Schlegel et al. 1993; Ahmad and Ahmad 2007).

6.2.1.2 Phosphate, Potassium, and Zinc Solubilization

Phosphorous is an important and vital element for growth and development of plant (Azziz et al. 2012). It is a second macroelement which is usually restraining the growth of land plants. It plays a dynamic part in plant metabolism, synthesis of proteins, and photosynthetic process. In earth phosphorous exists in organic or inorganic forms (rock phosphate, mineral salts, and calcium phosphate) which plants cannot utilize. However, plants are able to absorb phosphorous in monobasic (HPO_4^-) and dibasic (H_2PO4^-) soluble forms (Glass 1989). About 98% of Indian soil contains insufficient phosphorous and could not support plant growth and development. Phosphorus deficiency leads to poor root development, restricted growth, and low seed and yield production of plants. So, to overcome its deficiency in soil, synthetic P fertilizers are supplied to fields for better crop production. However, little portion of supplied synthetic P fertilizers are used by the plants, while the remaining portion gets converted into insoluble form. In this aspect, microorganisms present in the soil are supplied as biofertilizers which overcome the deficiency of phosphorous. These microbes convert the organic and inorganic forms of phosphorous into soluble form (Oteino et al. 2015) and are commonly called phosphate-solubilizing bacteria. Phosphate-solubilizing microbes are capable of solubilizing the phosphorous in free-living conditions in different types of soils and make it available to almost all types of crops. *Pseudomonas*, *Bacillus*, *Azotobacter*, *Agrobacterium*, *Rhizobium*, *Bradyrhizobium*, *Salmonella*, and *Thiobacillus* (Alori et al. 2017b) are few examples of phosphorus-solubilizing bacteria that have been reported to solubilize and mineralize the phosphorous in the soil (Liu et al. 2012). These microbes produce the mineral dissolving compounds, viz., protons, organic acids, carbon dioxide, hydroxyl ions, nitric acid, sulfuric acid, and chelating substances (Zhu et al. 2011). Besides bacteria, fungi also play an important part in solubilization of phosphorous and translocate it to the host plant. Arbuscular mycorrhizal fungi and ectomycorrhizal fungi are the widespread fungi that showed symbiotic relation with several land plants. They increase the uptake and solubilization of phosphorous and other elements to their host plant (Mallik 2000). Thus the application of potassium-solubilizing bacteria and fungi to the agricultural fields as biofertilizers reduces the consumption of chemical fertilizers and enhances sustainable crop productivity (Wei et al. 2017; Bakhshandeh et al. 2017).

Potassium is the third major nutrient element of plants. It plays an important role in the synthesis of protein, photosynthesis, as well as in enzyme activation. The deficiency of potassium is a chief limitation in agricultural crops as it also exists in insoluble form that living organisms cannot utilize (Meena et al. 2017). PGPRs are the best alternative for keeping the quantity of potassium in soil sufficient for plant growth and productivity. Soil microbes such as *Bacillus edaphicus*, *Ferrooxidans species*, *Burkholderia species*, *Acidithiobacillus species*, *Bacillus mucilaginosus*, and *Pseudomonas species* have been testified to secrete potassium in usable state (Liu et al. 2012).

For better plant growth and development, several other micronutrients are also required. Among them, zinc is also an important one for optimal growth. It plays a significant role in the synthesis of biomolecules such as carbohydrates, nucleotides, and phytohormones like auxin and chlorophyll. Zinc also provides the resistance of plants against high temperature (Singh et al. 2005). Plants face the deficiency of Zn due to its low solubility, pH conditions, magnesium to calcium ratio, etc. (Wissuwa et al. 2006). Large numbers of microbes were observed that are alternatives for zinc supplementation and potent to solubilize the insoluble form of zinc to usable form, thus making it available to plants (Barbagelata and Mallarino 2013). Microbes that have been reported to perform solubilization of zinc include *Pseudomonas species*, *Burkholderia cenocepacia*, *Bacillus thuringiensis*, *Gluconacetobacter diazotrophicu*s, etc. (Saravanan et al. 2007; Pawar et al. 2015; Abaid-Ullah et al. 2015)

6.2.1.3 Production of Siderophore

A plant needs iron for proper growth and development. Iron is used as a cofactor for proteins that are the important part of metabolic processes such as respiration and photosynthesis. Our earth contains an ample amount of iron, but maximum portion of that is present in ferric ionic form which living beings cannot easily assimilate (Ammari and Mengel 2006). In order to overcome this problem and make iron available to plants, microbes applied as biofertilizers develop several strategies and make iron available to plants. Among those strategies, siderophore production is one of them. Siderophores are tiny peptide molecules having side chains and functional groups. These functional groups offer ligands with high affinity to which ferric ions can bind. Large number of microbes has been reported that are involved in the production of siderophore. Thus, microorganisms are the key asset that provide plants the required quantity of iron through siderophore production and overcome the deficiency of iron through solubilization and chelation (Singh et al. 2017a, b). The prime function of these molecules is the chelation of ferric iron from diverse sources in order to make it available for plants. Depending on the chemical function and properties, siderophores are categorized into three groups, viz., catechol, hydroxycarboxylate, and hydroxamate. Boukhalfa and Crumbliss (2002) reported that *Pseudomonas* secrete different types of siderophore. A number of researchers confirmed that siderophore produced by microorganisms plays a tremendous role in the promotion of growth as well as biocontrol activity in plants (Kumar et al. 2017; Bindu and Nagendra 2016). Besides iron, siderophores are able to bind various other metals and are involved in different activities such as *Bacillus subtilis* obtained from the root zone of pepper plant that showed the biocontrol activity against *Fusarium wilt*. Similar report was also observed by Bindu and Nagendra (2016) in the rice field inoculated with *Pseudomonas aeruginosa*.

6.2.1.4 Phytohormone Production

Phytohormones or plant hormones are signal molecules made within plants in a very low quantity. Phytohormones are chemical molecules that promote growth, development, differentiation of cells, and various other functions at very low concentrations. They are directly involved in regular functioning of plants and also execute various other functions indirectly like providing defense against pathogens, abiotic stresses such as salt stress, temperature, and drought (Egamberdieva et al. 2017; Abd-Allah et al. 2018). It was observed that PGPRs in soil are able to produce various phytohormones including auxin, gibberellins, ABA, cytokinins, ethylene, brassinosteroids, strigolactones, and jasmonates. Bhardwaj et al. (2014) reported that a number of plant growth-promoting bacteria produced auxin and directly perform division, elongation, and differentiation of cells. *Pseudomonas* strains are said as strong producer of indole-3-acetic acid (IAA). PGPR producing IAA is believed to increase the root growth and length, enhance the surface area of root, and allow the plant to access more nutrients from soil. PGPRs, belonging to genera *Pseudomonas*, *Rhizobium*, *Azospirillum*, *Enterobacter cloacae*, *Bradyrhizobium japonicum*, *Bacillus cereus*, *Azotobacter*, *Burkholderia*, *and Mycobacterium* sp., have been reported to produce IAA and stimulate the plant growth.

Abscisic acid is a remarkable hormone providing defense to plants against various pathogens and controls the diseases in stress conditions (Masood et al. 2012; Nazar et al. 2014). It was observed that rice plants inoculated with *Bacillus* and *Pseudomonas* species grown in saline conditions produce maximum ABA and enhanced their growth characteristics as compared to the non-inoculated plants (Shahzad et al. 2017; Tuomi and Rosenquist 1995). Zhou et al. (2017) also proved that inoculation of *Chrysanthemum* with *Bacillus licheniformis* grown in salty soil decreased the salinity stress as well as increased the biomass and photosynthesis via mediating the level of ABA level at cellular level.

Gibberellin (GA) is an important hormone that too has been observed in some soil microbes. Gibberellins are the di-terpenoid derivatives with C20 or C19 carbon skeleton (Hedden and Thomas 2012). More than hundred structures of gibberellin have been observed till date. However, three to four strains of GAs have been observed that were produced by *Bacillus licheniformis* and *B. pumilus* (Gutierrez-Manero et al. 2001). Gibberellins induce seed germination, elongation of stem, and flowering and also increase the photosynthetic rate in plants (Khan et al. 2015). Inoculation of *A. lipoferum* to maize produces gibberellins and alleviates drought stress (Cohen et al. 2009).

Production of cytokinins in plants is involved in cell enlargement, cell division, and tissue expansion in several plants. *Paenibacillus polymyxa* and *Rhizobium leguminosarum* have been reported to produce cytokinin in wheat and soybean (Timmusk et al. 1999; Garcia de Salamone et al. 2001). Cytokinin stimulates plant cell division, controls root meristem differentiation, induce proliferation and inhibit lateral root formation and primary root elongation (Riefler et al. 2006). Few species of *Bacillus* were found to play a significant role in the signaling of cytokinin in shoot and roots of *Arabidopsis thaliana.*

Ethylene is a gaseous phytohormone that performs its activity in a very minute concentration. It is involved in the senescence as well as controls the growth of plant (Masood et al. 2012). *Penicillium cyclopium* inoculated to soil promote the production of ethylene. Inoculation of plants with 1-amino cyclopropane-1-carboxylate (ACC) deaminase plays a significant part in modulation of ethylene in plants, as ACC deaminase cleaves the ACC which is an immidiate precursor of ethylene in its biosynthetic pathway (Gamalero and Glick 2015). Ethylene is also called stress hormone as its level gets increased with the increase of stress conditions like pathogen attack, heavy metal, salinity, temperature, drought, etc. and provides defense to plant during these conditions. Moreover, excess ethylene level have negative impacts on plants, but there are few PGPRs that secrete ACC deaminase and are capable to decrease the ethylene levels by changing ACC to ammonia and α-ketobutyrate, thus maintaining the normal development of plants (Olanrewaju et al. 2017). Lim and Kim (2013) reported that inoculation of *B. licheniformis* to drought-stressed pepper plants increased the production of ACC deaminase and imparts tolerance against drought in these plants.

Brassinosteroids, strigolactones, and jasmonates are said as newly identified phytohormones. However, the role of PGPRs in the production of these molecules is yet to be discovered and needs further study. The microbes associated to produce phytohormones include the genera *Rhizobium*, *Herbaspirillum*, *Bacillus*, *Mesorhizobium*, *Pantoea*, *Arthrobacter*, *Pseudomonas*, *Bradyrhizobium*, *Rahnella*, *Enterobacter*, *Brevundimonas*, and *Burkholderia* (Montanez et al. 2012; Yadegari and Mosadeghzad 2012; Kumar et al. 2014a, b).

6.2.1.5 Production of Enzymes

Plants have numerous enzymes that regulate diverse metabolic activities. Some enzymes itself act as signal molecules such as hydrogen peroxide, regulate cell cycle and photosynthesis, and provide resistance against environmental stresses in plants. Superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), glutathione peroxidase (GPX), etc. are some enzymes present in plants. These enzymes regulate various metabolic processes as well as provide the defense against biotic and abiotic stresses in plants

(Dietz 2003; Mittler et al. 2004; Iqbal et al. 2006). An application of biofertilizer to agricultural crops enhanced the level of these enzymes and provides resistance against biotic as well as abiotic stresses up to some limit. Various microorganisms such as *Agrobacterium*, *Pseudomonas*, *Bacillus*, etc. were reported to produce proteases and lipases in plants which protect them from pathogen attack by degrading their cell wall (Ghodsalavi et al. 2013).

6.2.2 Indirect Methods

Indirect action refers to the capability of biofertilizers to diminish the harmful effects of phytopathogens on crop growth and productivity. The indirect methods include antibiotic synthesis, hydrogen cyanide synthesis, induced systemic resistance, cell wall degrading enzymes etc.

6.2.2.1 Antibiotic Synthesis

Antibiotics are low molecular weight substances generally produced as secondary metabolites by soil microbes. Antibiotic-producing microbes are distributed widely in nature and are involved in various functions. Microbes produce a number of antibiotics; out of them, very few are nontoxic and are used in medicinal purposes. The antibiotics produced by soil microbes have biocidal and biostatic effects on soilborne phytopathogens. Antibiotics produced by microbes are pathogen specific i.e. if a microbe produce an antibiotic control the growth of one pathogen in a plant but can not obstruct the growth of other pathogen present on the same plant (Olanrewaju et al. 2017). It was reported that *Penicillium*, *Streptomyces*, *and Bacillus* spp. produce numerous antibiotics such as sublancin, bacilysin, chlorotetain, iturin, subtilosin, fengycin, bacillaene, phenazine-1-carboxylic acid, zwittermicin A, cepaciamide A, karalicin, pseudomonic acid, kanosamine, rhamnolipids, cepafungins, azomycin, 2,4-diacetylphloroglucinol (DAPG), aerugine, pyrrolnitrin, oomycin A etc. These antibiotics are deleterious to metabolism of pathogens and constraining their growth (Kundan et al. 2015; Handelman and Stabb 1996). Antibiotics produced by the microorganisms damage the membranes of pathogens, for example, of *Pythium* species, and stop the formation of zoospores (de Souza et al. 2003). *Pseudomonas aeruginosa* produced phenazine which is a pyocyanin (5-N-methyl-1-hydroxyphenazine), cause the lipid damage, and also obstruct the electron transport in pathogens (Haas and Defago 2005). Various strains of *Bacillus* are used in agricultural crops as biocontrol agents and suppressed the growth of other microorganisms which are responsible for various problems such as root rot caused by *Rhizoctonia solani* and *Pythium* sp.

6.2.2.2 Hydrogen Cyanide Production

It is a secondary metabolite used as biocontrolling agent of weeds in agricultural systems as it showed significant toxicity against plant pathogens (Kundan et al. 2015). Large numbers of PGPR are able to produce HCN. It stops the energy supply of cells through the inhibition of electron transport chains and ultimately results in cell death. HCN have also antifungal activity as well as are responsible for the synthesis of some cell wall degrading enzymes. Nandi et al. (2017) proved that HCN produced by PGPR are able to inhibit the metalloenzymes and affect their toxicity. HCN produced by PGPB are used as biofertilizers and were reported to promote the plant growth and productivity (Rijavec and Lapanje 2016). *Pseudomonas fluorescens* is a hydrogen cyanide-producing microbe and a potent biocontrol agent. It's application enhanced the root and shoot length of barley and wheat as well as increased the rate of germination in *Secale cereale* (Heydari et al. 2008).

6.2.2.3 Induced Systemic Resistance

ISR is a resistance mechanism developed in plants due to the microbes. It is a signal transduction pathway stimulated by hormones such as ethylene and jasmonate and stimulates the resistance mechanism in plants against necrotrophic pathogens and behavior of insects (Verhagen et al. 2004; Pieterse et al. 2014). The importance of PGPR-mediated induced systemic resistance has been widely reported by various researchers (Pieterse et al. 2001; Siddiqui and Shaukat 2002). The mode of action of ISR does not directly assassinate the pathogen but somewhat creates the physical or chemical barrier such as chitinase, proteinases, peroxidases, etc. of the host plant. Various researchers revealed that PGPRs such as *Bacillus* and *Pseudomonas* species initiate the ISR in plants through the jasmonic acid-ethylene signaling pathway in an NRP-1-dependent manner (Van loon and Bakker 2005) and such type of pathway was reported in *Arabidopsis*.

6.2.2.4 Production of Cell Wall Degrading Enzymes

Besides above discussed functions, soil microbes are able to produce the enzymes that degrade the cell wall of another living organism. These enzymes degrade the cell wall of pathogens, change their structural integrity, and ultimately prevent their growth (Singh et al. 2017c). Several PGPRs such as *Paenibacillus*, *Streptomyces*, *Serratia marcescens*, *Pseudomonas aeruginosa*, etc. were reported by various researchers that secrete the cell wall degrading enzymes such as chitinase, β-1,3-glucanase, and chitin and degrade the N-acetylglucosamine and chitin which are constituents of fungal cell wall, thus inhibiting their pathogenic activity (Nelson and Sorenson 1999; Goswami et al. 2016).

6.3 Conclusion

Application of chemical fertilizers is effective as well as suitable for increasing crop productivity and disease control in agricultural practices, but at the same time they are potent intimidation for soil health and ecosystem. Therefore use of biofertilizers is a suitable technique for sustainable agricultural productivity. They were proven to be very effective and potent for elevating yield production, soil health, and sustainability. It releases an innovative way for industries, farmers, and researchers to use the microbial inoculants in the tolerance of biotic and abiotic stresses as they are able to manipulate plant growth hormones within plant tissues. Besides the use of beneficial microbes as biofertilizers, they are also used in disease management. Thus we can say that numerous benefits have been reached with the use of beneficial microbes in agriculture, but further opportunities need to be reconnoitered for future sustainable agricultural progresses.

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Chapter 7
Nano-agriculture: A Novel Approach in Agriculture

Mudasir Fayaz, Mir Sajad Rabani, Sajad Ahmad Wani, and Sameer Ahmad Thoker

Abstract Nanotechnology has a great potential to enhance the quality of life through its applications in various fields like agriculture and the food system. Around the world it has become the future of any nation. But we must be very careful with any new technology to be introduced regarding its possible unforeseen related risks that may come through its positive potential. However, it is also critical for the future of a nation to produce a trained future workforce in nanotechnology. In this process, to inform the public at large about its advantages is the first step; it will result in a tremendous increase in interest and new applications in all the domains will be discovered. There is great potential in nanoscience and technology in the provision of state-of-the-art solutions for various challenges faced by agriculture and society today and in the future. Climate change, urbanization, sustainable use of natural resources, and environmental issues like runoff and accumulation of pesticides and fertilizers are the hot issues for today's agriculture. Some of the potential applications of nanotechnology in the field of agriculture need many strategies for the advancement of scientific and technological knowledge currently being examined.

Keywords Agriculture · Nanoscience · Pesticides · Fertilizers · Natural resources

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7.1 Introduction

Historically, agriculture preceded the industrial revolution by around ninety centuries. However, while the seeds of research in nanotechnology started growing for industrial applications nearly half a century ago, the momentum for use of nanotechnology in agriculture came only recently with the reports published by Roco, the United States Department of Agriculture, the NanoForum, and Kuzma and VerHage, along with similar publications. These reports focused on identifying the research areas that should be funded and thus set the agenda for nanotechnology research in agricultural applications, which became the principal guiding force for many nations, especially those where agriculture is the primary occupation of the majority of the population. However, the conceptual framework, investigation pathways, and guidelines and safety protocols were left aside for scientific laboratories to innovate (Mukhopadhyay 2014).

Currently, the major challenges faced by world agriculture include changing climate, urbanization, sustainable use of natural resources, and environmental issues like runoff and accumulation of pesticides and fertilizers. These problems are further intensified by an alarming increase in food demand that will be needed to feed an estimated population of 6–9 billion by 2050. Furthermore, the world's petroleum resources are decreasing; there will be an additional demand on agricultural production as agricultural products and materials will soon be viewed as the foundation of commerce and manufacturing. At one fell swoop, there are new opportunities emerging, e.g., generation of energy and electricity from agricultural waste but pending workable economics and encouraging policy (Ditta 2012). This abovementioned scenario of a rapidly developing and complex agricultural environment and resource costs related with agricultural production. These technologies have the ability to conserve land and water by increasing yields through the application of the same or fewer inputs, ultimately conserving the environment. However, it will be very critical to support them, as these may not be commercially profitable and may also result in increase in the disparity between developing and developed countries. So their social and ethical implications should be considered. However, the need of the hour is to consider their efficiency in some fields, while these may not provide a solution to the existing problems associated with food production and its distribution around the world. Therefore, the developing countries should actively participate in research and development of these technologies while considering their ability to utilize these new technologies.

7.2 What Is Nanotechnology?

Profound interest and progress has been made since the invention of nanotechnology in 1959. The word nanotechnology was created in a lecture entitled "there's plenty of room at the bottom" given by physicist Richard Phillips Feynman at an

American Physical Society meeting at Caltech on December 29, 1959, and then its concept has been extended by Norio Taniguchi and Kim Eric Drexler, which imply a technology with tiny dimension of about 1–100 nm materials. Nano is derived from the Greek word meaning dwarf or extremely small. Today, nanobiotechnology (nanobiology) and bionanotechnology refers to the application of nanotechnology within the field of biotechnology and application of biotechnology within the field of nanotechnology, respectively. Nanotechnology has become an adjunct part of medicine, electronic, agriculture, clothing industry, and also food packaging. Therefore, the aforementioned technology is a part of progressing modern technologies that are received by mankind (Arora et al. 2008; Kumar et al. 2008). Today, nano-machines and nanomaterials are extremely in use. However, biological application of nanomaterials is in progress.

Nanotechnology, a vast field of the twenty-first century, is making a very significant impact on the world's economy, industry, and people's lives (Gruère et al. 2011; Scott and Chen 2013). It deals with the physical, chemical, and biological properties of matter considered at nanoscale (1–100 nm) and their implications for the welfare of human beings (Holdren 2011). According to the US EPA (US Environmental Protection Agency), nanomaterial is an ingredient containing particles with at least one dimension that approximately measures 1–100 nm. It has the ability to control and/or manufacture matter at this scale which results in the development of innovative and novel properties that can be utilized to address numerous technical and societal issues. Research work on nanotechnology-based delivery of agricultural chemicals has been quickly done by developing countries like China, and their field applications are expected in the next 5–10 years. However, their success depends on many factors like market demand, profit margin, environmental benefits, risk assessment, and management policies in the background of other competitive technologies. In the following section, some potential applications of nanotechnology for agriculture and food production and related issues are discussed. There are a number of applications in this field, but these are mostly at the benchtop exploration stage. However, it is very likely that in the near future, agriculture and the food sector will see large-scale applications. Some recent advances are discussed in the following section.

7.3 Overview of Nanotechnology Applications in Agriculture

Applications of nanotechnology in material science and biomass conversion technologies applied in agriculture are the basis of providing food, feed, fiber, fire, and fuels. In the future, demand for food will increase tremendously, while natural resources such as land, water, and soil fertility are limited. The cost of production inputs like chemical fertilizers and pesticides is expected to increase at an alarming rate due to limited reserves of fuel such as natural gas and petroleum. In order to overcome these constraints, precision farming is a better option to reduce production costs and to maximize output, i.e., agricultural production. Through advancement in nanotechnology, a number of state-of-the-art techniques are available for the improvement of precision farming practices that will allow precise control at nanometer scale.

7.4 Nanoscale Carriers

Nanoscale carriers can be utilized for the efficient delivery of fertilizers, pesticides, herbicides, plant growth regulators, etc. The mechanisms involved in the efficient delivery, better storage, and controlled release include encapsulation and entrapment, polymers and dendrimers, surface ionic, and weak bond attachments, among others. These mechanisms help improve stability against degradation in the environment and ultimately reduce the amount to be applied, which reduces chemical runoff and alleviates environmental problems. These carriers can be designed in such a way that they can anchor the plant roots to the surrounding soil structure and organic matter. This can only be possible through the understanding of molecular and conformational mechanisms between the delivery nanoscale structure and targeted structures and matter in soil (Johnston 2010). These advances will help in slowing the uptake of active ingredients, thereby reducing the amount of inputs to be used and also the waste produced.

7.5 Microfabricated Xylem Vessels

We are able to study the physicochemical and biological interactions between plant cell bodies and various disease-causing organisms, i.e., pathogens, through the advancement in nanofabrication and characterization tools. These tools have helped us in understanding the mechanisms involved and ultimately improved the strategies for the treatment of these diseases (Cursino et al. 2009). For example, in the past, to study xylem-inhabiting bacteria, changes in bacterial populations were monitored through destructive sampling techniques at different distances from inoculation sites, but this does not provide information about colonization, film development, and subsequent movement and recolonization at new areas because the same sample site cannot be followed temporarily. It has only been through the discovery of microfabricated xylem vessels with nano-sized features that we are able to study the above mechanisms which otherwise were not possible through traditional methods (Zaini et al. 2009).

7.6 Nanolignocellulosic Materials

Recently, nano-sized lignocellulosic materials have been obtained from crops and trees which had opened a new market for innovative and value-added nano-sized materials and products, e.g., nano-sized cellulosic crystals have been used as light-weight reinforcement in polymeric matrix (Laborie 2009; Mathew et al. 2009). These can be applied in food and other packaging, construction, and transportation vehicle body structures. Cellulosic nano-whisker production technology from wheat straw has been developed by Michigan Biotechnology Incorporate (MBI) International and is expected to make biocomposites that could substitute for fiberglass and plastics in many applications, including automotive parts. For the commercialization of this technology, North Dakota State University (NDSU) is currently engaged in a project.

7.7 Clay Nanotubes

Clay nanotubes (halloysite) have been developed as carriers of pesticides for low cost, extended release, and better contact with plants, and they will reduce the amount of pesticides by 70–80%, hence reducing the cost of pesticide and also the impact on water streams (Murphy 2008).

7.8 Photocatalysis

One of the processes using nanoparticles is photocatalysis (Blake 1999). It is a combination of two words in which “photo” means “light” and “catalysis” means “reaction caused by a catalyst.” So, it involves the reaction of catalyst (nanoparticles) with chemical compounds in the presence of light. The mechanism of this reaction is that when nanoparticles of specific compounds are subjected to UV light, the electrons in the outermost shell (valence electrons) are excited, resulting in the formation of electron-hole pairs, i.e., negative electrons and positive holes. These are excellent oxidizing agents and include metal oxides, like TiO2 (Bhatkhande et al. 2002), ZnO (Li and Haneda 2003), SnO2 (Ko et al. 2009), etc., as well as sulfides like ZnS (Feigl et al. 2010). Due to their large surface-to-volume ratio, these have very efficient rates of degradation and disinfection. As the size of particles decreases, surface atoms are increased, which results in tremendous increase in chemical reactivity and other physicochemical properties related to some specific conditions such as photocatalysis, photoluminescence, etc. So this process can be used for the decomposition of many toxic compounds such as pesticides, which take a long time to degrade under normal conditions (Malato et al. 2002), e.g., pathogens, etc.

7.9 Bioremediation of Resistant Pesticides

Nanoparticles can be used for the bioremediation of resistant or slowly degradable compounds like pesticides. These harmful compounds tend to join the positive holes, are degraded, and are converted into nontoxic compounds. Otherwise these harmful compounds enter the food chain and result in serious problems for the body. So nanoparticles can be used for environmental safety (Lhomme et al. 2008).

7.10 Disinfectants

The electron-hole pair, especially the negative electrons resulting from the excitation of nanoparticles, can also be used as a disinfectant of bacteria, as when bacteria make contact with nanoparticles, the excited electrons are injected into their bodies, which result in the bacterial removal from the object concerned, as in fruit packaging and food engineering (Melemeni et al. 2009).

7.11 Wastewater Treatment

In modern environmental science, the removal of wastewater is an emerging issue due to its effects on living organisms (Bauman et al. 2008; Mulligan et al. 2001). Many strategies have been applied for wastewater treatment, and of course the role of nanotechnology is also there. Photocatalysis can be used for purification, decontamination, and deodorization of air. It has been found that semiconductor sensitized photosynthetic and photocatalytic processes can be used for the removal of organics and destruction of cancer cells, bacteria, and viruses. Application of photocatalytic degradation has gained popularity in the area of wastewater treatment (Branton et al. 2010).

7.12 Nanobarcode Technology

In our daily life, identification tags have been applied in wholesale agriculture and livestock products. Due to their small size, nanoparticles have been applied in many fields ranging from advanced biotechnology to agricultural encoding. Nanobarcodes (>1 million) have been applied in multiplexed bioassays and general encoding because of their possibility to form a large number of combinations that make them attractive for this purpose. The UV lamp and optical microscope are used for the identification of micrometer-sized glass barcodes which are formed by doping with rare earth containing a specific type of pattern of different fluorescent materials

(Mathew et al. 2009). The particles to be utilized in nanobarcodes should be easily encodable, machine-readable, durable, submicron-sized taggant particles. For the manufacture of these nanobarcode particles, the process is semiautomated and highly scalable, involving the electroplating of inert metals (gold, silver, etc.) into templates defining particle diameter, and then the resulting striped nanorods from the templates are released. These nanobarcodes have the following applications:

7.12.1 Biological Applications of Nanobarcodes

Nanobarcodes have been used as ID tags for multiplexed analysis of gene expression and intracellular histopathology. Improvement in the plant resistance against various environmental stresses such as drought, salinity, diseases, and others has only been possible through advancement in the field of biotechnology at the nanoscale. In the near future, more effective identification and utilization of plant gene trait resources is expected to introduce rapid and cost-effective capability through advances in nanotechnology-based gene sequencing (Branton et al. 2010).

7.12.2 Nonbiological Applications of Nanobarcodes

Nanobarcodes serve as uniquely identifiable nanoscale tags and have been applied for nonbiological applications such as for authentication or tracking in agricultural food and husbandry products. This nanobarcode technology will enable us to develop new auto-ID technologies for the tagging of items previously not practical to tag with conventional barcodes.

7.13 Quantum Dots (QDs) for Staining Bacteria

Bacteria, the most primitive life forms present almost everywhere, are useful as well as harmful for life. There are numerous bacteria which are responsible for many diseases in humans like tetanus, typhoid fever, diphtheria, syphilis, cholera, foodborne illness, leprosy, and tuberculosis caused by different species. As a remedial process, we need to detect bacteria and for this, dye staining method is used. To stain bacteria, the most commonly used biolabels are organic dyes, but these are expensive and their fluorescence degrades with time. So the need of the hour is to find durable and economical alternatives. Fluorescent labeling by quantum dots (QDs) with bio-recognition molecules has been discovered through the recent developments in the field of luminescent nanocrystals. QDs are better than conventional organic fluorophores (dyes) due to their more efficient luminescence compared to the organic dyes, narrow emission spectra, excellent photostability, symmetry, and tunability according to the particle sizes and material composition. By a single excitation light source, they can be excited to all colors of the QDs due to their broad absorption spectra (Warad et al. 2004). Biolabeled *Bacillus* bacteria with nanoparticle consisting of ZnS and Mn^{2+} capped with bio-compatible "chitosan" gave an orange glow when viewed under a fluorescence microscope. For the detection of *E. coli* O157:H7, QDs were used as a fluorescence marker coupled with immune magnetic separation (Su and Li 2004). For this purpose, magnetic beads were coated with anti-*E. coli* O157 antibodies to selectively attach target bacteria and biotin-conjugated anti-*E. coli* antibodies to form sandwich immune complexes. QDs were labeled with the immune complexes via biotin-streptavidin conjugation after magnetic separation. A panel of QDs conjugated to molecules that label bacteria specifically according to strain, metabolism, surrounding conditions, or other factors would be extremely useful for a wide range of applications. One potential use is to study complex microbial populations, such as biofilms. Associations of microbes into biofilms result in properties that are very different from those of the individual cells, with resulting environmental, medical, and technological implications.

7.14 Biosensors

A variety of characteristic volatile compounds are produced by microorganisms that are useful as well as harmful to human beings, etc. Fermentation makes use of yeasts while alcohol is produced as a by-product when bacteria consume sugar. For rapid growth of a wide range of microorganisms, dairy products, bakery products, and other food products represent ideal media. The most common causal organisms of food rotting are bacteria. Foul odor is a clear indication of food rotting. The human nose can detect and distinguish a large number of odors, but sometimes it may be impractical and a further cause for poisoning. Therefore, it is more sensible to use an instrument like rapid detection biosensors for the detection of these odors.

Rapid Detection Biosensors These instruments are able to reduce the time required for lengthy microbial testing and immunoassays. Applications of these instruments include detection of contaminants in different bodies such as water supplies, raw food materials, and food products.

Enzymatic Biosensors Enzymes can act as a sensing element as these are very specific in attachment to certain biomolecules. According to Patel (2002), enzymatic biosensors on the basis of immobilization surface are classified into four groups: (i) controlled pore glass beads with optical transducer element, (ii) polyurethane foam with photothermal transducer element, (iii) ion-selective membrane with either potentiometric or amperometric transducer element, and (iv) screen-printed electrode with amperometric transducer element.

Electronic Nose (E-nose) It is a device based on the operation of the human nose and is used to identify different types of odors; it uses a pattern of response across an array of gas sensors. It can identify the odorant, estimate the concentration of the odorant, and find characteristic properties of the odor in the same way as might be perceived by the human nose. It mainly consists of gas sensors which are composed of nanoparticles, e.g., ZnO nanowires (Hossain et al. 2005; Sugunan et al. 2005). Their resistance changes with the passage of a certain gas and generates a change in electrical signal that forms the fingerprint pattern for gas detection. This pattern is used to determine the type, quality, and quantity of the odor being detected. There is also an improved surface area which helps in better absorption of the gas.

7.15 Gold Nanoparticles

Man has been fascinated by gold for a long time. It is one of the most widely studied and abundantly used nanoparticles like bulk gold. Due to several qualities, it has remained valuable both as a medium of exchange and for decorative use as jewelry throughout history. The gold nanoparticles, commercially used as rapid testing arrays for pregnancy tests and biomolecule detectors, are based on the fact that the color of these colloids depends on the particle size, shape, refractive index of the surrounding media, and separation between the nanoparticles. A quantifiable shift in the surface plasmon response (SPR) absorption peak results in a small change in any of these parameters. We can make these nanoparticles attach to specific molecules by carefully choosing the capping agent for stabilizing gold nanoparticles. These specific molecules get adsorbed on the surface of these nanoparticles and change the effective refractive index (RI) of the immediate surroundings of the nanoparticles (Nath and Chilkoti 2004). A few nanoparticles will be adsorbed if the detecting molecules (bio-macromolecules) are larger than the gold nanoparticles and result in the formation of lumps after agglomeration. Ultimately, color of gold nanoparticles is changed due to shift in SPR that results from the reduction of particle spacing.

7.16 Smart Dust

We can use the “smart dust” technology for monitoring various parameters like temperature, humidity, and perhaps insect and disease infestation to create distributed intelligence in vineyards and orchards.

7.17 ZigBee Mesh Networking Standard

ZigBee is a wireless mesh networking standard with low cost and utilizes low power. It has given the concept of “Smart Fields” and “SoilNet.” It consists of one or more sensors for environmental data (temperature, humidity, etc.), a signal conditioning block, a microprocessor/microcontroller with an external memory chip, and a radio module for wireless communication between the sensor nodes and a base station. It can be used for the identification and monitoring of pests, drought, or increased moisture levels in order to counterbalance their adverse effects on crop production (Kalra et al. 2010). Through this wireless sensor technology with nanoscale sensitivity, we can control plant viruses and level of soil nutrients, as the plant surfaces can be changed at nanoscale with specific proteins. This technology is important in realizing the vision of smart fields in particular. Wireless network sensor technology can also be used for monitoring the optimal conditions for mobile plants biotechnology.

7.18 Nanotechnologies in Animal Production and Healthcare

Livestock, poultry, and aquaculture are related with agriculture and have an important role and will continue to play an important role in human nutrition. There are a large number of constraints in animal production such as production efficiency, animal health, feed nutritional efficiency, diseases including zoonoses, product quality and value, by-products and waste, and environmental footprints. Nanotechnology can provide state-of-the-art remedies for these challenges (Kuzma 2010).

7.19 Improving Feeding Efficiency and Nutrition

The main challenge in sustainable agriculture is to minimize the inputs and to maximize the output. Feedstock is the most important input in animal production. Feeding efficiency is inversely related with demand of feed, discharges of waste, environmental burden, production cost and competing with other uses of the grains, biomass, and other feed materials. Nanotechnology has the potential to improve the profile of nutrients and their efficiency. In developing countries, animal feeds are mostly suboptimal in nutrient composition. To supplement them with nutrients is an efficient way of elevating the efficiency of protein synthesis and utilization of minor nutrients in animals. Similarly, cellulosic enzymes can help in better utilization of the energy in plant-derived materials. Moreover, micronutrients and bioactives can help improve the overall health of animals, ultimately achieving and maintaining optimal physiological state. For efficient supply of nutrients, a large number of nanoscale delivery systems like micelles, liposomes, nano-emulsions, biopolymeric nanoparticles, protein-carbohydrate nanoscale complexes, solid nano-lipid particles, dendrimers, and others have been developed. These systems not only have better adaptability against environmental stresses and processing impacts but also have high absorption and bioavailability, better solubility, and disperse ability in aqueous-based systems, i.e., food and feed, and controlled release kinetics (Chen et al. 2006). Sustainability can be achieved through the utilization of self-assembled and thermodynamically stable structures. So less energy is needed to process these structures. In addition, efficient veterinary drug delivery can be achieved through these systems which protect the drug in gastrointestinal tract and provide optimal rate and location for optimal action. These systems have helped in improved utilization efficiency of nutrients and product quality, as well as reducing the amount and financial burden of the producers, and ultimately production yield. Similarly, to food applications, it is the requirement of the system to be effective in its intended use and against adverse effects or unintended uses. There should be an accurate risk assessment of the nanoscale particles to be used in order to ensure safe and sound development and deployment in the products.

7.20 Zoonotic Diseases

Substantial losses in animal production are caused by diseases such as bovine mastitis, tuberculosis, respiratory disease complex, Johne's disease, avian influenza, and porcine reproductive and respiratory syndrome (PRRS). According to an estimate of the World Health Organization (WHO), 1/5 of animal production costs in the developed world and 1/3 in the developing world are represented by animal diseases. During the last 30 years, infectious diseases have emerged and 75% of them are zoonotic (WHO 2005), which not only cause economic loss but also serious danger to human health, e.g., variant Creutzfeldt-Jakob disease (vCJD). The zoonotic diseases include mad cow disease, avian influenza, H1N1 influenza, Ebola virus, and Nipah virus. For integrated animal disease management, detection and intervention are two important tools in order to reduce and/or to eradicate the disease. Nanotechnology has the potential to provide these strategies and has enabled revolutionary changes in this field, and new state-of-the-art strategies are expected to be developed in the near future (Emerich and Thanos 2006; Scott 2007). Numerous detection and diagnostic techniques have been offered by nanotechnology which are highly specific and sensitive; can detect multiple samples at a time; are time-saving; are robust; have onboard signal processing, communication, and automation; are convenient to use; and are economical. These help in quick, simple, and inexpensive treatment strategies that can be taken to remedy the situation. For agricultural field applications, portable and implantable devices have been developed. Drugs and vaccines developed through nanotechnology are relatively more effective and cheaper than those manufactured through previous technologies. This has enabled precise delivery and controlled release of drugs, resulting in a small footprint in animal waste and the environment, which would otherwise result in antibiotic resistance.

So it has reduced environmental concerns associated with the use of antibiotics and enabled new drug administrations that are easy, quick, nonintrusive to animals, and, most importantly, economical. Through advancement in nanotechnology, theragnostics has been developed in which both diagnostics and therapy are performed in a single step. But before deploying this innovative technology, pharmacokinetic and pharmacodynamic studies should be conducted under in vivo conditions in order to establish a relationship between dose, drug concentration at the site of action, and drug response (Morris 2009). Moreover, there should be collaboration between human and veterinary medical communities in the research and development for dealing with zoonotic diseases.

7.21 Animal Reproduction and Fertility

Animal reproduction is an important challenge for both developing and developed countries, as low fertility causes low production rate, increases in financial input, and reduced efficiency of livestock operations (Narducci 2007). To improve animal reproduction, many technologies have been developed, but microfluidic technology has ruled over the last two decades, and many nanoscale processing and monitoring technologies have been integrated, which include food and water quality, animal health, and environmental contaminations. These have enabled us to produce an automated and large number of embryos in vitro, and this has improved genetics and selection of livestock for human food and fiber production. In Brazil, fixed-time artificial insemination (FTAI) technology has been used to increase the cattle reproduction rate for many years, but its efficiency depends on the regulation of progesterone. Inefficient and irregular dispersion of hormone, disposal issues, being labor intensive, and requiring multiple animal handlings for each attempt are the drawbacks of this technology. Nanotechnology-based delivery systems help to improve bioavailability and release kinetics, reduce labor intensity, and minimize waste and discharge to the environment (Emerich and Thanos 2006; Narducci 2007). An implanted nanotechnology-enabled sensing device with wireless transmission ability is another strategy that can be used to control animal hormone level, thus providing information about the optimal available fertility period. This information is helpful for the livestock operators in decision-making for reproduction.

7.22 Animal Product Quality, Value, and Safety

Modification of animal feed improves not only the animal production but also product value and quality, which is helpful in producing animal-derived foods or products consistent with health recommendations and consumer perceptions, e.g., milk fatty acids, cis-9, trans-11 conjugated linoleic acid (CLA), and vaccenic acid (VA). These products help in the prevention of chronic human diseases such as cancer and atherogenesis (Bauman et al. 2008). Nanotechnology-based delivery of nutrients is helpful in efficiently controlling the biosynthesis and concentration of CLA and VA in the milk fat of lactating ruminants. It also helps in examining the biological benefits of functional foods with high CLA/VA contents and their relationship with human chronic diseases using biomarkers and biomarker triggered release mechanisms. Moreover, it has played an important role in economical sequencing of the mammalian genome within 24 h (Branton et al. 2010). In the next decade, if this technology is available, advances in biotechnology research and development will be substantially accelerated.

7.23 Nanotechnology and Animal Waste Management

In the animal production industry, animal waste is a serious concern, and its irresponsible discharge can only be prevented through strict environmental policies. It is also responsible for the production of unpleasant odors that adversely affect quality of air and, in turn, living conditions and the real estate value of the adjacent area. Animal waste could be used for the production of high-quality organic fertilizer when value is added and for improving environmental quality. Its bioconversion into energy and electricity can result in new revenue, renewable energy in the form of natural gas (Scott 2002). In efficient and cost-effective bioconversion, nanotechnology-based catalysts will play a critical role in electricity production and its storage which will be very helpful in the development of distributed energy supplies, especially in rural communities where infrastructure is lacking (Soghomonian and Heremans 2009). Such an approach could eliminate the need for a system of wide electricity grids, accelerate rural development, and improve productivity.

7.24 Nanotechnologies for Water Quality and Availability

Currently, provision of clean and abundant fresh water is one of the most important challenges faced by the world for human use and industrial applications such as agriculture (Vörösmarty et al. 2010). According to a survey, more than one billion people in the world are deprived of clean water, and the situation is getting worse. In the near future, it has been estimated that average water supply per person will drop by a factor of one third, which will result in the avoidable premature death of millions of people (Cross et al. 2009). A large amount of fresh water is required in agriculture, but, in turn, it contributes to groundwater pollution through the use of pesticides, fertilizers, and other agricultural chemicals. To combat this problem, novel, sustainable, and cost-effective technologies will be required for the treatment of this large amount of wastewater produced. During the treatment of wastewater, critical issues like water quality and quantity, treatment and reuse, safety due to chemical and biological hazards, monitoring, and sensors should be considered.

Research and development in nanotechnology has enabled us to find novel and economically feasible solutions for remediation and purification of this wastewater. Accessible water resources are mostly contaminated with waterborne pathogenic microorganisms like cryptosporidium, coliform bacteria, virus, etc., various salts and metals (Cu, Pb, As, etc.), runoff agricultural chemicals, tens of thousands of compounds considered as pharmaceuticals and personal care products (PPCPs), and endocrine disrupting compounds (EDCs) and radioactive contaminants, either naturally occurring or as the result of oil and gas production as well as mining activities due to natural leaching and anthropogenic activities. For improving water quality, nanotechnology has provided novel solutions which are discussed below.

7.25 Nanooligodynamic Metallic Particles

Physicochemical microbial disinfection systems like chlorine dioxide, ozone, and ultraviolet are being commonly used in developed countries, but most of the developing countries are lacking these systems due to the requirement of large infrastructure which makes them costly. The need of the hour is to search and develop alternative cost-effective technologies. Nanotechnology-based oligodynamic metallic particles have the ability to serve this function. Among these nanomaterials, silver is the most promising one as it is both bactericidal and viricidal due to the production of reactive oxygen species (ROS) that cleaves DNA and can be utilized for a wide range of applications. Other properties include low toxicity, ease of use, its charge capacity, high surface-to-volume ratios, crystallographic structure, and adaptability to various substrates (Melemeni et al. 2009).

7.26 Photocatalysis

Visible light photocatalysis of transition metal oxides, another nanoscale technological development, produces nanoparticles, nanoporous fibers, and nanoporous foams that can be used for microbial disinfection (Li et al. 2014) and for the removal of organic contaminants like PPCPs and EDCs. Moreover, tubular nanostructures, embedded into microbial cell wall, can disrupt its cell structure, resulting in the leakage of intracellular compounds and ultimately cell death.

7.27 Desalination

Due to limited resources of fresh water, it is likely that in the near future, desalination of seawater will become a major source of fresh water. Conventional desalination technologies like reverse osmosis (RO) membranes are being used, but these are costly due to the large amount of energy required. Nanotechnology has played a very important role in developing a number of low-energy alternatives, among which three are most promising: (i) protein-polymer biomimetic membranes, (ii) aligned carbon nanotube membranes, and (iii) thin film nanocomposite membranes (Hoek et al. 2014). These technologies have shown up to 1000 times better desalination efficiencies than RO, as these have high water permeability due to the presence of carbon nanotube membranes in their structure. Some of these membranes are involved in the integration of other processes like disinfection, deodorizing, defouling, and self-cleaning. Some of these technologies may be introduced in the marketplace in the near future, but scale-up fabrication, practical desalination effectiveness, and long-term stability are the most critical challenges to be considered before their successful commercialization.

7.28 Removal of Heavy Metals

Ligand-based nanocoating can be utilized for effective removal of heavy metals as these have high absorption tendency. It becomes cost-effective as it can be regenerated in situ by treatment with bifunctional self-assembling ligand of the previously used nanocoating media. Multiple layers of metal can be bonded to the same substrate using crystal clear technologies (Farmen 2009), and this technology is expected to be available in the near future. According to Diallo (2009), another strategy for the removal of heavy metals is the use of dendrimer-enhanced filtration (DEF), and it can bind cations and anions according to acidity.

7.29 Wireless Nanosensors

Crop growth and field conditions like moisture level, soil fertility, temperature, crop nutrient status, insects, plant diseases, weeds, etc. can be monitored through advancement in nanotechnology. This real-time monitoring is done by employing networks of wireless nanosensors across cultivated fields, providing essential data for agronomic intelligence processes like optimal time of planting and harvesting the crops. It is also helpful for monitoring the time and level of water, fertilizers, pesticides, herbicides, and other treatments. These processes are needed to be administered given specific plant physiology, pathology, and environmental conditions and ultimately reduce the resource inputs and maximize yield (Scott and Chen 2013). Scientists and engineers are working from dawn to dusk in developing the strategies which can increase the water use efficiency in agricultural productions, e.g., drip irrigation. This has moved precision agriculture to a much higher level of control in water usage, ultimately toward the conservation of water. More precise water delivery systems are likely to be developed in the near future. These factors critical for their development include water storage, in situ water holding capacity, water distribution near roots, water absorption efficiency of plants, encapsulated water released on demand, and interaction with field intelligence through distributed nanosensor systems (Cross et al. 2009).

7.30 Detection of Pollutants and Impurities

Sensing and detection of various contaminants in water at nanoscale under laboratory and field conditions has remained a hot issue over the last decade. In the near future, state-of-the-art nanotechnology-based techniques will help in developing many new technologies that will have better detection and sensing ability (Chen and Yada 2011).

7.31 Nanotechnology and Shelf Life of Agricultural and Food Products

Most of the agricultural commodities (fresh vegetables, fruits, meats, egg, milk and dairy products, many processed foods, nutraceuticals, and pharmaceuticals) are either perishable or semi-perishable. Research and development in nanotechnology can help to preserve the freshness, quality, and safety.

7.32 Green Nanotechnology

For sustainable development around the world, finding an inexpensive, safe, and renewable source of energy is the need of the hour. Green nanotechnology has been developed for a flexible and efficient source of energy in the form of solar cells which have long been an ambition for tropical countries. However, the use of glass photovoltaic panels is delicate and too expensive. A high priority of research in most industrialized countries has been given to the development of photovoltaic panels, energy storage, and other nanotechnology-enhanced solar thermal energy conversion systems. Economic feasibility is the critical factor for developing these photocatalysts and energy materials, and if we address this factor properly, we will be able to develop more and more “out-of-the-box” ideas. A substantial technical breakthrough has been made by Jennings and Cliffel at Vanderbilt University, who have explored the use of photosynthesis protein units derived from leafy vegetables and plants for direct conversion of solar energy to electricity, and has remained functional for about 1 year. A glass microscope slide that serves as the cell base is the most expensive component of this system. Capturing solar energy will be a great achievement that will serve humanity and is likely be persistent and intensified in the years ahead. Nanotechnology is also helpful for the conversion of biomass into fuels, chemical intermediates, specialty chemicals, and products including catalysts in order to reduce production cost while being economically feasible. These nanostructured catalysts have large surface area per unit volume and are capable of having precisely controlled composition, structure functionalization, and other important properties of catalysts.

7.33 The Role of Good Governance and Policies for Effective Nanotechnology Development

For about the last decade, nanotechnology has been actively pursued worldwide. Of course, it has made many advances in various fields, but the results are inconsistent across different scientific areas and geographic regions as these developments are at benchtop scale. Research on methodology, identification, and characterization of nanomaterials, testing priorities, and regulatory guidance on nanoparticle safety are still in their infancy; hence great efforts for their commercialization are required. In order to make advancement in the field, more research in potential risk assessment for responsible development by all the stakeholders will be required. There will also be the requirement of private-public partnership for getting substantial contributions and advancements in nanotechnology. However, engagement of the public to ensure a transparent and constructive discussion of the various issues will be mandatory. There should be fruitful discussion to establish good governance of nanotechnology-based applications in agriculture and food systems for sustainable financial investment, and these aspects include research and development, transfer models, intellectual property and efforts to understand and facilitate technology adoption, and sharing among industrialized and technologically disadvantage countries. For proper consideration of the abovementioned issues for development and innovation in nanotechnology, we need to enhance the role played by developing countries, encourage the development of innovative products addressing the current issues, and make these products safe, appropriate, easily accessible, and on a sustainable basis. For this purpose, a policy briefing published by the International Food Policy Research Institute should also be consulted. Partnerships and collaborations can play a key role in sustainable agriculture development. Nanotechnology is a multidisciplinary (engineering and the natural sciences, including such disciplines as physics, chemistry, biology, materials sciences, instrumentation, metrology, and others) approach requiring a high degree of cross sector collaboration among academic researchers, industry, and government. Progress in a number of tools for visualization, characterization, and fabrication, as well as methods for reproducing and controlling properties, scalability, and cost, will be required for the advancement of nanotechnology. Most developing countries continue to work on filling critical gaps in research infrastructure through contact and access to international research and development networks, despite the strong research capacity building efforts that have been made by these countries. So, an evaluation of possible collaboration and partnership mechanisms either between public and private or between developed and developing countries in order to meet global demands and expectations should be performed. Many developing countries, particularly Brazil, China, India, and South Africa, have already started significant investments in strategically conducting research in nanotechnology and its applications for agriculture and food systems. These investments are particularly made for research related to national interests like energy, health, water treatment, agriculture, and the environment. So in conclusion, there is a dire need for collaboration between public and private sector partnerships and between developed and developing countries.

7.34 Conclusion and Future Perspectives

Nanotechnology has great potential as it can enhance the quality of life through its applications in various fields like agriculture and the food system. Around the world it has become the future of any nation. But we must be very careful with any new technology to be introduced about its possible unforeseen related risks that may come through its positive potential. However, it is also critical for the future of a nation to produce a trained future workforce in nanotechnology. In this process, to inform the public at large about its advantages is the first step, which will result in tremendous increase in the interest and discovery of new applications in all the domains. With this idea in mind, this review has been written. The theme of the paper is based on the provision of basic knowledge about the applications of nanotechnology in agriculture and their prospects in the near future with reference to the current situation around the world. In this review, some of the potential applications of nanotechnology in agriculture for the welfare of humans and for sustainable environment, challenges, and opportunities for developing countries have been identified. Finally, for their solution, collaboration among developed and developing countries, public and private sectors, and between research institutions and international organizations has been identified and suggested. The future of nanotechnology is uncertain due to many reasons, such as negative reaction of the public toward genetically modified crops, lack of many of the requisite skills in public agricultural research organizations for this type of research, and ill-equipped and somewhat hesitant regulatory structures to deal with these new technologies. There is a dire need to tear down the sharp boundary present between the social and natural sciences, and if we succeed in discarding this boundary, we may be able to develop a more desirable and more democratic sociotechnical future.

Specifically, in agriculture, technical innovation is of importance with regard to addressing global challenges such as population growth, climate change, and the limited availability of important plant nutrients such as phosphorus and potassium.

Nanotechnology applied to agricultural production could play a fundamental role for this purpose, and research on agricultural applications is ongoing for largely a decade by now. This also touches on the issue of nanotechnology in developing countries.

7.35 Overview of Nanotechnology Research Activities in the Agricultural Sector

The application of nanomaterials in agriculture aims in particular to reduce applications of plant protection products, minimize nutrient losses in fertilization, and increase yields through optimized nutrient management.

Despite these potential advantages, the agricultural sector is still comparably marginal and has not yet made it to the market to any larger extent in comparison with other sectors of nanotechnology application.

Nanotechnology devices and tools, like nanocapsules, nanoparticles, and even viral capsids, are examples of uses for the detection and treatment of diseases, the enhancement of nutrients absorption by plants, the delivery of active ingredients to specific sites, and water treatment processes. The use of target-specific nanoparticles can reduce the damage to nontarget plant tissues and the amount of chemicals released into the environment. Nanotechnology-derived devices are also explored in the field of plant breeding and genetic transformation.

The potential of nanotechnology in agriculture is large, but a few issues are still to be addressed, such as increasing the scale of production processes and lowering costs, as well as risk assessment issues. In this respect, particularly attractive are nanoparticles derived from biopolymers such as proteins and carbohydrates with low impact on human health and the environment. For instance, the potential of starch-based nanoparticles as nontoxic and sustainable delivery systems for agrochemicals and biostimulants is being extensively investigated.

Nanomaterials and nanostructures with unique chemical, physical, and mechanical properties, e.g., electrochemically active carbon nanotubes, nanofibers, and fullerenes have been recently developed and applied for highly sensitive biochemical sensors. These nanosensors have also relevant implications for application in agriculture, in particular for soil analysis, easy biochemical sensing and control, water management and delivery, pesticide, and nutrient delivery.

In recent years, agricultural waste products have attracted attention as source of renewable raw materials to be processed in substitution of fossil resources for several different applications as well as a raw material for nanomaterial production (see, for instance, “New synthesis method for graphene using agricultural waste”). Nanocomposites based on biomaterials have beneficial properties compared to traditional micro- and macro-composite materials, and, additionally, their production is more sustainable. Many production processes are being developed nowadays to obtain useful nanocomposites from traditionally harvested materials.

7.35.1 Commercial Applications of Nanotechnology in the Agricultural Sector

From a commercial perspective, existing agrochemical companies are investigating the potential of nanotechnologies and, in particular, whether intentionally manufactured nano-sized active ingredients can give increased efficacy or greater penetration of useful components in plants. However, the nano-size so far did not demonstrate to hold key improvements in product characteristics, especially considering the interest of large-scale production and the costs involved in it.

Some specific nano-products for the agricultural sector have been put on the market by technology-oriented smaller companies, like soil-enhancer products that promote even water distribution, storage, and consequently water saving. However, the commercial market application of these products is so far only achieved at small scale, due to the high costs involved in their development. These costs are normally compensated by higher returns in the medical or pharmaceutical sectors, but so far there are no such returns in the agricultural sector. Research continues in the commercial agrochemical sector to evaluate potential future advantages.

Companies are also facing challenges derived from the definition of nanomaterials that is adopted by the EU. One crucial point related to the EU definition is the possibility that non-active substances already used for many decades in commercial products formulations will fall within the scope of the nano definition, although not intentionally developed as nanoparticles or having specific nanoscale properties. Nanoscale formulants (e.g., clay, silica, polymers, pigments, macromolecules) have been used for many decades and are also ubiquitous in many daily household products.

The concern is that the need for labeling of products that are already on the market since decades results in a scenario, in which the technology is stigmatized, preventing further and innovative applications of nanotechnology in agriculture.

7.35.2 Nanotechnology Risk Assessment and Regulation in the EU and Worldwide

Due to the variety of applications of nanotechnology, different pieces of legislation are concerned in the EU, including both horizontal legislation and product-specific legislation. The most comprehensive horizontal piece of legislation relevant to nanomaterials is the EU Regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), which addresses chemical substances, in whatever size, shape, or physical state. Substances at the nanoscale are therefore covered by REACH and its provisions apply. Some researchers, however, argue that REACH needs to be revised in three major areas (read more “Does the EU’s chemical regulation sufficiently address nanotechnology risks?).

Among product-specific legislation, some already explicitly address nanomaterials (cosmetics, food additives, provision of food information to consumers, and biocides), while others do not (toys, electrical equipment, and waste and environmental legislation). At international level, there are several activities in place on risk analysis of nanomaterials in the food and agricultural sectors, in particular by the governments of Australia/New Zealand, Canada, China, the EU, Japan, Switzerland, and the USA. Overall, definitions of nanomaterials developed in different countries result in different risk management measures. So far, apart from the EU, no country has set a regulatory framework for the mandatory labeling of nanomaterials in food, and current regulations do not cover all areas (see, for instance, “Gaps in U.S. nanotechnology regulatory oversight”).

7.35.3 Socioeconomic Issues of Agricultural Nanotechnology

The emergence of nanotechnology applications in consumer products has also raised a number of ethical and societal concerns in some countries, starting from health and environmental safety to consumer perception and intellectual property rights.

From different studies about consumer acceptance of nanotechnology products, it appears that the public opinion is generally not negative. The public seems to be unconcerned about many applications of nanotechnology with the exception of areas where societal concern already exists such as pesticides.

As for many emerging technologies, intellectual property in nanotechnology, and in particular freedom to operate, constitutes relevant issues for the development of new products. The number of patent applications in nanotechnology has increased more than tenfold during the last 20 years, demonstrating a great potential for commercial applications. Patenting on nanotechnology in general presents some important concerns (read more “Legal implications of the nanotechnology patent land rush”). Nanotechnology is pervasive in different fields of applications, and nano-based inventions could infringe existing granted patents in those fields. This risk of overlapping patents can also have consequences for the agri-food sector. Moreover, patent holders could lock up huge areas of technology. There are indeed already over 3000 patents worldwide for potential agrochemical usage of nanotechnology, but they are most likely patents with broad claims, filed with the scope of guarantee freedom to operate in the field in case of future commercial developments.

In developing countries, nanotechnologies can have important applications in several agri-food areas, such as food security, input delivery, rice production systems, agri-biotechnology, healthcare of animals, precision farming, food industry, and water use (read more “Small is beautiful? Nanotechnology solutions for development problems”). However, the main factors limiting the development of these applications are low investments in manpower training and in research infrastructure.

7.36 Applications of Nanotechnology in Agriculture

Nanotechnology applications are currently being researched, tested, and in some cases already applied across the entire spectrum of food technology, from agriculture to food processing, packaging, and food supplements. In the agricultural sector, nanotech research and development is likely to facilitate and frame the next stage of development of genetically modified crops, animal production inputs, chemical pesticides, and precision farming techniques. While nano-chemical pesticides are already in use, other applications are still in their early stages, and it may be many years before they are commercialized. These applications are largely intended to address some of the limitations and challenges facing large-scale, chemical, and capital-intensive farming systems. This includes the fine-tuning and more precise micro-management of soils; the more efficient and targeted use of inputs; new toxin formulations for pest control; new crop and animal traits; and the diversification and differentiation of farming practices and products within the context of large-scale and highly uniform systems of production.

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Chapter 8
Biofertilizers: Sustainable Approach for Growing Population Needs

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Abstract Inorganic chemical compounds which created a severe risk to human health and the atmosphere are primarily based on existing soil management policies. In agriculture it is important for their prospective function in meeting the safeguards and sustainable crops to exploit beneficial microbes as a biofertilizer. Biofertilizer contains microorganisms that promote adequate nutrient supply to host plants and ensure that their physiology is properly developed and regulated. Eco-friendly methods are used to encourage a broad variety of applications for crop growing to promote rhizobacteria (plant growth-promoting rhizobacteria, PGPR), ectomycorrhizal mushrooms, cyanobacteria, as well as many other helpful microscopic species. The chapter outlined biofertilizer translators to trigger multiple developments and defense genes for binding network of cellular tracts to produce cells and thereby enhance crop development. The vital characteristics are plant growth and productivity, nutrient profile, plant defense, and safety. It offers an economically attractive and environmentally sound route to increase the supply of nutrients. The information acquired from the literature assessed will assist us to comprehend biofertilizer's physiological foundation for viable farming in order to reduce the issues of the use of chemical fertilizers.

Keywords Biofertilizer · Crop improvement · Environmental stress · Mode of action of biofertilizers · Sustainable agriculture

8.1 Introduction

The traditional farming sector performs a key part in fulfilling an increasing human population's nutrition requirements, which also lead to an increase in reliance on chemical fertilizers and pesticides (Santos et al. 2012). Chemicals fertilizers are industrially handled, and their exploitation creates air and groundwater pollution

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through water body eutrophication by materials made of recognized quantities of nitrogen (N), phosphorus (N), and potassium (K) (Yousef and Eissa 2014). Recent attempts to guarantee sustainable conduct have been more channeled to the manufacturing of nutrient-rich, quality food. The creative approach in farm production draws increasing requirement for organic fertilizers based solely on organics (Raja 2013). In order to support new soil fertilization techniques in agriculture, organic materials are used to enhance nutrient availability and to conserve conservation of the area (Araujo et al. 2008). Organic agriculture is one of the methods which not only guarantees the safety of meat but also contributes to ground biological diversity (Megali et al. 2013). Additional advantages of biofertilization include larger life spans that have no negative effects on the environment (Sahoo et al. 2014). Organic farming depends in large part on the existing microflora of the earth, which contains various types of helpful bacterial and fungal products such as arbuscular mycorrhiza fungi (AMF). Biofertilizers maintain soil health with a variety of micro- and macronutrients, by fixating and mineralizing carbon, phosphate, and mineralizing potassium, by releasing regulated materials for plant development, by producing antibiotics, and by biodegrading the land (Sinha et al. 2014). In fact, between 60 and 90% of the complete fertilizer is wasted, and the rest of the crops account for between 10 and 40%. In this respect, the maintenance of agricultural productivity and good environment by microbial inoculants in embedded nutrient management schemes is crucially important (Adesemoye and Kloepper 2009). Co-inoculants for PGPR or PGPR + AMF can increase nutrient effectiveness (Adesemoye et al. 2009). It introduces latest field-management trends that show prospective use of biofertilizers and enhanced profiling of nutrients, crop development and efficiency, and enhanced sensitivity to economic stresses, particularly biofertilizers.

8.2 Nitrogen-Fixing Biofertilizers

A wide range of documents have been reported as normal endophyte in leguminous crops for rhizobia (*Rhizobium*, *Mesorhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Allorhizobium*, and *Sinorhizobium* species). The endophyte field is not confined to the Leguminosae category; however a huge community of endophytes from multiple nonleguminous crops has been recorded, varying from 106 to 107 neurons per gram of new material, in distinct areas of the globe (Yanni et al. 1997 and Mirza et al. 2001). Endophytes are not restricted to a particular room, but happen in the origins, stems, and branches of the conventional plant, in comparison to the legume-rhizobia interaction. Plant pads (stem, stem) are systematically sterilized to identify endophytes by using potassium hypochlorite or mercuric acid for the extraction of contaminating soil organisms. Reinhold-Hurek and Hurek (1998) looked at various soil sterilization methods and their efficiency in isolating endophytes from nonlegumes. The sample is then put on the surface-sterilized plant portion on nitrogen-free medium. Reinoculation of frozen plant seedlings (Yanni et al. 1997; Prayitno et al. 1999) confirms the capacity of the living bacteria as an endophyte. They are appointed as "real endophytes" by the detached endophyte (Reinhold-Hurek and Hurek 1998) to reinfect the parent plant and to meet Koch's premises. The postulate of Koch defines the requirements to determine whether the disease-causing officer is certain bacterium. One of them is to re-insulate the bacterium causing the disease of the patient. In the case of an endophyte-plant communication, bacteria accountable for encouraging development of a plant after inoculation should be re-isolated from affected crops. More recently, the sections of the bacterial genes produced from the original endophyte plant tissue are analyzed in immediate ways (Engelhard et al. 2000; Hurek et al. 2002). In contrast to those acquired using culturally dependent methods, Conn and Franco (2004) created a wider range of endophytes in wheat. The cultural methods offer the selective advantage of a certain bacteria group and therefore don't offer a full description of the population of endophytes. Knauth et al. (2005) developed a gene profiling for messenger ribonucleic acid (mRNA)-based nitrogenase (nifH) and achieved important distinction in the variation connected with nifH expressing populations. Likewise, Zhang et al. (2007) used a short oligonucleotide microarray based on nifH genes to analyze NF diazotrophs in Namibia's wild rice roots. Their results showed that nitrogen was actively fixed within the host by a small population of the total diazotroph identified.

8.3 Rhizobial Inoculation Enhances Plant Growth and Development

In agriculture, the significance of an enclosed endophyte relies on its efficiency in the growth and development of parent plants. Various studies carried on with gnotobiotic and pot-and-field condition (Yanni et al. 1997, 2001; Matthews et al. 2001; Muthukumaraswamy et al. 2005, 2007) illustrate the biofertilization characteristics of some of the secluded endophytes in the habitat purchased. Endophytes, used as a plant inoculant, have proven to be a cause of N that can partly substitute urea N in cereals and vegetables (Yanni et al. 1997; Govindarajan et al. 2007).

Increasing plant biomass, nitrogen content, grain output, and stroke transportation effects were engaged in the promotion of plant growth through rhizobial inoculation and led in inoculated stress retention in N-deficient and N-containing land (Yanni et al. 1997, 2001; Peng et al. 2002). Eleven *Rhizobium leguminosarum* species were separated by Yanni et al. (1997). In Egypt, rice trifolii has been cultivated for centuries with clover rotation. Giza's complete quantity of N (95% belief), seed N (99% belief), and ratio of maize yield (99% trust) improved substantially under circumstances of gnotobiotic and crop circumstances (Yanni et al. 1997, 2001; Biswas et al. 2000a, b). The complete crop output and N concentration rose 3.6 tons (1 ton) and 19–28% (Yanni et al. 1997, 2001; Biswas et al. 2000a), respectively. Rhizobial inoculation was noted in order to improve stomatal behavior, which increases the levels of photosynthesis of rice by 12%, with a rise in grain output of 16 percent. Therefore, the connection between enhanced seed output and the photosynthesis frequency of null N-level was favorable (Peng et al. 2002). They proposed, apparently, that some rhizobic species could encourage rice development and renewal by the system to improve the photosynthesis of the single-blade web. Some of the isolates of the rhizobium inhibited rice plant growth and development (Prayitno et al. 1999; Perrine et al. 2005). In the existence of nitrate/nitrite provided, this restriction was the only cause of N in the media. It is assumed that gene-encoded nitrogen absorption proteins in the bacteria plasmid pSymA induced the inhibitory impact noted in these species (2005, 2007). This exercise of the protein led to a nitrogen decrease and later concentration of nitrogen oxide (NO) that is inhibitory for crop development. Interestingly, plant growth-promoting rhizobacteria (PGPR) *R. trifolii* R4 is capable of further reducing NO to N2 due to the presence of additional reductases (Nitrous/NO; Perrine et al. 2007).

In most research, an original inoculum density of 108–109 cells per ml was enough to achieve optimum development in the inoculated crop (Yanni et al. 1997, 2001; Biswas et al. 2000a, b; Chaintruel et al. 2000). However, several methods for inoculating the plant in greenhouses or in areas have been implemented. Commonly used techniques include seed plunging, seedlings and sugarcane environments, seed layer coverings with inoculum species, and the bacterial suspension foliar spraying before seeding in broth plants, adding bacterial suspension straight to the soil (Yanni et al. 1997, 2001; Muthukumarasamy et al. 1999; Biswas et al. 2000a, b; Matthews et al. 2001; Feng et al. 2006). Unfortunately, the most efficient way to supply bacteria for plant growth and manufacturing is not understood of those methods. Earlier study now shows that many of the endophytic inoculations on marketable plants like maize, fruit trees, and corn can decrease entry into production of N fertilizer (Yanni et al. 1997; Govindarajan et al. 2006, 2007). Yanni et al. (1997) used one third of the recommended dose of N fertilization in addition to *R. trifolii*, in a rice field to produce equivalent grain yield as obtained by the full-recommended dose of fertilizer (144 kg N ha^{-1}). Likewise the inoculation of *Burkholderia* MG43 in sugarcane has led in a higher than half-to-full pace increase in fertilizer, reducing costs of <140 kg ha − 1 N (Govindarajan et al. 2006). *Herbaspirillum* is an endophyte that colonizes sugar corn, maize, corn, sorghum, etc. Baldani et al. (2000) studied 80 distinct types of *H. seropedicae* from cattle, corn, and sorghum originally separated for selection of the rice inoculums as they found rise of 12% in new grain weight over command led in a 100% rise in the species studied. Only a few types in consecutive tests were allowed to keep their efficiency. The crop output of *Herbaspirillum* in greenhouse (at 5% likelihood) was considerably improved to 7.5 g per crop (Mirza et al. 2000). The N quantity of rice varieties with Al-tolerant inoculates with *H. Seropedicae* Z67 showing a substantial increase in origins of 29–61% and in branches of 37–85% (Gyaneshwar et al. 2002a, b). Similarly, another endophyte, which is *Burkholderia* sp., has been widely studied in the field. In rice grain yields increased 0.5–0.8 tons, different forms of rhizospheric and endophytic (Baldani et al. 2000), while plant biomass increased by 22 mg per plane, respectively. This corresponds to a growth of 69% over the uninoculated yield of the power crop. The PsJN type needed a nadC-like gene for potato pipe development to encourage (Wang et al.

2006). Quinolinic acid phosphoribosyltransferase (QAPRTase). Enzyme activity of QAPRTase catalyzes the development of the de novo nicotinamide dinucleotide as a by-product of the creation of the nicotinic acid mononucleotide (NaMN). A nadC mutant could not synthesize the NaMN medium substratum and could not promote the development of the parent plant. However, a PsJN mutant's growth-fostering action has been returned to the press in vivo by adding the corporate NaMN (10–100 μM). And advanced B. Phytoformis PsJN also supplied inoculated cold-tolerant against non-bacterialized treatment (Barka et al. 2006). In freezing circumstances, B. Phytoformis PsJN has enhanced the photosynthesis and starch concentration in *Vitis vinifera* relative with non-inoculated crops ($P < 0.05$). The enhanced strength is a result of the rise, which performs an essential part in stress adjustment, in the prolines and phenolic material of the plant owing to the viral colonization (Barka et al. 2006). The parent plant has a phenolic increase which is also found in rice endophytes‘communication (Mishra et al. 2006) as a sort of stress response caused by bacterial attack. Endophytic bacteria can either be used discreetly or as a plant inoculation blend in containers or areas. The plant growth and growth lead of a combination of bacterial isolates used as an inoculum (Govindarajan et al. 2008). Although no assessment was made of the blend efficiency of Govindarajan et al. (2007) in maize, these trials underline the significance of sample choice in a blended inoculum to increase plant efficiency. Countries such as Brazil have already used crop growth-promoting organisms to develop the ability of endophytes in agribusiness in nonlegume cultivations. With the huge amount of insulated bacteria and the beneficial impact on rice, corn, corn, and sugarcanes on nonlegume growth and development, the possibility for improving these crops appears vivid. However, a critical assessment of productivity variation found at distinct locations in multiple plant rotations would be necessary for the broad implementation of this procedure.

8.3.1 Plant Growth Promotion by Endophytes: Proposed Mechanism

As a consequence of (1) colonizing BNF organisms and (2) rhizobacteria-producing plant-growing materials, benefits savored by the host plant were estimated at an endophyte-plant communication. In some instances, a combined participation of the above two systems was noted.

8.3.2 Nitrogen Accumulation

Nitrogen is the most important yield-limiting component in many agrarian manufacturing processes. It is recognized that the plant uses symbiotic bacteria to make a significant quantity of nitrogen in legumes through BNF (biological nitrogen fixation). In nonlegumes, the complete oxygen volume of the crop increases consistently when NF bacteria coexist as an endophyte. The concentration of nitrogen in inoculated nonlegumes can lead from methane groundwater uptake (Yanni et al. 1997; Prayitno et al. 1999; Elbeltagy et al. 2001). Over the years, a thorough research conducted by different employees in Brazil has shown that, given the N production deficiency, there is a decrease for some types of sugarcane cultivated in the last centuries or even one millennium (Boddey et al. 1995a, b). The first year output increased between 170 t and 230 t ha − 1 in some crop species, cultivated in well-irrigated and fertilized tanks, with a sufficient production of K and P, without N. In crop species CB45–3, SP70–1143, and Krakatau, the pattern of output development persisted for 3 following years. BNF made up 60–80% of the remaining oxygen in these variants (Boddey et al. 1995a, b). A wide variety of methods were used for an endophyte to solve ambient nitrogen in a subject: acid decrease test, 15 N isotope dilution tests, 15 N2 decrease tests, or natural abundance tests with 15 N. Dalton and Kramer (2006) addressed the laboratory information and weaknesses of these assays. The studies showed that rise of the donor plant N concentration in sugarcane to 30–45 mg of N per plant in rice to 170 kg of N per hectare per year in rice was the result of BNF (6-week-old crop) (Boddey et al. 1995a, b; Iniguez et al. 2004). Acetylene reductions and the addition of 15 N_2 gas to *Oryza officinalis* were implemented to determine nitrogen fixation in the plant following inoculation with endophytic *Herbaspirillum* sp. with strength B501. Another instance is *Burkholderia* colonization plants, with BNF obtained from fruit crop oxygen (Baldani et al. 2000).

8.3.3 Biofertilizer Relevance and Plant Tolerance to Environmental Stress

Abiotic and biotic pressures are the main limitations influencing crop productivity as much modern science equipment was widely used to improve plant stress, which has become the primary function for PGPRs as bio-protectors. *Rhizobium trifolii* inoculated with *Trifolium alexandrinum* indicates enhanced biomass in salinity conditions and nodulation (Antoun and Prevost 2005). *Pseudomonas aeruginosa* has proven to be resistant to biotic and abiotic stress (Pandey et al. 2012). *P. fluorescens* MSP-393 has been found by Paul and Nair (2008) that it produces salt stress-induced osmolytes and proteins which overcome the adverse effects of salt. The development level and several developmental parameters of the plant, new weight, and dry weight in alkaline conditions of the cotton *P. putida* RS-198 improved by raising the uptake levels K^+, Mg^{2+}, and Ca^{2+} and reducing uptake of Na^+. Few types of *Pseudomonas* obtained seed resistance via 2,4-DAPG (Schnider-Keel et al. 2000). Interestingly, the systemic reaction to *P. syringae* has been discovered in *Arabidopsis thaliana* by *P. fluorescens* DAPG (Weller et al. 2012). The Calcisol generated by PGPR in the following ways offers elevated temperature tolerances and stress salinity (Egamberdiyeva 2007), *P. alcaligenes* PsA15, *Bacillus polymyxa* BcP26, and *Mycobacterium phlei* MBP18. Plant inoculation with AM fungi has also shown that plant growth is increased under heat pressure (Ansari et al. 2013a, b). Interestingly, the systemic reaction to *P. syringae* has been discovered in *Arabidopsis thaliana* by *P. fluorescens* DAPG (Weller et al. 2012). The Calcisol generated by PGPR in the following ways offers elevated temperature tolerances and stress salinity (Egamberdiyeva 2007), *P. alcaligenes* PsA15, *Bacillus polymyxa* BcP26, and *Mycobacterium phlei* MBP18. Plant inoculation with AM fungi has also shown that plant growth is increased under heat pressure (Ansari et al. 2013a, b). The biomasses of tomatoes and peppers have also increased by *Achromobacter trochaudii*. Interestingly, the parent plant has been discovered to be protective from salt stress by the endophytic root fungus of *Piriformospora indica* (Ansari et al. 2013a, b). In one trial, either alone or together with AM as *Glomus intraradices* or *G. oxydans*, inoculation from PGPR was identified. In *Lactuca sativa*, *G. mosseae* has led to improved nutrient uptake and improved ordinary physiological procedures under stress. The osmotic stress resistance mechanisms using transcriptomic and microscophic approaches have disclosed a major shift in the reaction to transcriptome *Stenotrophomonas rhizophila* DSM14405 T (Gao et al. 2020), which was used to solve stress of the legumes through a mixture of AM and N2-fixant bacteria (Aliasgharzad et al. 2006). Combining Brazilian and AM enhanced herbal tolerance for various abiotic pressures. The addition of *Pseudomonas putida or Bacillus megaterium* and AM fungi was efficient in alleviating flood pressure (Marulanda et al. 2009). Under water strain, the use of *Pseudomonas* sp. has enhanced antioxidants and photosynthetic pigments in basil crops. Of interest, three bacterial species, paired, were the most active in leaves under air pressure (CAT, GPX, and APX activities and chlorophyll). It is interesting to note that the mixture of three bacteria has induced the most disturbance of CAT, GPX, APX, and chlorophyll in water deficiency (Heidari and Golpayegani 2012). *Pseudomonas* spp. were found to cause positive effect on the seedling growth and seed germination of *A. officinalis* L. under water stress (Liddycoat et al. 2009). After inoculation of arbuscular fungi (Ruiz-Sanchez et al. 2010), the photosynthetic efficiency and antioxidant reaction of rice plants exposed to drought stress have increased. Also under both cold and acidic circumstances, the positive impacts of mycorrhizae have been recorded (Aroca et al. 2013). *Glucanacetobacter* spp., phosphobacteria spp., and *Azospirillium* spp. isolated from rice and mangrove rhizosphere are heavy metal resistant especially iron was discovered to be more effective (Samuel and Muthukkaruppan 2011). The inhibitory effect of cadmium through IAA (siderophore and 1-aminocyclopropane-1-carboxylate deaminase (ACCD) can be protected against canola and strawberry crops (*P. putida* type 11 (P.P.11), P. putida type 4 (P.P.4), or *P. fluorescens* type 169 (P.F.169) (Baharlouei etc. Al2011). Rhizoremediation of contaminated soil with petrol has been revealed to be expedited by incorporating microbes into plant species such as cotton, ryegrass, heavy fescue, and alfalfa in the shape of an efficient microbial medium (EMA). PGPRs as biological agents have demonstrated their opposition to multiple pathogens as an alternative to chemical agents (Murphy et al. 2000). They can provide resistance against pathogens by generating metabolites besides serving as growth-promoting forces (Backman et al. 2008). The defense-related pathway may be induced by *Bacillus subtilis* GBO_3, namely, salicylic acid

(SA) and jasmonic acid (JA) (Ryu et al. 2004). *Paenibacillus polymyxa* SQR21 was another exciting trial which found *Fusarium* wilt biocontrol agents in watermelon (Ling et al. 2011). Moreover, the management of raised wilts in tomatoes, garlic and fruit composite turkey and banana-bunks of the highest varieties in bananas have been shown to be efficient in exploitation of PGPRs (Murphy et al. 2000); Harish et al. 2009).

In various studies, mycorrhizae can also confer resistant against fungal pathogens and inhibit the growth of many root pathogens such as *R. solani*, *Pythium* spp., *F. oxysporum*, *A. obscura*, and *H. annosum* by improving plant nutrients profile and thereby productivity (Ansari et al. 2013a, b). For instance, *Glomus mosseae* was effective against *Fusarium oxysporum* f. sp. which causes root rot disease of basil plants (Toussaint et al. 2008). *Medicago truncatula* also showed induction of various defense-related genes with mycorrhizal colonization. It was shown that addition of *Arbuscular mycorrhiza* fungi and *Pseudomonas fluorescens* to the soil can reduce the development of root rot disease and enhance the yield of *Phaseolus vulgaris* L. (Singh 2011)

8.3.4 Mechanism of Action of Various Biofertilizers

The fungus and higher plant stems are associated with mycorrhiza. While remaining an enigma, it is a model system for understanding the process behind stimuli to development of root cells as a result of mycorrhizal dwelling. In two DNA mapping EM plants (ectomycorrhizae), L. T, and 13 bicolor Melanosporum (white truffle) 14 enables to define variables regulating mycorrhiza growth and operate in plant cells. Fifteen genes were recognized as suspect hexotic conveyors in "L" that were symbiosis-controlled. It lost genes that were invertase encoding to make it glucose-dependent. However, Melanosporum has one invertase gene, as opposed to "L". "Bicolor" can use host saccharose (Bonfante et al. 2010). The upregulation on transporter genes during symbiosis stated the intervention by means of the symbiotic border to transport helpful compounds like amino acids, oligopeptides, and polyamines between organisms. Free working mycelium can take nitrogen and ammonium from land. These compounds then enter the cap and hartig membrane and transfer to the crops. The development of symbiotic connections as effector and facilitators cysteine-rich fungal protein (MISSP7) plays an important part (Plett et al. 2011). Many auxin-related mutations and plant morphogenesis were upregulated during mycorrhizal development (Splivallo et al. 2009). *G. versiforme* also has inorganic phosphate (Pi) carriers on their hyphae that aid in the direct absorption of phosphate from the earth and discover a glutamine synthase gene in *G. intraradices* strengthening oxygen absorption opportunities in the fungal phenomenon that may subsequently be transferred to the crop (Salvioli et al. 2012).

Nod variables, known as Myc, are suggested to be mycorrhizobial and rhizobial secreted by origins identified for the initiation of sensory transmission or prevalent symbiosis (SYM) mechanism (Kosuta et al. 2003). The paths of preparing crops for

AM as well as rhizobial infection are some popular highlights. The prevalent SYM path allows the household factory for modifications at the molecular and anatomical levels with the first fungal hyphae touch. Calcium should be the center of secondary messengers via Ca_2 plus spiking in the root-hair atomic area. Microarray tests indicate that *Rhizobium leguminosarum* biovar viciae can cause different mutations in crops, such as grain, alfalfa, and fruit beet. This in addition induces the development of nitric acid (NO), the second signal that triggers a complicated binding network that leads to better plant development and development procedures, by PGPR (Molina-Favero et al. 2007). Input is upregulated by manifestation of ENOD11 and many defense-related genes. This then allows the formation of a PPA (Bucher et al. 2009) or pre-penetration system. While the mechanism for the development of shrubs is unclear, when a protein called vapyrin is torn down, growth of shrubs decreases (Bapaume and Reinhardt 2012). Many other mutations are thought to be associated with the creation of arbuscles, including subtilisin protease 65, oxygen transporter 66, or two ABC carriers 67 (Tromas et al. 2012). Currently scientists are using genes to save nitrogen to create altered plants. Nif genes are caused in the rhizosphere to poor nitrogen and to small concentrations of oxygen in bacterial nitrogen fixators. Interesting are sugar maple plantlets of native form. *G. diazotrophicus* has demonstrated the fixation of radioactive N2 compared to *G. diazotrophicus* mutant of nif gene D, which has demonstrated significance of nif genes. Efficiency of nitrogen fixation is dependent on use of carbon (Sevilla et al. 2001). In cotton plant 247 mutations may be induced separately by a bacterium such as *Bacillus subtilis* (UFLA285) from the ones where no PGPR is given to the cotton plant. Induction of UFLA285 resulted in the different expression of many disease-based resistance genes which are characterized by signs of jasmonate/ethylene and osmotic regulation by proline chemistry. In rice-*H. seropedicae* connection, it was shown that the defense-related enzymes PBZ1 and thionins have been repressed, indicating that crop defense reactions during colonization are modified (Santos et al. 2012). The secretion of *Azospirillum* was suggested among PGPR species as gibberellins, ethylene, and auxins (Perrig et al. 2007). The roots have high IAA concentrations (Bent et al. 2001), and some plant-related organisms may also cause phytohormone production, as is the case with lodgepole oak. In a range of environmental circumstances such as pH, heat and existence of agro landfill, rhizobium and *Bacillus* were discovered to be a synthesis of IAA as a substrate (Sudha et al. 2012). Unlike other phytohormones, ethylene has an effect on the development of dicot crops. Glick (1995) discovered that plant growth can be enhanced by suppressing the activity of ethylene. Interestingly, a design was proposed showing that 1-amino cyclopropane-1-carboxylate (ACC) ethylene chemistry is also one of the processes for initiation of growth-friendly ethylene hydrolyzed with mammalian ACC deaminase enzymes that need the oxygen and carbon supply. Bacteria such as *Alcaligenes* sp., *Bacillus pumilus*, and *Pseudomonas* sp. have also been identified for ACC deaminase operation and the paradox of *Variovorax*. In canola, where genes in ACC deaminase have led a growth-promoting *Pseudomonas putida* to lose impact, the participation of ACC deaminases has been shown to have an immediate impact on plant development. In canola, where mutations of the ACC deaminase gene led to the failure of impact of development stimulating *Pseudomonas putida*, there was evidence of the participation of ACC deaminases in the negative impact on plant growth. Further, the possibility of PGPRs was further improved through the introduction into a certain helpful strain of PGPRs of genes participating in an immediate oxidation process and the solubilization of mineral phosphate (MPS). Gene encoding glucose dehydrogenase (gcd) involved in the DO pathway was cloned and characterized from *Acinetobacter calcoaceticus* and *E. coli* (Tripura et al. 2007). *Acinetobacter calcoaceticus* and *G. oxydans* have been cloned in a soluble type of gcd (Sashidhar and Podile 2010) (Fig. 8.1).

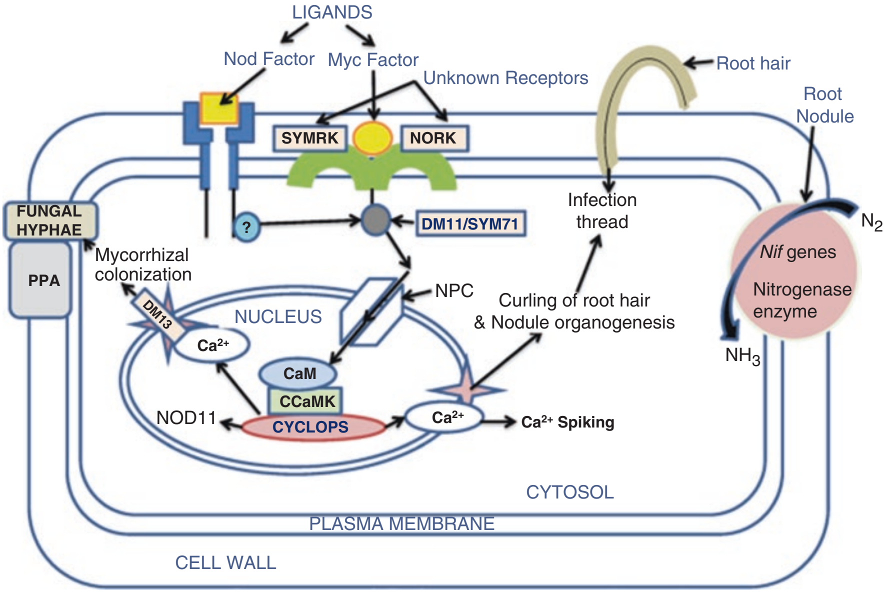

Fig. 8.1 A hypothesis of biofertilizer intervention system in the root cell. Host origins have been seen as the triggering gene transduction process. This triggers the further mechanism for gene transduction via unknown receptors (SYMRK and NORK), which triggers the discharge of Ca^{2+} into the cytosol. The entire path includes phosphorylated receptors such as kinases or other associated proteins such as DMI and SYM71 (Maillet et al. 2011). The nuclear pore complex (NPC) and some of its enzymes (NUP) participate in calcium spiking. Proteins from DM1 perform a part in maintaining periodic oscillation of calcium ions inside and outside the nucleus. Multiple protein canals (Ca^{2+} channel enzymes) also promote this method with the support of multiple transporters. CCaMK is a phosphorylated calcium-driven protein kinase result of CYCLOPS protein which initiates the activation of different genes affecting the development of pre-penetration system (Maillet et al. 2011) buildings such as noduli and PPA

8.3.5 Phosphate-Solubilizing Microorganisms

Microorganisms constitute a significant element of soil and immediately or indirectly impact soil health through their useful or damaging operations. Rhizospheric microorganisms mediate soil procedures such as decomposition, nutrient movement and mineralization, water retention, nitrogen fixation, and denitrification. Moreover, insoluble phosphatic compounds can, and will, be converted into soil-soluble formations by bacteria of phosphate-solubilizing capacity (Pradhan and Sukla 2006). The function of rhizospheric bacteria in mineral phosphate solubilization was already recognized in 1902. Ever since then, comprehensive tests have been undertaken with obviously rich rhizospheric microorganisms to resolve mineral phosphorus. The *Bacillus* and *Pseudomonas* (Illmer and Schinner 1992) and *Aspergillus* and *Penicillium* are important genera in the mineral oil solubilizers (Motsara et al. 1995). Nematophagous fungus *Arthrobotrys oligospora* is a stone phosphate resistant, Togolese stone phosphorus (Senegal TRS) and Tilemsi stone phosphorus (Mali TIRP), studied in vitro and in vivo lately. All three kinds of stone phosphates were solubilized, and stone phosphates could be solubilized in vitro from extra phosphates (Duponnois et al. 2006). Most of the influence of biofertilizer microorganisms was directed at the comprehension of biological nitrogen fixation. On the other side, there has been considerably less basic research on nodule bacterium phosphate solubility, though phosphorus is recognized to be a limiting factor in the symbiosis of rhizobium legume nitrogen fixation. Only few accounts of the solubilization of phosphate by *Rhizobium* (Chabot et al. 1996) and *Azotobacter*, a nonsymbiotic water fixer, have been revealed (Kumar et al. 2001). Omnipresent microorganisms which solubilize the phosphate differ from land to land. In land, 1–50% of the inhabitants is constituted by phosphate-solubilizing bacteria and 0.5% to −0.1% by plants. Generally, the organisms that solubilize phosphate are 2–150 times more than the fungal phosphate (Kucey 1983). The elevated share of PSM in the rhizosphere is considered to be more potent than from the habitats other than rhizosphere in the environment. In comparison, the rhizoplane has been recorded to have a peak of salt-, pH-, and temperature-tolerant phosphate solubilization organisms and to be accompanied by alkaline plants with root-free plants (Johri et al. 1999). The PSM species with these strained characteristics should therefore act as an outstanding template for the analysis of phosphate solubilization's physiological, biochemical, and molecular processes in strained habitats. Furthermore, after frequent subcultures, phosphate-solubilizers were noted to decrease phosphate solubilization exercise, but in phosphate-solubilizing fungi, no casualties were noted (Kucey 1983). Phosphate-solubilizing mushrooms usually generate more enzymes so that in the strong and fluid environment, there is higher phosphate solubilization action compared to fungi (Venkateswarlu et al. 1984). In the existence of ammonium salts, PSM's phosphate-solubilizing capability also depends on the complexity of the nitrogen supply used by the press and higher solubilization than when nitrate is used as nitrogen supply. The proton extrusion was ascribed to a reduction in extracellulary pH to offset ammonium intake (Roos and Luckner 1984). In some instances, however, ammonium may cause a decrease in phosphorus solubilization.

8.3.6 Phosphate-Solubilizing Microorganisms

Phosphate-solubilizing microorganisms may, through serial dilutions or methods for enrichment culture, in Pikov's medium be separated by non-rhizosphere and rhizosphere, rhizoplanes, and other environments, such as rock and phosphate depositions and soil or marine habitats. When the bacteria are incubated on the strong surfaces with insoluble phosphate, PSM is identified by the creation of transparent halos around the structures (Fig. 8.1). Several additional techniques for isolation and selection of PSM have recently been suggested (Nautiyal 1999).

Given the various species in phosphate-solubilizing bacteria, they are constantly subcultured for their phosphate-solubleness potentials to study their persistent phosphate-solubilizing behavior and vulnerability (Illmer and Schinner 1992). After selecting efficient phosphate-solubilizing bacteria, they are screened in order to solubilize insoluble phosphate under the liquid culture setting. Finally, inoculants are produced from the selection of effective phosphate solubilizators, and their efficiency is evaluated against multiple plants under pot/field circumstances.

8.3.7 Mechanism of Phosphate Solubilization

Many investigators have explored PSM's capacity to solubilize in a simple solution of fluid crops insoluble phosphorus (Narula et al. 2000). The microbial solubilization of soil phosphorus in fluid medium was often a consequence of excretion of fatty oxygen. For instance, oxalic acid, citric acid, lactic acid, etc. in fluid cultivation filtrate has been determined by paper chromatography, or liquid chromatography with a high degree of performance, and certain enzymatic techniques (Gyaneshwar et al. 1998). Such organic acids can dissolve by exchanging PO_4 2 by acid anions directly or dissolve the mineral phosphate and aluminum ions associated with the chelate. In some instances, phosphate hunger leads to solubilization (Gyaneshwar et al. 1999). But there is no clear link between PSM proteins and the amount of solubilized phosphorus (Asea et al. 1988). In the solubility of insoluble phosphorus, the function of organic acids generated by PSM can be attributable to reduced pH, the cation chelations, and the phosphate rivalry of adsorption sites (Nahas 1996). Inorganic acids, like hydrochloric acid, can also solubilize phosphates, but are less active than organic acids at the same photographs. Acidification appears, however, to be not the only process of solubility, because pH-reduced capabilities have not in some instances correlated to mineral phosphate solubility. The chelating capabilities of organic acids are important as it has been demonstrated that adding 0.05 M EDTA to the medium has the same solubilizing impact as the inoculation of Penicillium bilaii (Kucey 1988). Rhizobius was connected with the manufacturing of 2-ketogluconic acid, which has been eliminated by inclusion of NaOH, among nodule bacteria (e.g., *Rhizobium*/*Bradyrhizobium*), showing the phosphate-solubilizing exercise of that organism to be wholly owing to its capacity to lower the pH of the water (Halder and Chakrabarty 1993). However, the details of sugar solubilization biochemical and molecular processes by symbiotic nodule organisms are not understood.

8.3.8 Production of Phosphate-Solubilizing Microorganism Inoculants

As a microphone demand for landowners, efficient PSM plants are mass-produced. Microphosis manufacturing, which means preparing of microorganism, includes a three-stage process: first, phosphate-solubilizing strain choice and screening; secondly, the preparing and handling of inoculants, including product carrier choice and PSM mass culture; and, thirdly, the quality management and distributing procedure. Peat, farm yard manure (FYM), powdered land, and pig wood pie were proposed as appropriate transports for the manufacturing of microphones (Kundu and Gaur 1981). The crops are finally packaged in polybags and can be stored safely in 30 ± 2 ° C for approximately 3 months. In India, two effective phosphate-solubilizing bacteria (*Pseudomonas striata* and *Bacillus polymyxa*) and three phosphatesolubilizing fungi (*Aspergillus awamori*, *A. niger*, and *Penicillium digitatum*) had been created as a microbial preparing by Indian Agricultural Research Institute (IARI).

8.3.9 Importance of Biofertilizers in Conservation Agriculture

In cultivation, biofertilizers contribute to improved plant fertility, fluid composition, decay of plant residues, and the variety and habitat of plant microbials, eventually enhancing plant wellness and plant output. It also contributes to reducing the demands for chemical fertilizer during a specific harvest. On the other hand, the AMF produces glomalin, a heat-shock protein that increases soil regrowth and contributes to the carbon sequestration. This is known as glomalin. Glomalin and mycorrhizal hyphae combined cause a stable soil structure. The study presented by separate workers shows the elevated prospective importance of biofertilizer in the previous statements, as summarized below:

1. The implementation of rhizobium biofertilizer considerably enhanced agronomic output characteristics of pulse cultivation under temperate climates.
2. *Azospirillum* applied in agricultural plants improves the leaf area index, crop indices, and return characteristics.
3. Green gram inoculant of rhizobium was found at levels of fertility of less than 20 kg N + 45 kg P_2O_5 per hectare to enhance grain and straw output.
4. The beneficial impact of *Azotobacter chroococcum* in maize plants has been observed to significantly enhance output in comparison to treatment in biofertilizer-treated plants.

5. Enhanced soil formation by biofertilizer; the effect of the alkaline phosphatase was higher in *Azotobacter chroococcum* + P fertilizer than in the suppression of peach plants.

8.4 Conclusions

There are significant problems of environmental stress and a decline in productivity at an unparalleled pace. Our reliance on chemical fertilizers and pesticides has promoted the growth of sectors generating chemical substances that are life-threatening and which are not only dangerous to human consumption but also have an impact on the environment. At a moment when cultivation is faced with multiple economic pressures, biofertilizers can assist to resolve the issue of supplying a growing world population. The helpful elements of biofertilizers should be realized and their implementation to contemporary farmers' methods implemented. The fresh technique that has been created using the strong molecular biology instrument can improve phytohormone biological processes. These techniques can assist to alleviate economic pressures when recognized and transmitted to the helpful PGPRs. However there are few explanations why many helpful PGPRs still go beyond ecologists' and farmers' understanding about enhanced protocols for biofertilizer applications in the sector. However, progress in microbial science, plant-pathogenic interacting and genomics-related techniques will contribute towards optimizing the protocols needed. The achievement of biofertilizer research is determined by innovations of creative methods linked to PGPRs' tasks and their appropriate implementation in the agricultural sector. The great difficulty in this study region resides in the reality that the real working process of PGPRs must be dissected in order to ensure their efficiency in viable cultivation, together with the detection of different types of PGPR and its characteristics.

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Chapter 9
Role of Recombinant DNA Technology in Biofertilizer Production

Rohaya Ali

Abstract Nutrients present in plants are important for the maintenance of crops and production of healthy food for the constantly rising population of the world. For this efficient means of maintaining soil fertility are required. Soil maintenance approaches nowadays are largely reliant on chemical fertilizers, which may pose a severe threat to human well-being and the environment. In this backdrop, biofertilizers have been recognized as a substitute for elevating soil richness and hence crop productivity in sustainable agricultural practices. The utilization of advantageous microbial organisms as biofertilizers is extremely significant in agriculture as it aids in maintenance of food security and elevating crop produce. Furthermore, biofertilizers are highly significant in maintaining the quality of soil. Microbes which are frequently utilized as biofertilizers include potassium solubilizers, nitrogen fixers, mycorrhiza, cyanobacteria or blue green algae, plant growth-promoting *Rhizobacteria*. Biofertilizers aid in nutrient uptake by plants, offer forbearance to biotic and abiotic situations to plants and also maintain plant growth. Biofertilizers maintain nutrient richness by means of nitrogen fixation, solubilization of potassium, production of antibiotics, disintegration of organic substances and release of plant growth-promoting agents. Biofertilizers, when given in the form of seed or soil inoculants, contribute in nutrient cycling and lead to enhanced crop production. Biofertilizers, therefore, play a vital role in maintaining soil nutrients and hence agricultural produce. Furthermore, biofertilizer production by using the tools of molecular biotechnology like recombinant DNA technology can improve the metabolic pathways of production of important plant growth-promoting factors like phytohormones,, if recognized and transmitted to the useful plant growth-promoting microbes. Recombinant DNA technology offers numerous benefits, as explicit biological pathways can be controlled with high accuracy and entirely novel functions can be engineered into the microorganisms for producing efficient biofertilizers.

Keywords Biofertilizers · Solubilizers · Mycorrhiza · Phytohormones · Cyanobacteria

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9.1 Introduction to Biotechnology

The term "biotechnology" was coined by Karl Ereky and is blend of the two words, i.e. biology and technology. This field is tremendously divergent, enormous and multidisciplinary. Thus, a clear-cut description of the subject is slightly hard. Biotechnology is fundamentally the utilization of biological substances like microbes or cellular constituents in controlled fashion for the advantage of mankind (Okeno et al. 2012). In other words, biotechnology is an integrated utilization of biochemistry, microbiology and engineering knowledge for the utilization of microbes, cultured cells or tissues to their best. Human beings have sustained their search for enhancing the natural potential of microbes and making them competent for novel methods. In past, people exploited microbes for the production of cheese, bread production or brewing alcohol, even if the process of fermentation was not tacit comprehensively (Carpenter et al. 2002). At present, application of biotechnology is highly complicated. Now, scientists can manipulate living organisms and transfer genetic matter among them, producing transgenic organisms. The present relevance of biotechnology is largely in the area of biomedicine and agriculture. Current methods allow the construction of novel and enhanced food products. In biomedicine area, novel vaccines, antibiotics, etc. have been produced against various diseases like AIDS, cancer and many hereditary diseases. Biotechnology is also used in the area of bio-fuel production, mining and pollution control. Genetically modified microbes and plants are utilized to remove toxic chemicals from oil spoil spills or industrial effluents (Chen et al. 2007). Besides, improved superiority of life and still there exists a countless exhilarating opportunities in the field of biotechnology (Figs. 9.1 and 9.2).

9.1.1 Subfields of Biotechnology

Generally, biotechnology is categorized in three major subtypes:

- Green biotechnology
- White biotechnology
- Red biotechnology

9.1.2 Green Biotechnology

Green biotechnology is a vital field of contemporary biotechnology. Its foundation is on the crop enhancement and manufacture of new crop products (McAllister et al. 2012). This is achieved by introducing foreign genes into the plants having huge economic importance. It comprises of three major areas which include:

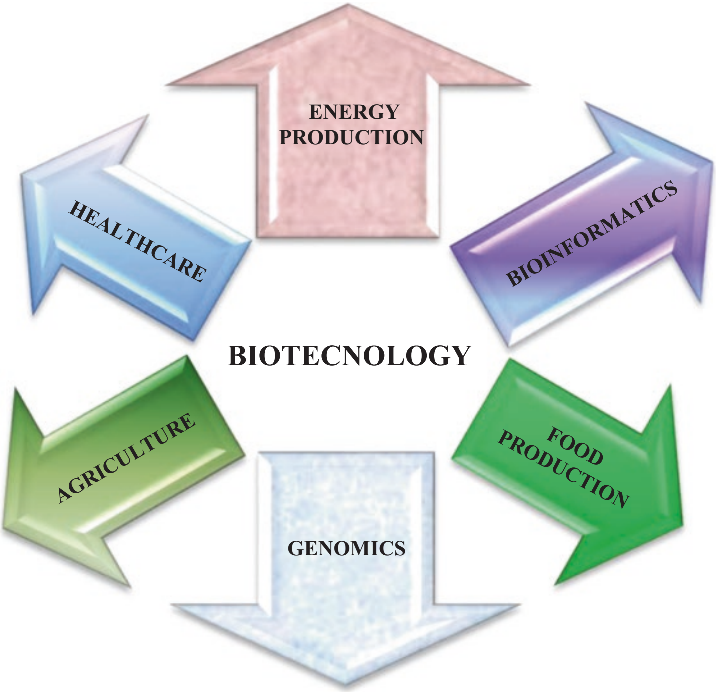

Fig. 9.1 Applications of biotechnology

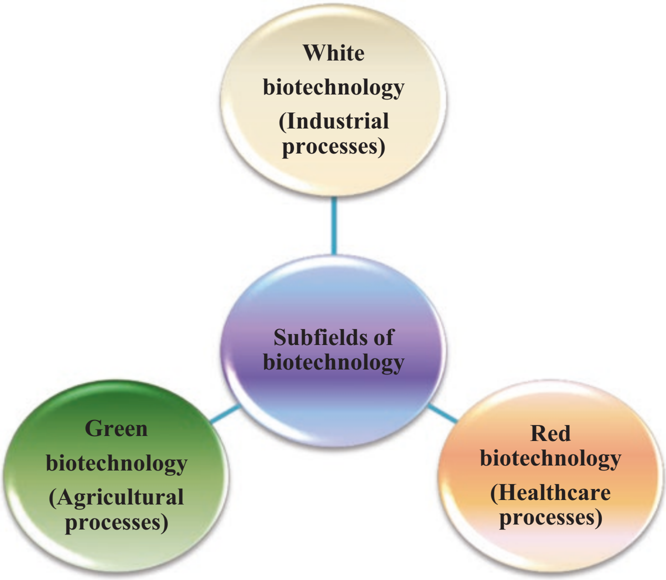

Fig. 9.2 Subfields of biotechnology

(a) Plant tissue culture
(b) Plant genetic engineering
(c) Plant molecular marker assisted breeding

Plant tissue culture involves the production of the whole plant or part of it under laboratory conditions. Its main advantage is the quick manufacture of plant materials like citrus fruits, banana, etc. On the other hand, plant genetic engineering involves the introduction of beneficial genes from one living organism to other. This generates improved varieties of crops with enhanced production (Brookes and Barfoot 2009).

In the case of plant molecular marker-assisted breeding, molecular markers (specific short sequences of DNA) are accountable for a preferred attribute. Thus, improved properties, like disease resistance, can be achieved (Horvath et al. 2012).

9.1.3 White Biotechnology

This area is concerned with industries. It utilizes enzymes, bacteria, yeast or moulds to produce valuable products. It results in the manufacture of wide range of bioproducts like vitamins, antibiotics, detergents, etc. (Bueno et al. 2016).

9.1.4 Red Biotechnology

It is concerned with medical biotechnology. It involves genetic manipulation of organisms to create antibiotics. Herein, the human body's own tools are utilized to eliminate the pathogens. It is of immense significance in the conventional drug discovery and also aids in improving the potential for cure, anticipation and analysis of diseases (Becker et al. 2008).

9.2 Recombinant DNA Technology

The growth and understanding of biological phenomena over the past few decades, both at molecular and cellular levels, is transfigured by the dawn of genetic engineering or recombinant DNA technology. This branch of biology is largely spawned under contemporary biotechnology that uses living organisms to generate enhanced and precious products for the betterment of human society. Chemical and biochemical engineering techniques are concerned with the production of recombinant DNA. Cultivation of microbes and their downstream processes rely on engineering techniques.

The history of recombinant DNA technology dates back to 1953, when the double helical structure of DNA was explicated by Watson and crick and the genetic code was cracked by Nirenberg. Afterwards, in 1973, the method of restriction digestion was invented by Cohen and Boyer which involve cut and paste of the DNA sequences (Ames and Martin 1964; Cohen et al. 1973).

Due to recombinant DNA technology, cloning of genes for production of polypeptides (growth factors, interferon, blood clotting factors, human insulin, viral coat proteins, etc.) has become achievable. Each of the polypeptide is unique in the context of its sequence or target. Now, with the advent of recombinant DNA technology, researchers can express a natural gene even in a very simple bacterium like *E. coli* (Brown et al. 2015).

Somatostatin was the first human protein produced in *E.coli* in 1977. Later in 1982, the first recombinant protein, i.e. human insulin, was available in the market. Kary Mullis in 1985 envisaged the idea of polymerase chain reaction (PCR) which revolutionized the field of recombinant biotechnology. Bimolecular archaeology, DNA fingerprinting, molecular ecology, forensics, etc. are novel branches that have become achievable due to PCR (Kakumanu et al. 2012; Huang et al. 2001).

9.3 Construction of the Recombinant DNA Molecule

To construct a recombinant DNA molecule, a DNA fragment (restriction fragment) is inserted in cloning vector at the corresponding restriction site. Then sticky ends of the vector and the foreign DNA are allowed to anneal. Then, by means of DNA ligase, they are joined covalently to create a chimeric DNA (Fig. 9.3).

9.4 Cloning of DNA by Recombinant DNA Technology

Comprehensive study of the working and construction of the gene at molecular stage needs a huge amount of individual gene in purest form. Recombinant DNA technology offers great advantage in cloning that permits researchers to create a huge quantity of matching DNA molecules. The DNA molecule so produced has sequences derived from diverse sources. In DNA cloning one of the important steps is to link the desired DNA fragment to a vector DNA that could duplicate within the host cell. As a result, the recombinant DNA molecule is produced that replicates together with the vector, producing a huge quantity of matching DNA molecules (Bonneau and Laarved 1999). The scheme of production of recombinant DNA is shown in the following diagram:

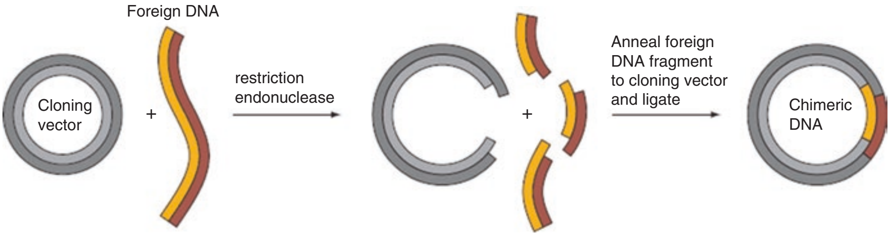

Fig. 9.3 Construction of recombinant DNA molecule. (Adapted from Biochemistry, 4th edition, Donald Voet and Judith G. Voet, 2011)

Vector DNA molecule + DNA molecule

↓

Recombinant DNA molecule

↓

Replication of Recombinant DNA

↓

Isolation and sequencing of the purified DNA molecule

9.5 Role of Restriction Enzymes in the Creation of Recombinant DNA Molecule

The main purpose of DNA cloning is to generate distinct, tiny regions of DNA molecule that comprises of definite genes. Practically very little amount of DNA molecules could be cloned in a vector. Thus, extremely lengthy DNA molecules must be cleaved into fragments that could be put easily into a vector DNA. To facilitate this process, restriction enzymes are used (Becker et al. 2008). Restriction enzymes are endonucleases that are obtained from bacteria and are characteristically able to distinguish and cleave specific four- to eight-base pair sequences, called “restriction site”. These sites commonly are short palindromes (sequence same on each DNA strand when read in the $5' \rightarrow 3'$ direction). One of the widely used restriction enzymes is from *E. coli* called *EcoR1* (Brown et al. 2015). This enzyme is able to make staggered cuts at the definite six-base pair (palindromic sequence) as shown in Fig. 9.4.

For every restriction endonuclease, bacteria also create a modification enzyme that protects the bacterium’s own DNA from cleavage. This modification enzyme adds a methyl group to one or more bases, generally within the restriction site. This methyl group prevents endonuclease from cleaving the DNA. Methylating enzyme and the restriction endonuclease together form the “restriction modification system”

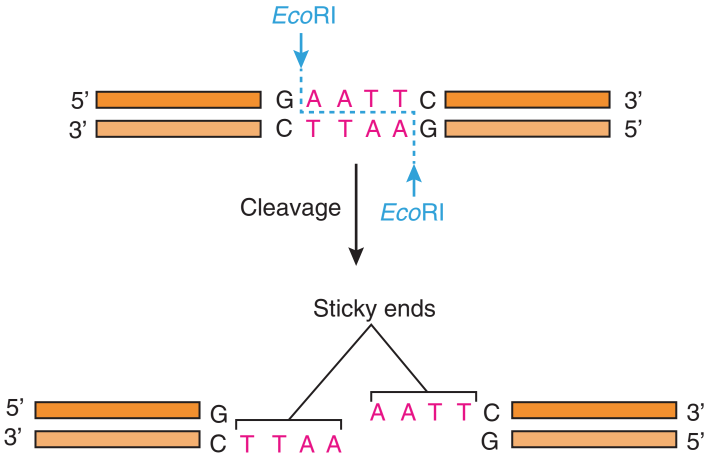

Fig. 9.4 Cleavage of DNA by *Eco*R1. (Adapted from Molecular Cell Biology, 4th edition, Lodish et al. 2002)

that protects the host DNA while it destroys incoming foreign DNA by cleaving it at all the restriction sites (Overton 2014).

9.6 Inserting DNA Fragments into Vectors

DNA ligases aid in the insertion of DNA fragments into the vector DNA. During normal DNA replication, these ligase take part in joining of short segments of DNA called *Okazaki* fragments. In case of DNA cloning purified DNA ligases covalently join the ends of restriction fragments and vector DNA that possesses complementary ends. This linkage is done through standard 3′ → 5′ phosphodiester bonds of DNA. The DNA ligase from the bacteriophage T4 can ligate both the blunt ends and the complementary sticky ends. Nevertheless, blunt-end ligation is intrinsically ineffective and needs huge amount of both DNA and DNA ligase (He et al. 2000).

9.7 Role of Recombinant DNA Technology in Agriculture

With the rise in demand for food, there is a huge demand to integrate biotechnology to enhance crop improvement strategies. The advent of biotechnology has revolutionized the whole crop improvement strategies by offering novel strains of crops, highly proficient, precise and selective pesticides and valuable fertilizers (Ewen and Pusztai 1999). Ancient communities maintained the crops by implementing selective breeding programs. By gathering seeds from the most advantageous crops, they were able to generate crops that were adapted to the changing environment and could offer higher yield. In agriculture, biotechnology has resulted in enhancement of crop productivity by controlling diseases through improved genetic resistance programs. Thus, it offers efficient tools for enhancing and sustaining food security. It offers an outstanding scenario for improving health by engineering the nutritional quality of food, bioremediation and genetic conservation (Buikema and Haselkorn 2001).

The development of protein engineering and synthetic biology has revolutionized the exploitation of proficient microbial systems for the generation of value added products. Environmentally friendly routes of manufacture, creation of smaller amounts of by-products and enhanced output of the target compounds are advantages of microbial biosynthesis over the chemical synthesis.

Extensive optimization of growth conditions is required while obtaining compounds from their native host. However, with the help of recombinant DNA technology, several natural products, their derivatives, or even unnatural compounds have been created within established microbial hosts. Alkaloids, terpenoids, flavonoids, amino acids, peptides, antibiotics, organic acids and vitamins are some examples of microbially produced compounds through modern recombinant biotechnology (Chaurasia et al. 2008).

9.7.1 Soil Fertility

Soil is that portion of the earth on which plant grows. It comprises of three main layers (top soil, sub soil and parent soil). Uppermost layer, i.e. top soil, contains minerals, water, air and inorganic or organic matter. Minerals include silica, aluminium oxide, calcium, potassium, magnesium and extremely little quantity of nitrogen, sulphur, boron, zinc, molybdenum, etc. (Huang et al. 2004). Among all the minerals, only 14 are essential minerals. Essential nutrients are further categorized into micronutrients and macronutrients. The macronutrients are further classified into primary macronutrient and secondary macronutrients. Primary macronutrients are frequently limited in the soil, while secondary macronutrients are rarely limited (Dash et al. 2016). Soil quality decides the quality and quantity of agricultural production. Besides, it also offers niche to a wide range of living organisms. For this reason, proper management of soil is one of the key factors for enhanced crop productivity (Sanahuja et al. 2011).

9.8 Biofertilizers

Chemical fertilizers offer convenient technique to supplement soil with valuable nutrients and therefore help to overcome the growing requirements of food. Nevertheless, they are reasonably very costly and risky to human well-being. On the other hand, they not only supply necessary nutrients to crops but also supply them in an easy accessible mode. Thus, chemical fertilizers can rapidly improve the development and yield of crops and are hence gaining fame around the world (Raja 2013). However, extensive employment of such fertilizers causes grave ecological problems. Nitrate leaching and contamination of groundwater are due to augmented exploitation of fertilizers. Inorganic fertilizers like calcium nitrate, ammonium chloride and sodium nitrate produce greenhouse gases that result in pollution. Elevated levels of greenhouse gases and heavy metal uptake by plants are major causes of environmental damage. Eutrophication of freshwater is also due to chemical fertilizers. Furthermore, chemical fertilizers can eradicate the advantageous microbial or insect community of the soil. Fortunately, nature has bestowed the soil with a variety of microbes with specific mechanisms to overcome this challenge. This mechanism besides maintaining soil quality also works in tandem with plants as an element of ecosystem. Such mechanism is what constitutes "biofertilizers" (Khosro and Yousef 2012). Biofertilizers constitute a central part of green agriculture. Biofertilizers contain proficient strains of microbes, organic products and departed and rotten parts of plants which supply nutrients to soil. It progressively elevates crop yield by means of enhancing soil fertility. They change the unavailable form of nutrients to the accessible form by escalating the population of microbes in the rhizosphere (Leonardo et al. 2006). Microbes are accountable for delivering soluble nutrients to crops (Chang and Yang 2009). These are helpful in a variety of ways that include solubilization of plant nutrients and fixing of atmospheric nitrogen. They also encourage the formation of growth-promoting phytohormones like cytokinins and auxins. They also defend the plant against various abiotic and biotic stresses (Mitragotri et al. 2014).

Biofertilizers aid plants in accessing the nutrient present in its surroundings. The microbes frequently employed as the biofertilizers include *Rhizobium*, *Azotobacter*, *Anabaena* (nitrogen fixers), *Pseudomonas putida*, mycorrhizal fungi, etc. (Liu and Golden 2002). Likewise, phytohormone-/auxin-producing bacteria could also be utilized as biofertilizer (Somasegaran and Springer 1994). All of these microbes enhance growth and development of plants (Table 9.1). The grievance from agriculturalists regarding the effectiveness of biofertilizer is their improper storage and the larger time period between field application and production. This restricts their employment due to compatibility and constancy issues under diverse soil environments. For this reason, improved shelf life is the basis for the popularization of biofertilizers (Adesemoye and Kloepper 2009).

Table 9.1 Plant growth-promoting substances associated with various microbes

Microbe	Plant growth promoting substance
Azotobacter	Vitamins, gibberellins
Azospirillum	Indole acetic acid, gibberellins, indole lactic acid
Cyanobacteria	Vitamins
Phosphate-solubilizing bacteria	Vitamins (thiamin, biotin, riboflavin)
Mycorrhizae	Cytokinin, gibberellins

Presently, a variety of marketable biofertilizers are obtainable and a variety of mechanisms have been formulated to guarantee maximum viability of the microbes used in such formulations (Bhattacharyya and Jha 2012). These strategies include:

- Optimization of biofertilizer formulation
- Usage of thermo-resistant or drought-resistant and genetically modified strains
- Employment of liquid biofertilizer

For dexterity, a carrier substance is utilized as a vehicle for the microbes which are to be used as biofertilizer. Carrier substances include clay, vermiculite, peat, seed, lignite powder, rice bran, charcoal, etc. For enhanced shelf life of biofertilizer formulation, a combination of these carriers is employed. Likewise, pre-sterilization of carriers is done to enhance the shelf life of microbes (Wani et al. 2013; Liddycoat et al. 2009). Liquid biofertilizer formulation is an important aspect to improve shelf life. These formulations enclose an adequate amount of cell protectants and nutrients that are responsible for the extended shelf life of biofertilizers. Besides, these formulations can endure huge temperature range (Santos et al. 2012; Ruiz-Sanchez et al. 2010).

Biofertilizers got commercialized with the launch of "nitrogen" by Hiltner and Nobbe. This preparation was for legumes. Later microbial inoculants for legumes were made like "Alnit". It proved advantageous for the development of non-leguminous plants. These bacteria were recognized to be local ammonifiers. Discovery of *Azotobacter* and *Clostridium* developed a new field for investigating economical bacterial fertilizers. The rhizosphere of these plants contains a range of species of soil bacteria that enhance plant growth by numerous ways. Such bacteria are jointly known as plant growth-promoting *Rhizobacteria* (PGPR). One of the ways is through fixing of atmospheric nitrogen which enhances the accessibility of exploitable form of nitrogen in the rhizosphere. They also promote symbiosis between plants and microorganisms (Mfilinge and Mtei 2014).

There are diverse modes of interactions between biofertilizers and plants, taking into account the extent of association between microbes with plant roots which are mentioned as follows (Youssef and Eissa 2014):

- Microbes living in the soil near the root, utilizing nitrogen and carbon metabolites leaking from the root (rhizosphere)
- Microbes colonizing the root surface (rhizoplane)
- Microbes colonizing the root tissue inhabiting intercellular spaces (endophytes)
- Microbes living inside cells in specialized root structures or nodules (symbionts)

9.8.1 Types of Biofertilizers

Biofertilizers are categorized into various types on the basis of microorganisms they contain (Chun-Li et al. 2014). The different types of biofertilizers are discussed below:

9.8.1.1 Symbiotic Biofertilizers

Symbiotic microbes infect root tissues and form new structures. In many cases, the application of molecular biology tools allows the discovery of the genes and signals involved in the beneficial interaction between the microorganism and the plant. The main symbiosis relating to agricultural application as biofertilizers is considered below.

9.8.1.2 Rhizobia

Rhizobium is an illustration of a symbiotic association colonizing legume roots and fixes the atmospheric nitrogen. It has a capability to fix atmospheric nitrogen in leguminous and non-leguminous plants. The different genus and species inhabiting legume root nodules are usually referred to as *Rhizobia*. These involve *Alphaproteobacterias*, e.g. *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Azorhizobium*, *Allorhizobium* and *Agrobacterium*, and *Betaproteobacteria*, e.g. *Burkholderia*. The best model describing the interaction between rhizobia and legume roots includes flavonoid/isoflavonoid molecules released by the plants which induce bacterial genes and consequently the synthesis of the lipochitin oligosaccharide (LCO) molecules, which in turn control infection and nodule growth in the root tissue (Rajaram and Apte 2008). Usually, it pierces the root hair and multiplies there in special root structures called root nodules. The quantity of nitrogen fixed depends on host, strain of *Rhizobium* and existing environmental conditions. They are very proficient biofertilizers for legumes as far as the magnitude of nitrogen fixation is concerned. The nod, nif and fix genes control the nodulation and nitrogen fixation by the bacterium (Lavakush et al. 2014).

9.8.1.3 Blue Green Algae

Blue green algae (BGA) are the most ancient organisms possibly the first among those that started evolving oxygen. These appear in numerous shapes (single celled, branched or unbranched with filaments). The majority of them possess special structure called heterocyst whose role is to fix nitrogen. The algae that are frequently applied in fields belong to *Anabaena*, *Nostoc*, *Scytonema*, *Tolypothrix*, etc. (Joseph and Meeks 1987). These are widely used in rice fields (Zhou et al. 1998). BGA secrete numerous growth-promoting substances like amino acids, vitamins, polysaccharides, sugars, etc. which boost the yield of crops (Schiefer et al. 2002; Hussain et al. 2002).

9.8.1.4 Mycorrhiza

Mycorrhiza is the best example of the symbiotic association between fungi and plant roots (higher plants). The fungi enhance the growth of plants and protect them from various stresses. These fungi colonize the root cortex and mycelia of the plants and help them to obtain nutrients from soil. These fungi are cosmopolitan in soil and are seen in the roots of thallophytes, gymnosperms, pteridophytes and angiosperms (Stewart 1980). Plants, on the other hand, protect fungi from root pathogens and also provide them with carbohydrates, hormones, nutrients, etc. The mycorrhizal plants have better forbearance to poisonous metals, salinity, elevated soil temperatures and unfavourable pH. Such plants also resist transplantation shocks. They play a significant task by enhancing growth and nutrient uptake in plants (Vessey 2003).

9.8.1.5 Free-Living or Non-symbiotic Biofertilizers

Since the description of PGPR by Kloepper and Schroth (1978), many different bacteria genera have been described as PGPR: *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Gluconacetobacter*, Herbaspirillum, *Bacillus*, *Burkholderia*, *Erwinia*, *Caulobacter*, *Azotobacter*, *Chromobacterium*, *Serratia*, *Micrococcus*, *Flavobacterium*, *Actinobacteria*, *Enterobacter*, *Arthrobacter*, *Agrobacterium* and *Hyphomicrobium* and fungus such as *Trichoderma*, among others (Gupta 2004).

Many PGPR have been described as endophytic bacteria. It is not clear if the plant growth promotion effects are a consequence of plant-microbe interaction in the external part of the rhizosphere or if an endophytic state is necessary (Hayat et al. 2010). Many different mechanisms have been claimed to be responsible for the plant growth promotion effect after in vitro experiments under controlled conditions. In some cases, the use of appropriate mutants helps in the definition of these mechanisms. But since different mechanisms are always present in a single strain, it is almost impossible to know which are the main mechanisms operating and driving the plant growth promotion. Irrespective of the real mechanisms operating in PGPR with a positive effect in the field, the use of these micro-organisms has dramatically increased in recent years and will probably continue to grow because biofertilizers appear as a valuable opportunity for future sustainable agriculture (Gonzalez et al. 2015). Many commercial products already exist which are based on *Pseudomonas* or *Azospirillum* strains in the market (Yang et al. 2009; Scalenghe et al. 2012). The different mechanisms operating in PGPR can be classified as N_2 (nitrogen) and P (phosphorus) nutrition effects and plant root development and fitness mediated by phytohormones (Fig. 9.5**).**

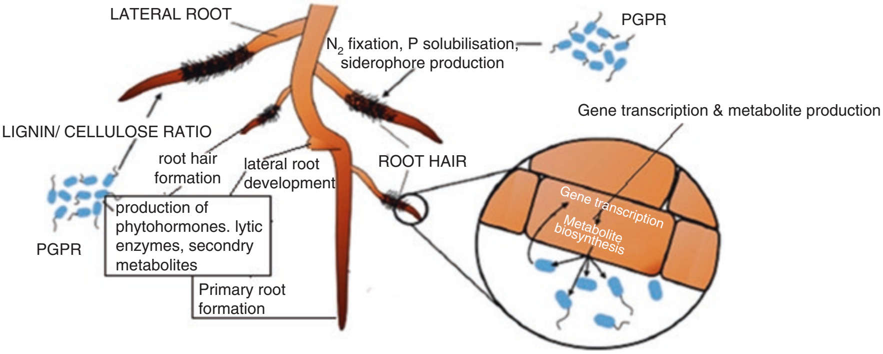

Fig. 9.5 Mode of action of PGPR

9.9 Phytohormone-Mediated Mechanism of Plant Growth-Promoting Microorganisms/Bacteria (PGPB)

One of the most visible effects on plants after inoculation with PGPB is the huge development – and sometimes changes in the architecture – of the root of the plant. This general improvement of root growth, including root-hair development, is one of the characteristic phenotypes of the interaction plant-PGPB.

It is likely that water and mineral uptake is consequently improved because of the increase in the root system, although the specific mechanism is not completely clear. Changes in hormone balance, enhancement of proton-efflux activity and modification in a wide range of related enzymatic activities would be part of the mechanisms behind this phenotype (Backman and Sikora 2008; Joo et al. 2005).

9.9.1 Auxins

The general root improvement phenotype can be reproduced by replacing phytohormones with PGPB. Auxin-related substances, such as indole acetic acid (IAA), appear to be involved in one of the most important mechanisms regarding the general root development improvement. Nevertheless, bacterial production of IAA in plants has not yet been demonstrated. There are no IAA completely deficient mutants, but IAA attenuated mutants were ineffective as PGPB, compared to parental strains (Ahmed and Hasnain 2010; Chen 2006).

9.9.2 Cytokinins

The role of cytokinins in the promotion of root development is not clear, but cytokinin-producing PGPB stimulate nodulation in legumes when co-inoculated with *Rhizobia*. Besides, it has been demonstrated recently that there is a Nod factor-independent mechanism for infection and nodulation, probably mediated by rhizobial cytokinin. This particular area deserves more attention in the future (Riefler et al. 2006; Sokolova et al. 2011).

9.9.3 Ethylene

Ethylene is related to general plant responses when a stress condition appears, even if it is a very low stress situation. When this happens, the plant synthesizes ethylene and stops its growth temporarily. This is because of the regulatory effects of ethylene on different cell functions. 1-aminocyclopropane-1-carboxylate is a precursor of ethylene synthesis. The enzyme ACC deaminase is present in some bacteria which can even use ACC as C (carbon) and N sources. When ACC deaminase is expressed by rhizospheric bacteria, root growth and development are enhanced. It is probably because of the elimination of the inhibitory concentrations of ethylene produced by the plant. This enzyme is not present in every bacteria, and its activity is codified by a single gene acdS. The introduction of this gene from *Pseudomonas putida* into other bacteria species confers plant growth-promoting functions to the recipient bacteria that are absent in the parental strain. This represents a potential biotechnology-based tool to improve microorganisms to be used as biofertilizers (Reid 1981).

9.9.4 Nitric Oxide

Nitric oxide (NO), a plant regulator volatile phytohormone, is also produced by some PGPB. Bacterial nitric oxide is a mediator in IAA-induced root development. NO can also mediate plant growth-promoting action in *Azospirillum brasilense* Sp245 inducing morphological alterations in tomato roots irrespective of the full bacterial capability for IAA biosynthesis (Butterbach-Bahl et al. 2013).

9.9.5 Helper Bacteria

In the studies of plant microbe interaction which induced some kind of plant growth promotion, there are other cases that do not fit into the previous definitions but which can be considered as another kind of biofertilizer. That is the case of bacteria which improve a plant-microbe interaction as a third partner in the interaction. An example can be found in rhizospheric actinomycetes isolated from legumes or actinorhizal nitrogen-fixing nodules which are able to stimulate nodulation, consequently nitrogen fixation in the plant and finally plant growth. This tripartite plant-microbe interaction is not well known in terms of mechanism. However, it clearly shows that biofertilizers can be improved by the use of more than one microorganism at a time (Egamberdiyeva 2007).

9.10 Genetically Modified Microbes as Biofertilizers

Genetically modified organisms (GMOs) symbolize a genetic reserve. Such microorganisms may find a use as donor or recipient of desirable genes. Microorganisms play an important role in various sectors of agriculture, food processing, environmental management and pharmaceutical industries. Genes of microbes can be optimized or improved by means of various genetic modifications using recombinant DNA technology (Tabashnik et al. 2011). Usually, this is dependable on the recognition and selection of the mutants with favourable traits. In numerous cases, the usage of molecular biology tools or recombinant DNA technology allows the discovery of the genes and signals concerned in the advantageous interaction (endophytic, mycorrhizal and diazotrophic) between the microbe and plant (Fig. 9.6). These symbiotic interactions can assist plant growth and development through nitrogen uptake, siderophore production, phosphate solubilization, etc. (Ritika and Uptal 2014).

Recombinant biotechnology offers an advantage to decrease the employment of synthetic fertilizers. Biofertilizer technology has significantly developed in the market. The nature of multiple mechanisms discovered for PGPR actions and the option of genetically modifying a specific strain relating to a particular plant growth-promoting activity imply that the use of genetically modified organisms like biofertilizers will be an area of diverse potential in the coming times (Tabashnik et al. 2011). Further, the knowledge of microbial ecology and its dynamics will surely enhance the biofertilizer technology. Microbes are particularly targeted for genetic improvement since they are given huge importance in modern agriculture as they are used as biofertilizers. Biofertilizers represent an alternative to synthetic fertilizers which are facing lots of disparagement due to their negative impact on the ecology and human well-being. There is an important requirement to build up eco-friendly control using existing microbes. Such microbes would offer protection to plants against pathogens and would be economical, reliable and effective (Pishchik et al. 2002). To obtain this target, better-quality strains are needed. Thus, genetically modified microbes could be used for this purpose. Efforts are in progress to formulate proficient biofertilizers compatible with a broad choice of plants and soil by means of genetically engineered techniques. For example, biofertilizers have been formulated based on nitrogen-fixing rhizobial bacteria occurring naturally in the nodules of leguminous plants. Nevertheless, these microbes are not competent

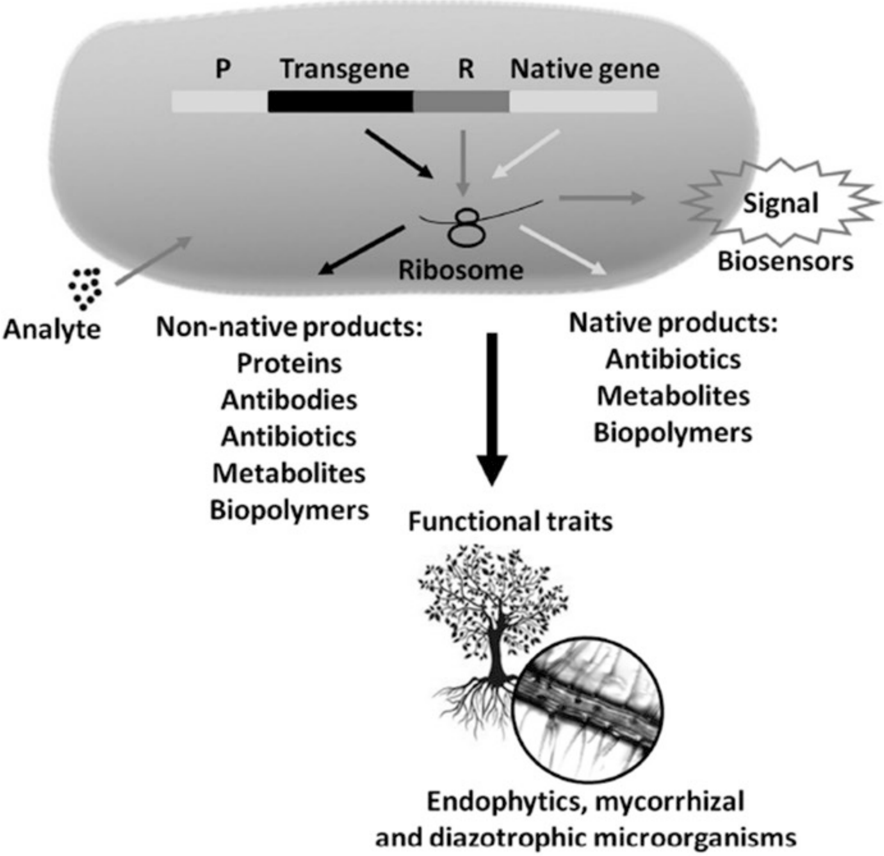

Fig. 9.6 The use of recombinant DNA technology in the generation of products assisting in the symbiotic interactions. (Adapted from Vitorino and Bessa 2017)

enough to supply nitrogen to non-legumes. In such cases, genetic engineering is of special importance, as it assists in the development of efficient delivery systems. In this way non-legumes could be grown together with symbiotic rhizobial root nodules devoid of externally applied nitrogen fertilizers (Aloni et al. 2006; Ruiz-Sanchez et al. 2010). The foreign genes used for transforming microbes could be integrated into the host genome or plasmid. To express a heterologous gene in bacteria and fungi, the regulatory area of this gene should be modified in promoter and terminator regions in order to optimize the function of the inserted gene in the new host. Adding specific genes which can bestow biocontrol ability could improve the biocontrol ability of microbes lacking such genes (Dash et al. 2016). For example, many *Rhizobacteria* with biocontrol activity produce chitinases. However, few *Rhizobacteria* like *Rhizobium meliloti* and *Pseudomonas putida*, both of which are outstanding root colonizers, are deficient in synthesizing chitinase (Bagwan et al.

2010). Incorporation of chitinase gene into their genome has enabled them to defend the plant against fungi.

Nitrogen-fixing property of *Rhizobium* inoculants could be augmented by means of genetic engineering tools. An additional way is by planting the crops that use nitrogen more proficiently. An example of such crops is genetically modified Canola which exhibits a noteworthy decline in the amount of nitrogen fertilizer that is leached into soil or lost into the atmosphere, and hence it improves the economies of farmers through the enhanced profitability. Moreover, biofertilizers when formulated by means of molecular biotechnology can improve the biological pathways of production of phytohormones like auxin, cytokinin, etc. which assist in plant growth and development (Nautiyal et al. 2008). Similarly, many pseudomonads in the rhizosphere manufacture siderophores which can chelate iron ions and thus escalate iron uptake by the plants. The genetically modified strain (RMBPC-2) of *Sinorhizobium meliloti* has added genes that control nitrogenase enzyme from the plant to the bacterium (Boccia and Sarnacchiaro 2015). Likewise, Trichoderma species are extensively found in the soil and are antagonistic to other fungi. *Trichoderma harzianum* is an efficient rhizosphere colonizer and is able to parasitize pathogenic fungi. Many extracellular enzymes like glucanases, chitinases, lipases and proteases synthesized by *Trichoderma* species have been improved with the transfer of chitinase genes, notably from *Serratia marcescens* (Awais et al. 2010). Thus, such genetically modified strains could act as efficient biofertilizers and will aid in crop improvement.

9.11 Conclusion

Our reliance on chemical fertilizers has encouraged the flourishing of factories or industries that are generating lethal chemicals that are not only dangerous for human utilization but can also perturb the normal environmental equilibrium. Now, attention is diverting towards consuming food grown with organic fertilizers than with chemical fertilizers (Leonardo et al. 2006). Biofertilizers can assist in solving the problem of food crises of the ever-rising worldwide population. It is essential to recognize the positive aspects of biofertilizers so as to apply it to modern agriculture. The employment of biofertilizers containing advantageous microbes improves the crop productivity to a larger extent (Kanchiswamy et al. 2015). Biofertilizers play an important role in maintenance of soil quality. This would in turn protect the environment and would require less expenditure. Besides, biofertilizers when formulated using the tools of molecular biotechnology can improve the biological pathways of production of plant growth-promoting substances, if identified and transferred to the useful plant growth-promoting microbes (Goswami et al. 2014). Recombinant biotechnology offers numerous advantages in this area, as particular metabolic processes could be tackled with additional accuracy, and entirely novel functions could be introduced in microbes.

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Chapter 10
Root-Associated Ectomycorrhizal Mycobionts as Forest Biofertilizers: Standardized Molecular Methods for Characterization of Ectomycorrhizal Wood Wide Web

Rezwana Assad, Zafar Ahmad Reshi, and Irfan Rashid

Abstract Ectomycorrhizal (ECM) fungi play a crucial role in nutrient mobilization and cycling, particularly in temperate forests dominated by coniferous species. The belowground ectomycorrhizal Wood Wide Web interconnects innumerable host plants and serves as a sustainable continuum for plant and soil health in forest ecosystems. Conifers, particularly conifer roots harbouring ectomycorrhizal fungi, are rich in phenolics and other secondary metabolites, which interfere and hamper their DNA extraction and inhibit all downstream processes like amplification and sequencing. The present study was projected for presenting the standardized molecular methodology for characterization of ectomycorrhizal fungi from conifer roots, starting from extraction of high-quality DNA and its PCR amplification, followed by DNA purification and loading, to final sequencing, all things reflected in a chronological manner. This chapter highlights the role of root-associated ectomycorrhizal fungi as biofertilizers in forest ecosystems and efficient molecular methods specially optimized for characterization of ectomycorrhizal fungi associated with conifers.

Keywords Biofertilizers · Ectomycorrhiza · Forestry · Microbiota · Molecular characterization · Soil health

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10.1 Introduction

Belowground microbiota is an imperative constituent of forest ecosystems. The rhizospheric microbiota comprises of diverse microorganisms including actinomycetes, algae, archaea, bacteria, fungi, protozoa, and viruses (Tarkka et al. 2018; Adeleke et al. 2019). Amongst these ecologically important communities, ectomycorrhizal (ECM) fungi are essential acquaintances of this intricate forest microbiota that function as biological linkages between diverse assemblage of forest organisms and serve as a sustainable continuum for plant and soil health in forest ecosystems (Molina 1994). Root-associated ECM fungi are a ubiquitous group of microorganisms that correlate plants through a huge belowground hyphal network, which facilitate interplant metabolite passage (Chatterjee et al. 2019; Domínguez-Núñez et al. 2019). This belowground diverse mycorrhizal network that interconnects rootlets of innumerable plants has been named as "common mycorrhizal networks (CMN)" or "Wood Wide Web" (Giovannetti et al. 2006; Simard 2012; Martin et al. 2016; Adeleke et al. 2019). ECM fungi through these hyphal networks enhance nutrient acquisition capability of host plants by extending their root surface area and facilitate interplant communications (Bücking et al. 2012; Adeleke et al. 2019).

The species-level identification of individual ECM mycobionts is a prerequisite for understanding the ecological significance of this symbiotic association (Gil-Martínez et al. 2018). However, these plant-fungal species interactions are poorly studied, primarily because of methodological limitations to the accurate identification of these mutualists. The study of taxonomy and structural and functional diversity of ectomycorrhizal fungi has proven reasonably exigent (Gil-Martínez et al. 2018). Isolation of DNA from ectomycorrhizal roots is intricate due to the presence of tough chitin cell wall, co-occurrence of host plant cells along with fungal cells, and co-precipitation of secondary metabolites of host plant which obstruct downstream processes like PCR and sequencing (Janowski et al. 2019).

Initially, ECM diversity studies were primarily based on sporocarp analysis (Horton and Bruns 2001; Domínguez-Núñez and Albanesi 2019). However, in view of the poor correspondence between the diversity of ECM based on survey of aboveground ECM fruiting bodies and ECM actually colonizing roots of host species, sampling and screening of conifer roots for associated ECM fungi employing morpho-anatomical and standard molecular methods was undertaken. Furthermore, recent advancements in molecular research and DNA sequencing technologies have made exceptional contribution to our understanding of ECM fungal diversity, ecology, and biogeography (Horton and Bruns 2001; Nilsson et al. 2011; Smith and Peay 2014; Domínguez-Núñez and Albanesi 2019; Janowski et al. 2019).

This chapter highlights the role of root-associated ectomycorrhizal fungi as biofertilizers in forest ecosystems and efficient molecular methods for characterization of ectomycorrhizal fungi associated with conifers. Moreover, this chapter also divulges a comprehensive deliberation of chemical concentrations, their preparations, and corporations from which these chemicals were procured.

10.2 Root-Associated Ectomycorrhizal Fungi as Forest Biofertilizers

Biofertilizers encompass a live formulation of beneficial microbes which, on application to soils, plant surfaces, or seeds, colonize rhizosphere or plant interior and elevate growth by escalating the supply or accessibility of crucial nutrients to the host plant (Vessey 2003; Malusá et al. 2012; Mahanty et al. 2017; Thomas and Singh 2019). *Amanita* spp., *Hebeloma* spp., *Laccaria* spp., *Pisolithus tinctorius*, *Piriformospora indica*, *Rhizopogon luteolus*, *Suillus luteus*, and *Tuber melanosporum* are some ECM fungi that have been used as forest biofertilizers (Marx and Cordell 1989; Domínguez et al. 2006; Schwartz et al. 2006; Chavez et al. 2014; Pal et al. 2015; Sharma 2017; Domínguez-Núñez and Albanesi 2019; Domínguez-Núñez et al. 2019). ECM fungal biofertilizers serve as a natural, effective, economic, non-bulky, productive, and eco-friendly substitute of synthetic chemical fertilizers and pesticides (Pal et al. 2015; Bhat et al. 2017; Mahanty et al. 2017; Thomas and Singh 2019).

In forest ecosystems, ectomycorrhizal fungi are directly involved in nutrient cycling (Domínguez-Núñez et al. 2019). One of the most effective forest management strategies for regeneration and reinstatement of degraded forest ecosystems is the use of ECM fungi as promising biofertilizers to improve survival, growth, health, and establishment of seedlings (McAfee and Fortin 1986; Adeleke et al. 2019).

Ectomycorrhizal biofertilizers boost plant health and concurrently improve sustainability and health of the soil (Bhardwaj et al. 2014; Nuti and Giovannetti 2015; Pal et al. 2015; Bhat et al. 2017; Vecstaudža et al. 2018; Chatterjee et al. 2019), through processes like solubilization/mobilization of soil nutrients (mainly phosphorus and nitrogen), escalation of long-term soil fertility and soil aeration, mounting efficient uptake of water and nutrients by increasing surface area of host plant roots, repression of soil borne diseases, and production of plant growth-promoting substances into the soil (Mridha 2003; Malusá et al. 2012; Nuti and Giovannetti 2015; Pal et al. 2015; Mahanty et al. 2017; Frąc et al. 2018; Chatterjee et al. 2019; Thomas and Singh 2019).

The common ECM species associated with different coniferous hosts can be utilized for mass production of inoculum for in vitro mycorrhization of conifer seedlings in forest nurseries. This in vitro mycorrhization of tree seedlings has become the essential ingredient of successful reforestation programme because anthropogenic activities, like deforestation, urbanization, altered land-use pattern, pollution, etc., severely impair the ECM diversity in soil, which results in reduced natural regeneration.

Main sources of ectomycorrhizal inoculum are forest soil, chopped ECM roots, spores of fruiting bodies, and pure mycelial inoculum (Sim and Eom 2006; Domínguez-Núñez et al. 2019). Several biofertilizers consist of single ECM strain; however, numerous strains in the form of consortium have been also employed, which promote plant growth through diverse mechanisms (Pal et al. 2015; Vecstaudža et al. 2018). However, the appropriate choice of apposite host-mycobiont is indispensable for the success of mycorrhization (Olivier 2000).

Various polymicrobial formulations like mycorrhizal helper bacteria (MHB) and other beneficial rhizospheric microbes have the capability to facilitate ectomycorrhiza formation and act in synchrony with ectomycorrhizal symbionts (Saravanan and Natarajan 1996, 2000; Schrey et al. 2012; Chatterjee et al. 2019; Domínguez-Núñez et al. 2019). A better understanding of mycorrhizospheric microbiota is crucial for comprehending the functional aspect of these symbionts for their exploitation in sustainable forest management and environmental protection (Tarkka et al. 2018; Janowski et al. 2019).

10.3 Standardized Molecular Methods for Characterization of Ectomycorrhizal Wood Wide Web

10.3.1 Sampling of ECM Root Tips

ECM root samples of various conifer species like *Abies pindrow* (Royle ex D. Don) Royle, *Cedrus deodara* (Roxb. ex D. Don) G. Don, and *Picea smithiana* (Wall.) Boiss. were collected from Naranag, Ganderbal province (N34° 22′ 16″; E74° 59′ 45″), of Kashmir Himalaya, for molecular characterization of associated ECM diversity. A spade was used to collect soil cores, starting from the organic layer, after removal of litter layer. All the samples were collected in sealed polybags and were stored at 4 °C before being processed, but were not held longer than 2 weeks.

Prior to analysis, the soil cores were immersed in water and soaked carefully in water until saturated. Fine ECM-infected root tips (short lateral roots) were gently rinsed under a cold running water in a 1 mm sieve to limit damage to the ectomycorrhizas. Cleaned lateral roots were placed in a Petri dish containing double distilled water, to avoid drying up of delicate root tips (Fig. 10.1). Fine root tips were carefully sorted from the main roots and processed for isolation of fungal DNA.

10.3.2 Scanning Electron Microscope and Compound Microscope Study of Ectomycorrhizal Roots

To observe surface features of ectomycorrhizal roots with scanning electron microscope (SEM), tertiary roots of the studied species (showing a well-developed mycorrhizal sheath) were excised and prepared for SEM study by the method of Kinden and Brown (1975) and Chung et al. (2003).

The ectomycorrhizal roots were rinsed twice with 0.1 M sodium phosphate buffer solution (pH 7.3) and fixed in 2.5% glutaraldehyde solution at 4 °C for 2 h, then washed it off with 0.1 M sodium phosphate buffer solution (pH 7.3), and post fixed

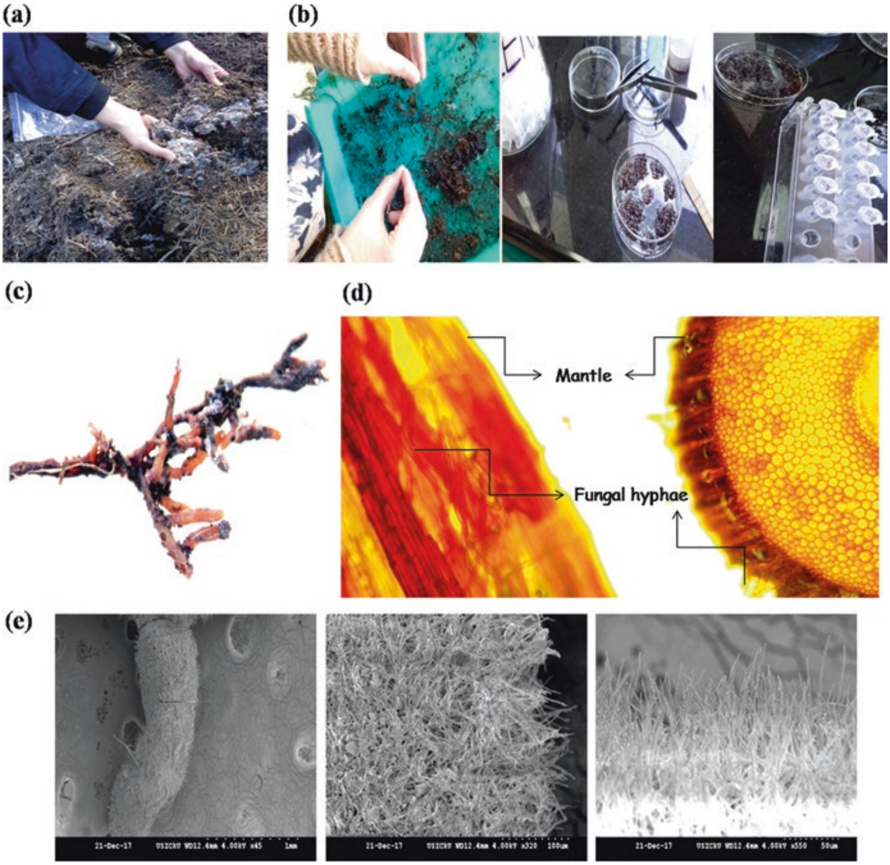

Fig. 10.1 (**a**) White-coloured ECM mycelium in forest soil, (**b**) processing conifer ECM root material in laboratory, (**c**) association of ectomycorrhizal fungi with conifer roots, (**d**) mantle peel with fungal hyphae and transverse section of ectomycorrhizal root showing distinct mantle and emanating hyphae, (**e**) scanning electron micrographs showing external surface features of ectomycorrhizal roots with projected fungal hyphae

the sample with 1% osmium tetroxide (1% OsO_4 in 0.1 M cacodylate buffer). Post-fixed specimens were rinsed thrice with 0.1 M sodium phosphate buffer solution (pH 7.3) for around 40 min. These samples were then dehydrated with 50%, 70%, 80%, 90%, 95%, and 100% ethanol for 20 min, followed by treatment with 100% isoamyl acetate for 40 min. Subsequently, these samples were dried up. After attaching the carbon tape on aluminium stub, these samples were pasted carefully on it and were coated with gold using a sputter coater and were then observed with SEM (Hitachi S-3000H).

The morpho-anatomical structural details of mantle, Hartig net, and emanating hyphae of ECM roots were observed via mantle peels and thin hand-cut transverse sections of ectomycorrhizal root. These sections were gently cleared in hot alkali

(10% KOH), stained overnight with Aniline blue (0.5%) or Trypan blue (0.5%), followed by destaining with 10% lactic acid. These were then examined at 4x, 10x, and 40x magnification under a compound microscope (Magnus MLX LED).

10.3.3 Molecular Methods for Characterization of Ectomycorrhizal Fungi

Molecular characterization of root-associated ECM was done by extracting genomic DNA from the root tips of the studied species, followed by the amplification of the ITS region using universal primers and universal fungal-specific primers. The amplified products were sent for sequencing. The sequences were then identified by performing BLAST searches on GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and UNITE (https://unite.ut.ee/).

10.3.3.1 Protocol for ECM Root Tip DNA Extraction

Modified CTAB (cetyltrimethylammonium bromide) protocol was employed for DNA extraction of ectomycorrhizal roots of selected conifer species:

(i) **Mechanical lysis:** 2–3 frozen root tips were taken in a 2 ml microcentrifuge tube (having a flat base). This material was crushed by a sterilized cold Micro Pestle (made of polypropylene, designed for crushing a material inside a microcentrifuge tube), without using liquid nitrogen.

(ii) **Chemical lysis:** 1 ml prewarmed isolation buffer (freshly prepared) was poured immediately into each microcentrifuge tube and mixed well. The tissue was completely homogenized in buffer. (Optional step: Samples can be pulverized further after addition of isolation buffer.) The composition of isolation buffer is given in Table 10.1.

Table 10.1 Composition of isolation buffer

S. No.	Stock	Working (for five reactions)
1.	CTAB (10%)	1 ml
2.	NaCl (5 M)	1.4 ml
3.	EDTA (0.5 M) (pH = 8)	0.2 ml (200 μl)
4.	Tris-HCl (1 M) (pH = 8)	0.5 ml (500 μl)
5.	β-Mercaptoethanol (2-mercaptoethanol) (99.0%)	0.005 ml (5 μl)
6.	Double distilled water	To make the volume up to 5 ml (like 1.9 ml was added)
7.	PVP (1%) or powdered – optional	–

All the reagents of isolation buffer and other requisite material like microcentrifuge tubes, tips, etc. were autoclaved, prior to use. Use of prewarmed and freshly prepared isolation buffer provided better results. Preparation and concentration details of CTAB reagents are stated in the supplementary material.

(iii) **Incubation:** The samples were then incubated at 65 °C for 1 h (in a water bath) with occasional mixing by gentle inversion of the microcentrifuge tubes.

(iv) **DNA purification:** For purification purposes, the tubes were centrifuged at 12,000 RPM for 10 min, and the supernatant (upper layer) was collected in a fresh microcentrifuge tube. The cell debris was discarded along with the tube.

(iv-a) **Protein denaturation and removal step:** Equal amount of chloroform:isoamyl alcohol (CI-Mix) (24:1, v/v) was added to each tube (like 500 μl CI-Mix was added to the 500 μl collected supernatant). The mixture was emulsified by inversion of the tube. The mixture was then centrifuged at 12,000 RPM for 5 min, and the upper layer was pipetted out to a fresh tube. Repeat CI treatment if samples seem unclean. (The upper aqueous phase must be taken off carefully without touching the lower layer or else the DNA will get contaminated; furthermore, wide-bore tips should be used in this step; otherwise, the DNA will get sheared.)

[Optional step: Chloroform- or Tris-saturated phenol:chloroform:isoamyl alcohol (PCI-Mix) (25:24:1, v/v) can be used instead of CI-Mix.]

(iv-b) **RNA removal step:** For removal of RNA, 3–4 μl of RNase A (Sigma or Qiagen) was added to each tube, followed by incubation at 37 °C for 1 h (in an incubator). The tubes were occasionally mixed by gentle inversion. To remove additional unused RNase, 500 μl of chloroform:isoamyl alcohol was added and mixed by inversion. Then the samples were centrifuged again at 12,000 RPM for 5 min. The supernatant (upper phase) was collected in a fresh tube.

(v) **DNA precipitation:** The DNA was precipitated in these tubes by adding 750 μl (or two-third volume) of ice-cold isopropanol. These tubes were gently inverted for proper mixing and precipitation of DNA and were then kept overnight at −20 °C in a deep freezer. These samples were then centrifuged at 13,000 RPM for 20 min. The upper phase (most of the supernatant) was discarded by pouring, and the pellet was collected. Nucleic acid concentrations are minute and transparent in nature, so even if there is not any noticeable pellet in the tube, DNA can be still there. (The samples can be even kept for only 1 h in a deep freezer; increase the time according to the quantity of pellet required; however, keeping tubes for longer duration for DNA precipitation also co-precipitates impurities and inhibitors with it, which further hinder the downstream processes.)

(vi) **DNA washing:** The pellet was washed with 200 μl of 70% ice-cold ethanol and was centrifuged at 8000 RPM for 5 min. The upper phase was discarded, and the pellet was dried in an oven at 37 °C in an incubator (do not overdry the pellet; otherwise, it will not get dissolved).

(vii) **DNA pellet solubilization:** The dried pellet was dissolved in 10–20 μl nuclease-free water. The samples were kept at 4 °C for an hour or so, for proper DNA pellet solubilization. These samples were then stored at −20 °C until further use.

10.3.3.2 Polymerase Chain Reaction (PCR) Protocol

The fungal ITS region of rDNA was amplified by polymerase chain reaction (PCR) by using gene-specific primers (ITS1/ITS2; ITS3/ITS4; ITS1/ITS4; ITS1F/ITS4) in a thermal cycler (Table 10.2). The amplified fragments include ITS1, 5.8S, and the ITS2 of rDNA.

The 20 μl reaction mixture for each PCR reaction contained 2 μl PCR buffer (with $MgCl_2$), 0.5 μl of 10 mM dNTPs, 0.4 μl of each primer (10 μM), 2 μl template DNA, 0.5 μl of 5% DMSO, 0.5 μl of 0.1% BSA, and 0.2 μl of 5 U/μl Taq polymerase (Table 10.3). Preparing master mix is more convenient and time-saving as compared to setting up each reaction independently.

Amplifications were performed in a thermal cycler (Applied Biosystems) with an initial denaturation step of 95 °C for 10 min, followed by 35 cycles of 95 °C for 1 min, 55 °C for 30 s, and 72 °C for 1 min, and a final extension of 72 °C for 10 min. A negative control reaction was performed with same reaction components and conditions except that nuclease-free water was added instead of DNA template. PCR amplification was confirmed on 1.5% agarose gel. Preparation of PCR reagents is reflected in the supplementary material.

10.3.3.3 Agarose Gel Electrophoresis

The integrity of DNA was checked on 0.8% agarose gels (for genomic DNA) or 1.5% agarose gels (for PCR amplicons). Electrophoresis was performed at room temperature in 1X TAE buffer with an applied voltage of 8–10 V cm^{-1}. The compo-

Table 10.2 Primers used for amplification of ITS region

Forward primer	Reverse primer	Tm (°C)	GC (%)
TCCGTAGGTGAACCTGCGG	GCTGCGTTCTTCATCGATGC	**ITS1:** 68.5	**ITS1:** 63.1
(ITS1)	**(ITS2)**	**ITS2:** 68.2	**ITS2:** 55
GCATCGATGAAGAACGCAGC	TCCTCCGCTTATTGATATGC	**ITS3:** 68.2	**ITS3:** 55
(ITS3)	**(ITS4)**	**ITS4:** 61.5	**ITS4:** 45
TCCGTAGGTGAACCTGCGG	TCCTCCGCTTATTGATATGC	**ITS1:** 68.5	**ITS1:** 63.1
(ITS1)	**(ITS4)**	**ITS4:** 61.5	**ITS4:** 45
CTTGGTCATTTAGAGGAAGTAA	TCCTCCGCTTATTGATATGC	**ITS1F:** 56.6	**ITS1F:** 36.3
(ITS1F)	**(ITS4)**	**ITS4:** 61.5	**ITS4:** 45

Table 10.3 Composition of 20 µl PCR reaction

Component	Basic concentration	Volume
PCR buffer	10X	2 µl
dNTPs	10 mM	0.5 µl
Forward primer	10 µM	0.4 µl
Reverse primer	10 µM	0.4 µl
Taq polymerase	5 U/µl	0.2 µl
Template DNA	10 µg	2 µl
DMSO	5%	0.5 µl
BSA	0.1%	0.5 µl
Nuclease-free water		To 20 µl

Note: Using concentrated DNA template with high DNA content hindered the PCR. So, in order to overcome this, DNA template was diluted by nuclease-free water up to 1:10 (1 µl DNA:9 µl water); 1:100 (1 µl DNA:99 µl water), or even 1:1000 (1 µl DNA:999 µl water)

Table 10.4 Composition of loading dye and TAE buffer

Buffer	Composition
6X DNA loading dye	Glycerol
	Bromophenol blue
	Xylene cyanol
1X TAE buffer	Tris base
	Glacial acetic acid
	EDTA

sition of loading dye and TAE buffer are given in Table 10.4. The gels were visualized on a gel imager after ethidium bromide (0.5 µg ml^{-1}) staining.

10.3.3.4 Purification of PCR Products

PCR products were purified prior to further downstream analysis using Gene-Jet Gel Extraction Kit (Thermo Fisher Scientific; Cat No. K0691), following manufacturer's instructions. PCR products were resolved on 1.5% agarose gel, and each of the separating bands was cut and placed in separate 1.5 ml Eppendorf tubes. The gel solubilization buffer was added to gel pieces and incubated at 50 °C for 10 min, till the gel dissolved completely. The mixture was then passed through a binding column after addition of 1 volume isopropanol. After proper washing, samples were eluted with elution buffer and stored at −20 °C until use.

10.3.3.5 Sequence Analysis and Identification of ECM Species

The purified PCR products were sent for sequencing (in both directions with respective primers) to Xcelris, Gujarat, India. Each sequence was then separately identified by performing individual nucleotide BLAST searches on GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and UNITE (https://unite.ut.ee/).

10.4 Findings and Inferences

10.4.1 Morpho-Anatomical Characteristics of ECM Roots

ECM roots are characterized by the incidence of mantle, Hartig net, and extraradicular mycelium. The surface of these ECM root tips was found to be harbouring fine fungal hyphae. ECM fungi grow and extend their hyphal network (mycelial strands or rhizomorphs) into the rhizospheric soil, forming dense mycorrhizal mycelial mats with specialized conducting hyphae (Fig. 10.1). The major distinction between mycorrhizal and dead roots is that mycorrhizal roots are living, coloured, and swollen with a growing mycelium attached to the surface whereas the dead roots are shrivelled and black (Fig. 10.1).

10.4.2 Molecular Characterization of Ectomycorrhizal Fungi

10.4.2.1 Analysis of ECM Root Tip Genomic DNA

The purity and integrity of ECM root tip genomic DNA was checked on 0.8% agarose gel (Fig. 10.2). The DNA quality and quantity was determined by measuring the optical density (OD) at 260/280 nm on spectrophotometer, and samples with OD ranging between 1.7 and 1.9 were selected for PCR amplification of ITS region.

10.4.2.2 PCR Amplification Analysis

The purity and integrity of amplified DNA was checked on 1.5% agarose gel (Fig. 10.3). The amplicons ranged between 250 and 300 bp for ITS1/ITS2; 350 and 450 bp for ITS3/ITS4; 600 and 755 bp for ITS1/ITS4, and 700 and 800 bp for ITS1F/ITS4 primer combinations (Table 10.5). Negative control in which nuclease-

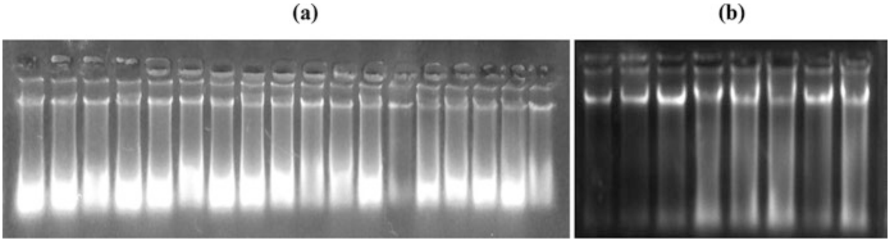

Fig. 10.2 Representative gel for ECM root tip genomic DNA loaded on 0.8% agarose gel. DNA extracted by (**a**) CTAB method (with RNA) and (**b**) CTAB method (without RNA after RNase treatment)

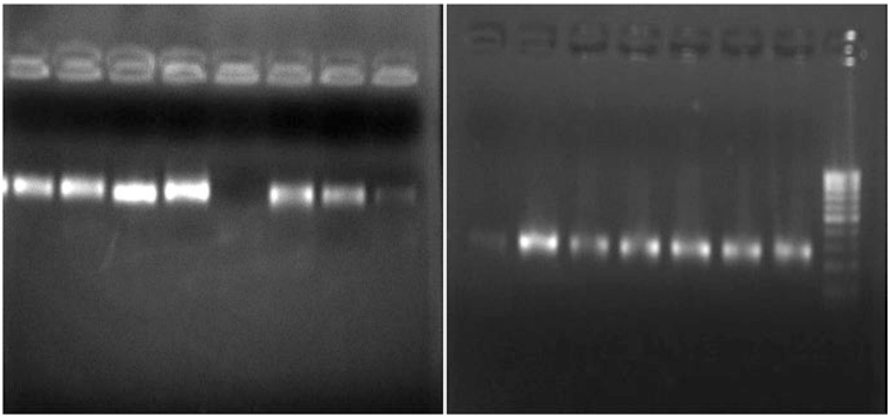

Fig. 10.3 Representative gel for PCR-amplified DNA loaded on 1.5% agarose gel with 100 bp ladder

Table 10.5 Amplicon size obtained using various primer combinations

S. No.	Primer combination	Amplicon size (in bp)	Annealing temperature (°C)
1.	ITS1/ITS2	250–300	55
2.	ITS3/ITS4	350–450	55
3.	ITS1/ITS4	600–755	55
4.	ITS1F/ITS4	700–800	53/55

free water was used instead of DNA template showed no band. Each band was then gel purified for further downstream analysis.

10.4.2.3 Role of Dimethyl Sulphoxide (DMSO)

Various enhancing agents can be employed in PCR reactions to increase specificity and yield. One of those enhancing agents is dimethyl sulphoxide (DMSO), which is used in PCR to interrupt the formation of secondary structures in DNA template or in DNA primers. DMSO formulates hydrogen bond with template and consequently distorts the double helix structure of DNA. This is predominantly useful in templates with high GC content because augmented hydrogen bond strength enhances intricacy of denaturing template and leads to the formation of intermolecular secondary structures, which then compete with primer annealing (Chakrabarti and Schutt 2001). Moreover, DMSO decreases melting temperature needed for separation of both strands of DNA. Thus, addition of DMSO can significantly improve specificities of PCR priming reactions (Frackman et al. 1998; Hardjasa et al. 2010; Jensen et al. 2010).

10.4.2.4 Role of Bovine Serum Albumin (BSA)

Bovine serum albumin (BSA) facilitates specific fragment amplification (Nagai et al. 1998) and enhances PCR amplification yields from low purity templates (Farell and Alexandre 2012). Addition of BSA improves yield of PCR product, by binding fatty acids and phenolic compounds that can inhibit PCR reaction. It also checks adhesion of various PCR reagents to tip and tube surfaces.

10.4.2.5 Why DNA Template Dilutions?

DNA template was diluted by nuclease-free water to 1:10 (1 μl DNA:9 μl water); 1:100 (1 μl DNA:99 μl water), or even 1:1000 (1 μl DNA:999 μl water). Using concentrated DNA template with high DNA content hindered PCR. One of the reasons of diluting DNA template before PCR is to counteract the effect of inhibitors (less DNA: less inhibitors). In addition to this, by limiting the quantity of template, it confines non-specific binding of primers and thus results in the formation of only one specific PCR band.

10.4.2.6 Sequence Analysis and Identification of ECM Species

The sequences were identified by performing BLAST searches on GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and UNITE (https://unite.ut.ee/) (details in the supplementary material). The outputs from the BLAST searches were sorted on account of the maximum identity and were recorded according to their coverage. Sequence similarity with a cut-off of 90% or greater was considered significant, while others which showed similarity of 80–90% were considered as low confidence score species.

10.4.2.7 Advanced Techniques for the Study of Ectomycorrhizal Microbiome

The Sanger sequencing resulted in the identification of only one fungal species per sample (the most abundant one). The conifer root samples are categorized as environmental samples (contain a mixture of fungi).

Amongst different techniques like cloning, terminal restriction fragment length polymorphisms (T-RFLP), single-strand conformation polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), and next-generation sequencing (NGS) for sequencing of environmental samples, NGS is the most advanced, accurate, and reliable technique. Globally, metagenomic NGS techniques like Illumina sequencing technology are apposite techniques for the study of samples taken directly from diverse environments and plant-associated ectomycorrhizal communities.

10.5 Conclusions

Root-associated ECM fungi, informally named as "Wood Wide Web", are a ubiquitous group of microorganisms that correlate plants through a huge belowground hyphal network and serve as sustainable continuum for plant and soil health in forest ecosystems. Ectomycorrhizal fungi like *Amanita* spp., *Hebeloma* spp., *Laccaria* spp., *Pisolithus tinctorius*, *Piriformospora indica*, *Rhizopogon luteolus*, *Suillus luteus*, and *Tuber melanosporum* serve as a natural, effective, economic, and eco-friendly substitute of synthetic chemical fertilizers and pesticides. One of the most effective forest management strategies for regeneration and reinstatement of degraded forest ecosystems is the use of ECM fungi as promising biofertilizers, to improve survival, growth, health, and establishment of seedlings. Ectomycorrhizal biofertilizers boost plant and soil health through processes like mobilization of soil nutrients, escalation of soil fertility and aeration, and mounting efficient acquisition of water and nutrients by increasing the surface area of host plant roots. However, the appropriate choice of apposite host-mycobiont is indispensable for the mycorrhization success.

The species-level identification of individual ECM mycobionts is a prerequisite for understanding the ecological significance of this symbiotic association. However, the study of taxonomy and structural and functional diversity of ectomycorrhizal fungi has proven reasonably exigent. Subsequently, sampling and screening of conifer roots for associated ECM fungi employing morpho-anatomical and standard molecular methods was undertaken in the present study.

This chapter highlights the role of root-associated ectomycorrhizal fungi as biofertilizers in forest ecosystems and efficient molecular methods specially optimized for characterization of ectomycorrhizal fungi associated with conifers. A better understanding of mycorrhizospheric microbiota is crucial for comprehending the functional aspect of these symbionts for their exploitation in sustainable forest management and environmental protection.

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Conflict of Interest The authors declare that they have no conflict of interest.

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Chapter 11
Plant Growth-Promoting Rhizobacteria (PGPR) as Biofertilizers and Biopesticides

Umair Riaz, Ghulam Murtaza, Wajiha Anum, Tayyaba Samreen, Muhammad Sarfraz, and Muhammad Zulqernain Nazir

Abstract Plant growth-promoting rhizobacteria (PGPR) play an important role in sustainable agriculture through the improvement of plant growth via different processes like biological nitrogen fixation, phosphate solubilization, siderophore production, and phytohormone synthesis. The use of PGPR is potentially increased in sustainable farming due to its ecofriendly and efficient nature. It is being used as an alternative source to minimize the increasing use of synthetic fertilizers and pesticides. Biofertilizers are the substances containing living microbes, helping to improve plant growth and development. These living microorganisms enhance the nutrient status of soil through the expansion of root surface area, nitrogen fixation, phosphate solubilization, and combination of all these mechanisms. The market of the biofertilizers is expected to reach 3.8$ billion by 2025 from 2$ billion in 2019. Some *Pseudomonas* species also improve the plant growth through the production of water-soluble vitamins like niacin. PGPR have the potential to work as phytostimulators through the production of various phytohormones like indole acetic acid (IAA), cytokinin, gibberellins, and ethylene. But some bacteria and fungi have ability to improve plant growth by restricting the growth of plant pathogens are known as biopesticides. Cyanide biosynthesis, siderophore production, and induction of systemic resistance genes in plants are the different mechanisms for the PGPR to work against the plant pathogens. PGPR can also work as biocontrol agents providing protection to the plants, enhancing the plant growth through the synthesis of antibiotics. The use of the biopesticides is increasing slowly at a rate of 8% annually based on the different types of microbial pesticides.

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11.1 Introduction

Plant growth-promoting rhizobacteria (PGPR) are the beneficial crowd of rhizosphere microorganisms that can increase the plant growth through various processes such as nutrient uptake, synthesis of siderophore, phytohormone synthesis, N_2 fixation by the living organisms, solubilization of insoluble phosphorous, introduction of systemic tolerance genes, synthesis of ACC deaminase, various volatile organic compounds (VOC), etc. The PGPR utilization is potentially increased in sustainable farming because of its eco-friendly and practical nature to substitute the increasing usage of synthetic nutrients and insecticides. Rhizobacteria produce a large number of substances that affect plant growth promotion through a direct or indirect way. The use of commercial biofertilizers containing best PGPR strains is increasing rapidly, and for this reason, the importance for the search of PGPRs and their mode of action is increasing day by day (Bhattacharyya and Jha 2012). PGPR can be divided into three categories as biopesticides, biofertilizers, and phytostimulators (Table 11.1).

Table 11.1 Types of PGPR and mode of working promoting crop growth and development

Types of PGPR	Definition	Mechanism of action	References
Phytostimulator	Microorganisms having an ability to make phytohormone like IAA and ethylene, etc.	Synthesis of phytohormones	Lugtenberg et al. (2002)
			Somers et al. (2004)
Bioinsecticides or biopesticides	Fungi and bacteria enhancing plant growth and development by restricting the growth of plant pathogens	Synthesis of siderophore and HCN	Vessey (2003)
		Hydrolytic enzymes production	Somers et al. (2004)
		Introduction of systemic tolerance genes	Chandler et al. (2008)
Biofertilizers	A material that contains living microorganisms that make colonization with the roots in the rhizosphere to improve plant development via enhanced availability of nutrients	BNF	Vessey (2003)
		Insoluble phosphate solubilization	Somers et al. (2004)

11.2 Plant Growth-Promoting Rhizobacteria as Biofertilizers

Bhattacharyya and Jha (2012) observed that the substances made from living bacteria and fungi, when inoculated to the soil or seedling roots make colonization in the inner sections of the plants or in the rhizosphere, thus enhancing plant growth and development. Vessey (2003) reported that the PGPR strains like *Rhizobium*, *Mesorhizobium*, *Azorhizobium*, *Bradyrhizobium*, etc. have the capability to act as biofertilizers. Two primary types of relationship are found between the PGPR and their host: (1) endophytic and (2) rhizospheric. The relationship in which PGPR lives inside the host plants is called an endophytic relationship. He also proved that endophytes are present in the apoplastic intercellular places of the parenchyma tissue. PGPR increase the fertility in the rhizosphere through five different areas like enhancing nutrient status in the exceptional vicinity of roots, BNF, promoting useful symbiosis of the host plants, expanding the root surface area and the combination of all the above mechanisms of action. McCully (2001) stated that PGPR could colonize plant roots in the rhizosphere relationship. The capacity of PGPR colonizing to the plant roots in soil rhizosphere is mainly affected by the soil pH and plant exudation.

11.2.1 Increased Availability or Nutrient Solubilizing Ability in the Rhizosphere

Nitrogen fixation with the help of living organisms (BNF) and phosphate solubilization are the two significant methods increasing the nutrient availability in the rhizosphere. Phosphorous (P) is essential for crop growth and productivity. Phosphorous deficiency or lower availability to plants is due to the abundant amount of insoluble forms. Only two water-soluble forms like ($H_2PO_4^-$) and (HPO_4^-) are available to plants (Vessey 2003). Richardson et al. (2009) suggested that the ability of various phosphate solubilizing bacteria is the conversion of unsolvable soil P into plant-available form, through organic acid production, secretion of protons, and acidification. These rhizospheric bacteria can solubilize the inorganic insoluble soil P and increasing the availability of nutrients for plant growth promotion. The capacity of PGPR to solubilize the inorganic P can increase the P accessibility for efficient crop production. Verma et al. (2001) observed that PGPR have the potential for the solubilization of precipitated phosphates and increase its availability for crop growth promotion under field trials. Atmospheric nitrogen is fixed by different PGPR either symbiotically or non-symbiotically. *Azotobacter* and *Bacillus* species are the two significant and most effective PGPR having the potential to fix atmospheric nitrogen in a symbiotic way, when inoculated to the leguminous crop plants (Esitken et al. 2006). Free-living diazotrophs like *Azospirillum* are potentially able to fix atmospheric nitrogen non-symbiotically (Bashan and de-Bashan 2010).

11.2.2 Biosynthesis of Plant Growth-Regulating Substances (PGRS)

Different types of PGPR produce different phytohormones like cytokinins, IAA, etc. which can change the root structure and enhance the plant growth (Kloepper et al. 2007). PGPR producing IAA and gibberellins in rhizosphere soil play an essential function in enhancing the number of root tips and increasing the root surface area in several herbaceous plants (Han et al. 2005). Werner et al. (2003) explained the increase in root surface area, root initiation, cell division, and cell enlargement through increased growth of lateral and adventitious roots by the use of PGPR-formulated cytokinins.

11.2.3 Importance and Regulation of Ethylene Level in Plant

One of the gaseous plant growth regulators is ethylene which is most famous for healthy plant growth. Due to its higher amount, it can produce harmful effects on crop performance through the induction of premature removal of leaves and other cellular processes (Desbrosses et al. 2009). The beneficial group of rhizospheric bacteria helps in lessening the accumulation of ethylene and makes a strong root structure which is mandatory to survive with ecological abiotic stresses. Destruction of ethylene level through *Rhizobium* and *Pseudomonas* rhizobacteria with ACC-deaminase producing ability is the primary mechanism (Duan et al. 2009). The application of ACC-deaminase producing bacteria decreased the amount of salt-induced ethylene and increased the salt resistance in canola plants and enhanced plant growth and crop productivity (Cheng et al. 2007).

11.2.4 Siderophore Biosynthesis

Siderophores are the lower molecular weight organic substances produced by the microorganisms, chelating Fe^{+3} ions during the reduced accessibility of iron (Fe). Fe is required by the bacteria and fungi for heme formation, ATP production, and other essential purposes. Microbially produced siderophores are playing a central role in agricultural farming on a sustained basis due to their major function for detoxifying the heavy metal polluted soils and increasing Fe supply to plants (Saha et al. 2016). Tripathi et al. (2018) explained that Fe supply to plants is greatly affected under saline, drought, and heavy metal stresses. Bacterial siderophores are the significant source of Fe^{+3} supply to plants under abiotic stresses or reduced Fe conditions (Grobelak and Hiller 2017).

11.2.5 Biosynthesis of Vitamins

The study for producing water-soluble vitamins through bacterial, mainly some *Pseudomonas* species, is limited to some extent, and its important role in enhancing plant growth is not cleared yet (Tazoe et al. 1999). *Pseudomonas fluorescens* strain 267 produced a large number of B vitamins like pantothenic acid (0.75 μg ml^{-1}), niacin (0.92 μg ml^{-1}) in a minimal medium with various carbon sources and at different pH values, and also other essential vitamins like pyridoxine, biotin, etc. The production of B vitamins is greatly varied with carbon source and pH values of the medium (Marek-Kozaczuk and Skorupska 2001).

11.2.6 Production of Antibiotics

The provision of protection to plants is a vital function played by PGPR. The synthesis of antibiotics such as pantocin, oomycin, etc. which is the primary method of PGPR acts as biocontrol agents. *Pseudomonas*, *Bacillus*, and *Rhizobium* are some significant PGPR strains identified. Antibiotics trigger to introduce systemic resistance (ISR) genes in crop plants through direct antipathogenic action. So, plant disease management is the major function of antibiotics (Fernando et al. 2005).

11.2.7 Cyanide Biosynthesis

A volatile compound, suppressor of weeds, formed by the antagonistic gram-negative biocontrol bacteria, is called hydrogen cyanide (Haas and Keel 2003). Some beneficial group of rhizobacteria is also active against weeds. One of the groups of bacteria which act as biocontrol agents against weeds and can make colonization with the plant roots in soil rhizosphere is known as deleterious rhizobacteria (DRB). Ahmad et al. (2008) stated that the ability of HCN production is the common trait of *Bacillus* (50%) and *Pseudomonas* (88.89%) in plant root nodes and rhizosphere. The functioning of pathogens living in roots can be restricted by the involvement of certain fluorescent pseudomonads through HCN production.

11.2.8 Improvement of Plant Resistance Against Abiotic Stresses

Biopesticides play a significant function for the improvement of crops under different ecological stresses. Resistance against heat stress and salt stress is resulted due to calcisol produced by different PGPRs. The application of AM fungi, along with the nitrogen-fixing microorganisms in leguminous plants, can be used to overcome the problem of drought stress. Some pseudomonas species like *P. putida* enhance the sprouting rate and also increase the plant physical growth parameters like the fresh and dry weight of cotton under high pH and saline conditions through decreasing the Na^+ ion absorption and by increasing the uptake of calcium, magnesium, and potassium. (Bhardwaj et al. 2014).

11.3 Plant Growth-Promoting Rhizobacteria (PGRP) as a Biopesticides

Plant growth-promoting bacteria (PGPB) are a class of bacteria associated with plant growth. They are linked with numerous plant species and prosper efficaciously in an inclusive range of environments (Compant et al. 2005). PGPR are the most studied group of PGPB that usually colonized on the surface of the root of plants and thrived in the rhizosphere (Bashan and Holguin 1998; Kloepper 1978; Kloeppe et al. 1999). PGPR is a very broader term because it not only consists of bacteria that are promoting visible plant growth but also contributing in increased yield by strengthening plant against disease attacks (Banerjee et al. 2006). Kloepper introduced PGPR acronym for the first time in 1978. when a study was conducted to know the effect of a subset of rhizobacteria that was used as seed inoculum to induce plant growth positively. The bacterium used as seed inoculants (biopesticides, biofertilizers, and phytostimulators) achieve higher crop yield and provide crop protection in an environmentally friendly way, therefore, providing a substitute to reliance on synthetic fertilizers and pesticides (Banerjee et al. 2006). *Pseudomonas* and *Bacillus* strains are expansively studied PGPR (Prasad et al. 2019) and reflected as two very significant genera in disease management attributable to their effective antibiotic mechanism (Jayaprakashvel and Mathivanan 2011; Dominguez-Nuñez et al. 2016).

PGPR have the aptitude to improve plant performance and nutrient uptake capacity by natural means resulting in reduced agrochemical inputs (Kloepper et al. 1980; Lucy et al. 2004; Richardson and Simpson 2011). Diverse products of PGPR are available in market such as biofertilizers that boosts plant's ability to acquire more nutrients from the surrounding (Richardson et al. 2009), bioagents to control pathogens (Hol et al. 2013) and biostimulants to increase growth of different plant parts, e.g., roots (Lugtenberg and Kamilova 2009). A plant-microorganism endures a complex mechanism behind such events. Their efficiency is reliant on a number of abiotic factors like climatic conditions, soil pH and minerals, and biotic factors such as pathogen pressure and competency of the bacterial rhizosphere (Benizri et al. 2001; Ortíz-Castro et al. 2009; Dutta and Podile 2010). Host plant traits such as root morphology, exudation, and nutrient acquisition are entirely dependent on its genotype and are decisive factors for their compatibility of PGPR strains (Bais et al. 2006; Yang 2016).

Table 11.2 PGPR strains against certain diseases

Targeted disease	PGPR strain	References
Rice blast (rice)	*Azospirillum* strains	Naureen et al. (2009)
Maize rot (maize)	*Burkholderia* strains	Gijon-Hernandez et al. (2010)
Fusarium avenaceum (chickpea)	*Enterobacter sp.*	Hynes et al. (2008)
Blue mold (tobacco)	*Streptomyces marcescens*	Zhang et al. (2002)
Fungal disease (sesame)	*Paenibacillus polymyxa*	Ryu et al. (2006)
Downy mildew (pearl millet)	*B. pumilus*	Chandrashekhara et al. (2010)
Banana bunchy top virus	*Pseudomonas fluorescens*	Kavino et al. (2010)
White fly, thrips	*Verticillium lecanii*	Hynes and Boyetchko (2006)
Powdery mildew	*Pseudozyma flocculosa*	Hynes and Boyetchko (2006)

Similarly, PGPR showed promising outcomes in weed control of economically important crops. Hydrogen cyanide (HCN) was identified from antibiotics produced by antagonistic gram-negative biocontrol bacteria (Haas and Keel 2003); they are volatile compound that suppress weeds. Some rhizobacteria are directly active against some insects and weeds (Flores-Vargas and O'Hara 2006; Péchy-Tarr et al. 2008), e.g., *Meloidogyne incognita* (Siddiqui et al. 2005). Similarly, commonly occurring field carabid beetles, like *Harpalus pensylvanicus*, are helpful in controlling weeds by consuming on weed seeds. Kamei et al. (2014) stated that there are natural weed predators present in most of the fields. A list of PGPR strains effective against certain diseases is depicted in Table 11.2.

11.4 EPA Categorization of Biopesticides

Environmental protection agency (EPA) deals with scrutiny of chemicals that may pose threats to the environment. EPA only permits those chemicals/pesticides which are harmless to use in agricultural products; hence, it highly encourages biopesticides. A few of the fungus taxa have been efficaciously commercialized and promoted as biopesticides approved and registered by EPA (Arora et al. 2010). In the United States, the registered biopesticides contain the microorganisms belonging to genera *Candida*, *Trichoderma*, *Coniothyrium*, and *Ampelomyces* (Gardener and Fravel 2002).

Conventional pesticides are risky as compared to biopesticides; henceforth EPA generally entails less data for PGPR registration. A conventional pesticide may register in 3 or more years; however, biopesticides generally undergo the registration process in less than a year. However, EPA conducts rigorous reviews for guaranteeing that registered pesticide will not produce detrimental effects to the wellbeing of the people. A registrant proved requisite results associated with compositions, degradation, toxicity, and other parameters to pass through the authorization process (EPA 2019). The EPA classifies biopesticides into microbial, plant-based, and biochemical ones.

11.4.1 Microbial Biopesticides

Microbial biopesticides contain microorganism like bacteria, fungi, viruses, or protozoan as the active ingredient for controlling phytopathogens. They primarily make use of microorganisms from the subspecies and strains of *Bacillus thuringiensis* (Bt) (Marrone 2009).

11.4.2 Plant Biopesticides

Plant biopesticides are generally those substances produced by the plant for subduing phytopathogen. They likewise comprise the genetic modification and introduction of a specific gene. For instance, Bt contains a pesticidal protein that kills the pest. EPA in this case considers the gene of interest and its effects on the environment for its registration/use (Leahy et al. 2014).

11.4.3 Biochemical Biopesticides

Those naturally occurring nontoxic mechanism that controls phytopathogens are called biochemical biopesticides; in contrast, the synthetic pesticides kill or make the insects inactive (Marrone 2009). Sex pheromones that interfere with mating are one of the examples of biochemical pesticides. Similarly, there are various scented plant extracts that attract insect pests towards the traps (Environmental protection agency 2007).

11.5 Mode of Action of PGPR as Biopesticides

The plant growth-promoting bacterium may possess one or more mechanism for the beneficiary effects on the plants (Compant et al. 2005; Siddiqui 2005). PGPR control viral and bacterial diseases, fungi, and nematodes. The organisms utilized in biopesticides are capable of producing the siderophores which limit the iron availability to plant pathogens. In this way, indirect plant growth is promoted, while directly, they release antibiotics that kill the plant pathogen, antibiosis, and induce systemic resistance in the plant. PGPR produce specific proteins and chemicals involved in plant defense. They also lead to cell wall structural modification and physiological changes impacting the plant growth positively (Akhtar and Siddiqui 2010). Biological control is the use of PGPR either to control or minimizing the harmful effect of phytopathogens. PGPR play a role in controlling the detrimental effects of pathogens on plants by making the host plant resistance against disease or by producing specific growth inhibitors like antibiotics, lytic enzymes, and bacteriocins. In the different ongoing mechanisms in regulating the antagonistic activity of PGPR, such mechanism includes productions of antibiotics, parasitism, competition, and siderophores. Different reports have confirmed the positive influence of rhizobacteria strains on increased plant growth and antagonistic effect on plant pathogens. Most effective rhizobacteria belong to genera *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Bradyrhizobium*, *Burkholderia, Enterobacter*, *Flavobacterium*, *Klebsiella*, *Mesorhizobium*, *Pseudomonas*, *Rhodococcus*, *Streptomyces*, *Serratia*, etc. (Tariq et al. 2014; Ahmad et al. 2008).

The mechanism behind the pathogen proliferation prevention is due to the production of antibiotics. Many reports reveal that bacteria produce of antifungal metabolites in vitro which also showed activity in vivo (Akhtar and Siddiqui 2010). Numerous studies are present that reveal the presence of metabolites like amphisin, 2,4-diacetylphloroglucinol (DAPG), cyclic lipopeptide butyrolactones, HCN, oligomycin A kanosamine, oomycin A, pyoluterin (Plt), phenazine-1-carboxylic acid (PCA), tropolone, zwittermicin A, pyrrolnitrin (Pln), tensin, viscosinamide, and xanthobaccin, as produced by PGPR (Milner et al. 1996; Whipps 1997; Kang et al. 1998; Kim et al. 1999; Nakayama et al. 1999; Raaijmakers et al. 1999; de Souza et al. 2003; Compant et al. 2005).

PGPR reduce the pathogen by reducing their proliferation as siderophores have the ability to bind the Fe(III) in root vicinity (Akhtar and Siddiqui 2010). The pathogens do not flourish in that area (Siddiqui 2005). The siderophore synthesis in bacteria is generally regulated by iron-sensitive fur proteins, global regulators (GasS and GasA), sigma factors (RpoS, PvdS, and Fpv1), quorum-sensitive autoinducers (N-acyl homoserine lactone), and many site-specific recombinases (Ravel and Cornelis 2003; Compant et al. 2005)

The antagonistic mechanism takes place after the interaction of pathogen and rhizobacteria. Cyclic lipopedtides (CLPs) are produced as a result, and it forms ion channels in the plasma member of the targeted host that leads to cytolysis. Pores are formed, and many cellular compounds are released along with the alkalization of intercellular fluids. During the process, modification is made in the permeability of the cell membrane that permits polysaccharides, lipids, and nucleotide proteins to escape outside of the cells. At higher concentrations, direct solubilization of the plasma membrane may also occur (Quan et al. 2010). A brief description of the mode of action is depicted in Fig. 11.1.

The *Bacillus* genus comprises of bacteria that are widely used as biopesticides, posing antifungal and antibacterial properties. The compounds produced are of various origins; for example, sublancin, TasA, subtilosin A, and subtilin are of

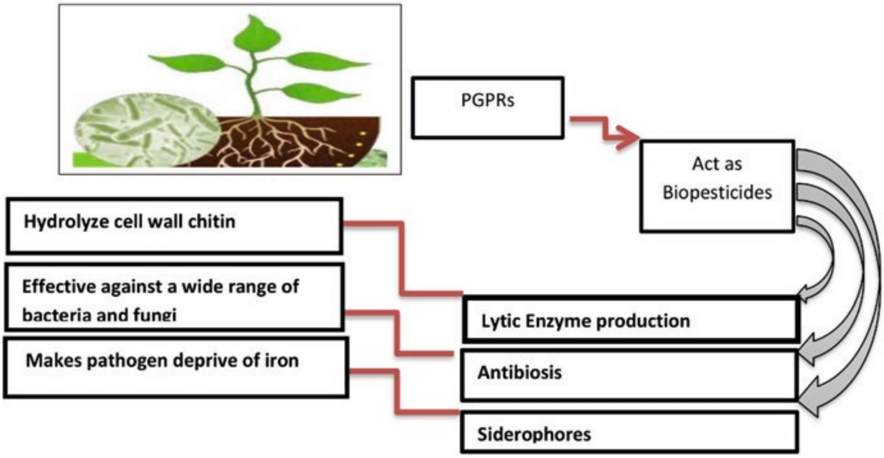

Fig. 11.1 PGPR mode of action as biopesticides

ribosomal origin. Similarly, few others are formed through nonribosomal peptide synthetases or polyketide synthases (PKS). These include bacilysin, mycobacillin, chlorotetain, bacillene, difficidin, rhizocticins, and lipopeptides belongings to the families iturin, surfactin, and fengycin (Leclère et al. 2005).

Plant root-colonizing *B. amyloliquefaciens* (FZB42) produces antifungal and antibacterial compounds. It has gene clusters that biosynthesize antibiotics (Chen et al. 2007). Nine clusters have been identified as dfn, mln, dhb, nrs, fen, bmy, and srf, involved in bioactive peptides and polyketides under the action of mega enzymes, nonribosomal peptide synthetase (NRPSs) and PKS (Quan et al. 2010).

11.6 Viability and Shelf Life of PGPR

Biopesticides face problems for its storage and shelf life and to how to extend it if they are kept viable. As microorganism require specific sets of conditions for their growth and survival, it becomes difficult for their effective marketing. However according to Kosanke et al. (1992), the survival time problem is adjustable through air-dried and lyophilized preparations. In this way the lowered water content ensures long-term survival during the storage, making the bacteria inactive, insensitive to the contamination, and resistant to the environmental stresses (Arora et al. 2010) They are also more compatible with fertilizer applications (Bashan and Holguin 1998). During the biopesticide formulation, the drying process is the most crucial step especially in case of non-spore-forming bacteria (Shah-Smith and Burns 1997). However, the accurate formulations are formed on the basis of culture medium for growing bacteria, the physiological state of bacteria after its harvest, the protective material, drying technology, and the dehydration rate used in the preparation (Paul et al. 1993).

11.7 Benefits of PGPR

The use of chemical pesticides can be altered by using PGPR with biochemical traits that can reduce the pathogens and diseases severity to the same extend as chemical pesticides (Prasad et al. 2019).

Synthetic pesticides undergo a lot of processes in their formulations requiring a lot of inputs, while the biopesticides microorganisms are naturally occurring and nature-free-of-cost raw material. Their multiplicity is the primary advantage over other phytosanitary products. PGPR biopesticides show a direct antagonism of pathogen growth and host plant immunization. It is better accepted by consumers as compared to genetically modified organisms (GMOs). Moreover, because they establish themselves photosphere with little efforts and produce antibiotics in a persistent manner with direct contact of the plant, a little amount of the required compound is sufficient for achieving the efficacy (Cawoy et al. 2011). Biopesticides only affect the exact target species or closely related species. In this way, there is no threat to the non-targeted organisms such as birds, mammals, and beneficial insects (Marrone 2009).

11.8 Limitations

The experiments conducted for testing the efficiency of rhizobacteria revealed that Pr for root no doubt acted as a growth stimulant and improved the acquisition of macronutrients. However, it reduced the acquisition of zinc and copper (Weber et al. 2018). *P fluorescens* is capable of releasing pseudofactin (Becker et al. 1985), pyoverdine (Meyer and Abdallah 1978), and siderophores which cause chelate complex formation, such as copper, zinc, and iron (Brandel et al. 2012). Nevertheless, unlike phytosiderophores, released by *Poaceae* for Fe uptake, metal complexes with microbial siderophores seem to be a poor metal source for plants (Walter et al. 1994).

Integrated pest control with the inclusion of *Bacillus thuringiensis* (Schnepf et al. 1998) or other biopesticides based on natural systems can be a promising tool to reduce pathogenic events. However, under practical conditions, lower financial achievement has not been repeated with many of the registered biopesticides particularly in forestry and public health protection (Hynes and Boyetchko 2006). There are many biopesticides which had been successfully registered; but due to difficulties like formulation, fermentation of microorganisms, and market research, they were not able to produce popularity and ultimately overlooked (Auld 2000). Market constraints relevant to inconsistence performance of biopesticides are identified as the major constraint. A rapid decline in population size of active cells has led to this failure.

11.9 Future Perspectives

With the advent in technology and development, the beneficial microorganism and their importance have been well documented; however, many areas also remain unidentified or little knowledge is present. The effect of type of soil on the mechanism related to ecology and physiology on PGPR at micro-level is limited. For this in vitro and vivo studies related to inoculant cell physiology, their response under different soil types, monitoring the bacteria after inoculation, and their systemic changes need to be more elaborated. In this regard, there is a need for advancement in visualization technology, molecular analysis (boot at the root level and microorganism pore level), signaling in the rhizosphere, microorganism engineering, biotechnology, and functional genomics studies.

11.10 Conclusion

PGPR as biofertilizers and biopesticides are attractive as well as an economic approach for sustainable agriculture. In the new climate change scenario, there is a dire need to lower down the chemical fertilizers, thus the abrupt shift towards the environmentally safe, more productive, use of natural resources to reduce the pest attacks is the demand of time. PGPR not only protect the plant against phytopathogens but also enhance plant growth and performance. After the successful implementation, food safety will be ensured, thus making trades movement more reliable with minimum harmful residues. In this way, a win-win situation can occur but through proper management practices. However, barriers like lack of awareness and different perception are still prevalent.

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Chapter 12
Halotolerant Microorganism Reclamation Industry for Salt-Dominant Soils

Shakeel Ahmad Dar, Zulaykha Khurshid Dijoo, Rouf Ahmad Bhat, and Mohammed Tauseef Ali

Abstract The extent of salinization of environmental resources affects almost 1 billion hectares worldwide (7% of the total continental area of planet earth). Salt-affected area numbers are escalating due to intrusion of saline water in arable land in coastal areas besides increased evaporation rates and decreased rainfall rates. Agricultural crops are usually intolerant to salinity. The bacterial domain halophiles are usually considered moderately tolerant and are a good choice for reclamation of salt affected soils. The presence of *Bacillus* species in plant root zone changes the metabolism of stressed plants and accelerates plant development. Salt-stressed plant with *Bacillus* increases plant growth, water, nutrients (nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, manganese, copper, and iron), antioxidants, and pigments plus hormones (IAA and GA) and reduces the Na, Cl, and ABA plus caspase activity which is responsible for programmed death of cells during stress conditions. Plant growth promoter bacteria's like rhizobacteria reduce the level of ethylene, restricts Na^+ uptake, increases K^+ and Ca^{2+} uptake, regulates sodium transport, increases exopolysaccharide production, and enhances enzymatic activity and phytoharmonic activity. Genetically engineered crops with salt-tolerant genes can be used in salt-affected areas for crop production. A positive influence in nutrient cycling by halotolerant microorganisms in salt-effected soils could be boon for plants under such environmental conditions.

Keywords Halotolerant · Soil · *Bacillus* · Reclamation · Environmental conditions

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12.1 Introduction

Salt-affected soils are normally considered physiologically dry soils due to the harsh nature for plant growth. Salinity is among the various effects resulting in degrading quality of land in terms of fertility and agricultural production. Saline soils are described as those soils with electrical conductivity (EC) of the saturation extract (ECe) in the root zone exceeding 4 dS m^{-1} (approximately 40 mMNaCl) at 25 °C and having exchangeable Na of 15% (Munns 2005; Jamil et al. 2011). Globally, estimations have shown that 20% of absolute cultivated and 33% of irrigated agricultural lands are distressed by unbalanced salt content. The annual increase in salinity areas is 10% due to less precipitation, increased evaporation, breakdown of local rocks, irrigation with saline water, and unscientific traditional practices. Rough estimates have shown that by 2050, higher than 50% of the total arable land would be affected by increased salt concentrations (Jamil et al. 2011). Salt stress is recognized to be a factor that affects plant development. Plants in their normal habitat are inhabited by endocellular as well as intracellular microbes (Paul 2012; Gray and Smith 2005). Stress due to salinity, its duration, as well as severity affects several physical, biological, and metabolic crop developments (Fig. 12.1). Primarily soil salinity is recognized to represses plant development by changes in osmotic stress commenced by high ion concentrations that are toxic to plant growth (James et al. 2011). During the early stages of salinity stress, water absorbing capability of root systems declines, and water loss from leaves is enhanced because of osmotic stress due to elevated salt buildup in soil and plants and consequently salinity stress considered as hyperosmotic stress (Munns 2005).

The increase in salinity of soil is a leading cause of the diminishing spread of plants in natural habitats. It is an escalating problem in arid and semi-arid areas.

Fig. 12.1 Crop affected by salinity and waterlogging (Satellite image of IRS 1D LISS III)

According to Fisher and Turner's (1978), they approximated that arid and semi-arid areas are occupying 40% of the total planet area. Salt tolerance is an aggregate of numerous features resulting from different physiological interactions. The threat of salinization to environmental resources affects approximately 1 billion hectares globally, which represents almost 7% of the total continental area of the planet, i.e., around 10 times the size of Venezuela or 20 times France's size (Yensen 2008). Physical and chemical methods are not as sustainable as that of the biological remediation of saline soil reclamation. Numerous microorganisms have the ability to live under severe conditions and are being under study for various applications. Halophiles essentially inhibit hyper-saline environment. These are categorized as slight halophiles with optimal growth at 2–5% NaCl, moderate halophiles showing the same at 5–20% NaCl, plus extreme halophiles that need 20–30% NaCl concentration for their optimum growth (Kushner 1993). The application of halophilic bacteria aids in reclamation of saline soils by assisting the vegetation growth. All halophilic microbes consist strong transport mechanisms, largely dependent on Na^+/H^+ antiporters for removing Na ions from the inner cell portions (Oren 2002). A variety of halophilic microbes such as bacteria, AM fungi, some cyanobacteria, etc. are beneficial in the reclamation of soils affected by salinity. Microbes can be used in association with FYM or in isolation for the adaptation of agricultural crops to salt stress. Genetic engineering techniques for improvement of crops in salinity-affected soils are also among the current available choices.

12.2 Sensitivity of Crops to Salt Level

Crops may be intolerant to salinity level, moderately tolerant to salinity level, or highly tolerant to salinity level of soils. Among cereals, rice (*Oryza sativa*) has been found to be most sensitive to salinity level, and barley (*Hordeum vulgare*) is found to be highly tolerant. Bread wheat (*Triticum aestivum*) is moderately tolerant. Some halophytic cotyledons need high sodium chloride content (100–200 mM) for optimal growth (Munns and Tester 2008).

12.3 Diversity of Halophilic Soil Microbes

Soil environment is full of microbes, and microbes can be found in isolation or in micro-colonies. Microbes have a fundamental role in biological restoration and conservation of higher organisms. Bacterial domain includes a large number of halotolerant microorganisms (Oren 2002; Arora et al. 2014). The bacterial domain halophiles are usually considered moderately tolerant and potent for reclamation of saline soils. Halophilic bacterial activities promote plant growth in salt-stressed soils and can also be used as bio-indicators in saline wells (Arora et al. 2014).

12.4 Application of Bacillus Species for Improving Plant Health in Salt-Affected Soils

Agricultural land salinity is increasing worldwide because of reduced rainfall and increased evaporation and faulty irrigation exercises. Salt accumulation in soil results in reduced water potential and reduced rates of nutrient intake (Al-Karki 2006). Under saline conditions, non-halophytic plants face severe disorders and dysfunctioning of metabolic pathways. Microbes particularly *Bacillus* strains can be used in sustainable way to reduce salt stress of plants (Hashem et al. 2015). Several plant growth-stimulating characters like phosphate-solubilizing capacity, NH_3, IAA, and "siderophore" production of *Bacillus* licheniformis A2 alleviate the disadvantageous impacts of salt stress and enhance plant growth in stressed peanut plants (Goswami et al. 2014). *Bacillus* species presence in root zone of salt-affected plants accelerates their metabolism resulting in enhanced plant growth and increased water holding capacity; higher content of nutrients, antioxidants, pigments, as well as increased hormone activity (IAA + GA) reduces Na, Cl, ABA, as well as caspase activity, and up- or downregulated the salt stress-responsible genes (Ashraf et al. 2004; Radhakrishnan et al. 2017). In order to thrive well in salt-affected soils, plants must avoid excessive intake of Na^+ and Cl^- ions. *Bacillus* species that are able to produce exopolysaccharide (EPS) such as *Bacillus insolitus* MAS17 strain shields the root zone of plants cultivated in saline soil with soil covers and limits the flow of Na^+ into the steal, thereby minimizing the consequences of salt stress (Ashraf et al. 2004). *Bacillus* strains minimize toxicity created by saline environment by influencing lipid peroxidation (Han et al. 2015). Caspase is the causal agent for programmed cellular death in plants, and when *Bacillus pumilus* is introduced in rice fields with high salinity, it reduces caspase activity, and as a result, cell death is reduced and increases the activity of antioxidants to increase plant tolerance. Salt-tolerant *Bacillus subtilis* RH-4 enhances seed germination as well as plant growth by improving the activity of photosynthetic pigments, sugars, proteins, and osmolytes like proline, and glycine betaine besides choline, in salt-stressed chickpea (Qurashi and Sabri 2013). Monitoring of metabolic activities increases salt-tolerating capacity of plants in the saline soils (Fig. 12.2). Using microbes on saline soils enhances the production of some secondary metabolites like gallic acid, caffeic acid, syringic acid, vanillic acid, ferulic acid, cinnamic acid, and also of quercetin, which improves salinity-related stress responses of plant (Tiwari et al. 2011).

12.5 Screening of PGPR for Saline Tolerance

From saline desert, rhizospheric region of a halotolerant *Suaeda fruticosa* with salinity of 4.33% was used for isolation of PGPR (Goswami et al. 2014). Out of 85 isolates, 23 isolates succeeded insolubilizing phosphates and 11 isolates produced IAA. Seven of them were found to have capability of solubilization as well as IAA

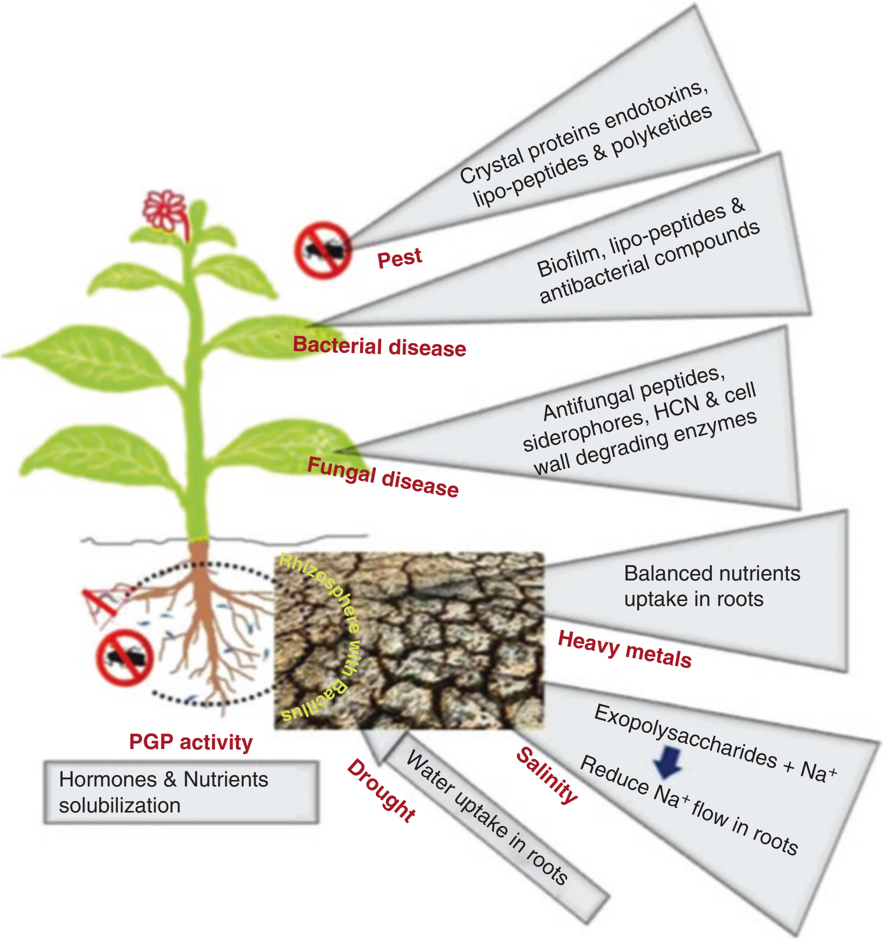

Fig. 12.2 Various effects of *Bacillus* secretion as plant safeguard against unfavorable conditions (Radhakrishnan et al. 2017)

production. All traits were screened for PGP properties such as production of ammonia, siderophore, chitinase, HCN, and antifungal action. Among all the screened isolates, *Bacillus licheniformis* A2 was able to enhance plant growth in groundnut (*Arachis hypogaea*) under salt-stressed surroundings in vitro as well as in vivo. Using salt-tolerant phosphorous solubilizing bacteria, *B. megaterium* A12 can result in increased growth of rice plus yield fractions (Sapsirisopa et al. 2009). Islam et al. 2013 was successful in isolating as many as five rhizobial strains, (i) L-19, (ii) L-68, (iii) L-292, (iv) L-304, and (v) L-335, having salt-tolerant ability from salty soils, and also observed their functioning in lentil (*Lens culinaris*) in saline soil. Strains L-19 and L-304 proved to be efficient for nodule formation, enhanced growth of plant, increased production, and enhanced nitrogen fixation in lentil in addition to N build up. At different levels of NaCl salinity stress, inoculating the soil by AM fungi plus *Azospirillum brasilense* boosted nitrogen and phosphorus nutrition in treated cowpea plant (Rabie et al. 2005).

12.6 Role of PGPR in Plant Stress Mitigation

The presence of plant growth-promoting rhizobacteria in plants has an essential function of stress alleviation. The separation of native microbes from the salt-stressed soils followed by their assessment based on stress tolerating limits and PGP attributes can prove beneficial as an option for use in prompt and proficient strain selection for bio-inoculants for salt-stressed crops (Shrivastava and Kumar 2015). A few of the recent researches related to evaluation of the functioning of rhizobacterial strains as salt stress remediation options have been tabulated in Table 12.1.

Table 12.1 Functioning of PGP bacteria as salt stress mitigation measures in plants (Shrivastava and Kumar 2015)

PGP bacterial species	Plant studied	Noted effects	References
Achromobacter piechaudii	Tomato	Dwindle ethylene levels	Mayak et al. (2004)
Aeromonas hydrophila	Wheat	Exopolysaccharide production	Ashraf (2004)
Pseudomonas syringae	Maize	ACC deaminase action	Nadeem et al. (2007)
Pseudomonas fluorescens	Groundnut	Improved ACC deaminase action	Saravana Kumar and Samiyappan (2007)
Bacillus subtilis	*Arabidopsis thaliana*	Regulation of Na transporter HKT1	Zhang et al. (2008)
Rhizobium	Maize	Decrease "electrolyte" loss, rise "proline productivity" and maintain virtual water level	Bano and Fatima (2009)
Bacillus pumilus	Rice	Enhanced content of "glycine betaine"	Jha et al. (2011)
Pseudomonas putida	Cotton	Upsurge in absorbing Mg^{2+}, K^+ and Ca^{2+}	Yao et al. (2010)
Pseudomonas fluorescens	Wheat	Improved germination and nutrient limit	Nadeem et al. (2013)
Pseudomonas pseudoalcaligenes	Salt-sensitive rice GJ-17	Diminution in lipid peroxidation	Jha and Subramanian (2014)

12.7 Remediation of Sodic Soils to Enhance Agricultural Yield

There are two hypotheses for reclamation of saline soils. The first one states that the microbial actions in salt-affected soils can promote the growth of plants with resistance to salinity states, while the second one states that microbes can be used as indicators of salinity in salt-affected water wells. The ability of microbes to grow at varying salt concentrations can be employed for choosing indicator species. Halophilic microbes have proven to be successful in removal of salt from highly affected soils (Arora et al. 2013; Bhuva et al. 2013). Halophilic bacteria as agents of salt removal also support vegetative growth and also improve the crop yield. Arora et al. (2014) used two halophilic bacterial strains, viz., CSSRO2 and CSSRY1, for salt removal from agricultural fields. Halophilic bacterial strain, CSSRO2, is quite effective in decreasing Na^+ level from 11.2% in supernatant to 10.0% at 24 rs, while strain CSSRY1 decreased Na^+ levels to 9.3% at 48 h in halophilic broth consisting of 15% NaCl. The outcome shows that using bacterial strains resulted in removal of 1.2 and 1.95% of Na^+.

12.8 Effect of Use of Halophilic Bacterial Strains on Soil Properties

Halophilic bacteria alone or in association with FYM are good at reclamation of saline soils. Influence of inoculation on saline soils using Na^+ content as indicator was examined. The results indicated that Na^+ content was reduced by inoculation in surface soil layers (Arora et al. 2014). The soil's biochemical properties also showed improvement upon addition of microbial biomass C up to 137 μg/g in surface soil in comparison to 82 μg/g in control.

12.9 Influence on Plant Growth by Halotolerant and Halophilic Microbes

The study on the positive effects of halophilic bacteria for enhancing the growth of stressed plants has been done extensively in many parts of the world (Essghaier et al. 2014). Using microbial strains having stress tolerance while being associated with agronomic crop roots can be helpful in improving the productivity of soil besides providing the plant with resistance to detrimental environmental settings (Wu et al. 2009). Orhan (2016) explored 18 halotolerant as well as halophilic bacteria to study the effects of these on growth, improving capabilities both in vitro and in hydroponic cultures. The results showed that in vitro, the bacterial strains under study had variable number of activities related to plant growth promotion. Under salt stress conditions (200 mM NaCl), the bacterial strains considerably boosted the root as well as shoot length. In addition to this, the total plant fresh weight also increased. The growth rates of plants inoculated with bacterial strain was in the range of 62.2% to 78.1%.

12.10 Effect of Inoculation of Halophilic Bacterial Strains on Wheat

PGPR such as *Rhizobium*, *Pseudomonas*, *Acetobacter*, *Bacillus*, *Flavobacterium*, and *Azospirillum* are helpful for increasing the plant growth in salt-stressed environs. Halophilic bacterial strain CSSRO2 (*Planococcus maritimus*) and CSSRY1 (*Nesterenkonia alba*) have plant growth promotion capabilities; therefore, these strains were isolated from rhizosphere of prevailing halophytes from coastal saline soils. The results showed that these strains were able to alleviate salt stress (Arora et al. 2012; Trivedi and Sanjay 2013).

Arora et al. (2014) tested halophilic PGP bacterial isolates in saline soils for field experiment at a pH of 9.4. Results revealed that the production of wheat (*T. aestivum*) in the fields showed an increase from 3497 kg ha-1 to 4129 kg ha^{-1}, while consortia of halophilic N-fixers plus P solubilizers were inoculated in seeds. Likewise, straw production showed increase from 5.03 to 6.24 t ha^{-1}, while the halophilic inoculates was put to use. This favorable influence of *Azospirillum* and halophilic N-fixers inoculation was witnessed in wheat seeds, where alleviating influence of salt stress was seen (Creus et al. 1997; Arora et al. 2015). Wheat plantlets exposed to osmotic stress resulted in substantially better coleoptiles, increased fresh weight, as well as improved water status due to *Azospirillum* inoculation.

12.11 Bioengineering as a Tool Against Soil Salinity

Genetic engineering is the influential tool in the hands of scientists for transfer of salt-tolerant genes to plants that can be later grown in salt stress conditions. Several studies for tolerating salinity in plants focuses on genes governing ion transportation, because regulating uptake and compartmentalization of Na$^+$ is an essential means for plant existence (see Table 12.2 (Gupta and Huang 2014)).

Table 12.2 Plant salt-tolerant improvement by engineered genes for membrane antiporters (Gupta and Huang 2014)

Transgenic host	Gene engineered	Source	Improved functions under salt stress	References
Arabidopsis	Vacuolar Na^+/H^+ antiporter Ms. NHX1	Alfalfa	Enhanced osmotic balance. Upsurge in MDA	Hanzawa et al. (2002)
Rice	Vacuolar Na^{+}/H^{+} antiporter Pg NHX1	*Pennisetum glaucum*	Extensive root spread	Takahashi and Kakehi (2010)
Wheat	Vacuolar Na^{+}/H^{+} antiporter at NHX	*Arabidopsis thaliana* L.	Improvement in grain yield, biomass productivity	Cona et al. (2006)
Tobacco	Vacuolar Na^+/H^+ antiporter GhNHX1	*Gossypium hirsutum*	Na^+ compartmentalization	Tisi et al. (2008)
Tomato	Vacuolar Na^{+}/H^{+} antiporter AtNHX1	*Arabidopsis thaliana* L.	Increased productivity of vacuolar Na^+/H^+ antiporter	Navakoudis et al. (2003)
Tobacco	Vacuolar Na^+/H^+ antiporter AlNHXI	*Aeluropus littoralis*	Compartmentalization of Na^+ in root system. Constant K^+/Na^+ ratio in the leaves	Roy et al. (2005)
Brassica	Vacuolar Na^+/H^+ antiporter AtNHX1	*Arabidopsis thaliana* L.	Improved proline concentrations, enhanced growth rate. Alleviation of lethality by Na+	Roychoudhury et al. (2011)
Arabidopsis	Plasma membrane Na^{+}/H^{+} antiporter SOS1	*Arabidopsis thaliana* L. (wild type)	Increased rates of germination, improved root system growth, and increase in chlorophyll quantity. Reduction in Na^+ aggregation	Wang et al. (2007)

12.12 AM Fungi in Saline Environments (Effects on Plant Growth and Development)

Arbuscular mycorrhizal fungi (AM) are fungi present in soil that are involved in the development of symbiotic relationship with many plants. In this mutually advantageous relationship, the host plant supplies the fungi with required hydrocarbons for its growth and development (Miransari 2014). The fungi in return provide water plus nutrients to the host by its widespread web of hyphae, linking soil and host roots (Smith and Read 2008). For the beginning and extension of symbiosis, the existence of the host plant is compulsory, though the fungal spores can germinate in the absence of the host plant (Smith and Read 2008; Smith et al. 2010). In certain saline soils, moderately enormous populations of AM fungi have been encountered,

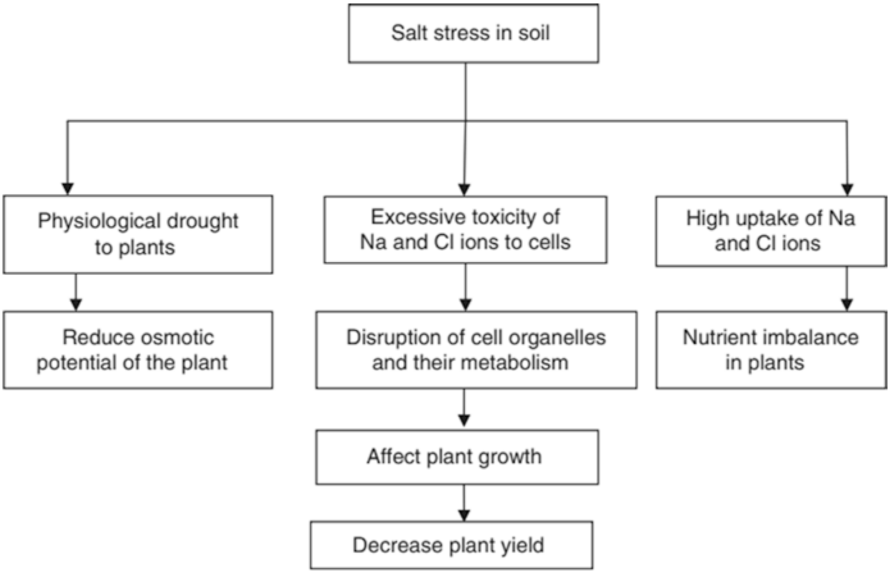

Fig. 12.3 Salt stress responses by plants (Evelin et al. 2009)

despite low mycorrhizal affinity of numerous halophytes (Aliasgharzadeh et al. 2001). Enhancement in capability of certain plant species to stand salt stress (Fig. 12.3) with mycorrhizal symbioses has been reported by Al-Karaki (2001) and Ruiz-Lozano et al. (1996). Salt-treated AM plants have resulted in increased shoot and root dry weights than non-AM controls in some studies. AM fungi stimulated growth of *Zea mays* in salty environments, with the effect enhancing with the increase in stress (Feng et al. 1998) and elsewhere (Bhoopander and Mukerji 1999; Cantrel and Linderman 2001). Under glasshouse environs, enhanced shoot content of phosphorus and potassium was reported by inoculating with AM fungi (Asghari 2008). Inoculating plants with AM fungi in salty conditions usually enriched the buildup of leaf and root sugars, proline besides total free amino acids (Ezz and Nawar 1994). In addition to this, Ruiz-Lozano et al. (1996) has reported that AM fungi stimulate the hormones in plants plus enhancing of water intake capability causing improvement in growth followed by dilution of lethal effects by ions (Al-Karaki 2001). Also, AM fungi have been reported to influence the enzyme activity of polyphenol oxidase, but it had no influence on peroxidase activity (Santos et al. 2001). A constructive effect on phosphorus by AM fungi on the plants cultivated in salt-stressed environments is by improving selective intake of nutrients (Al-Karaki and Clark 1998). The amount of nutrient supply to the root system is principally controlled and improved (absorption and/or translocation) by AM fungi (Sharifi et al. 2007). K^+ absorption under saline conditions can enhance under mycorrhizal colonization (Zuccarini and Okurowska 2008) while preventing Na^+ translocation to shoot tissues. Synthesis plus storage of polyphosphate also influences Na^+ and especially K^+ uptake.

12.13 The Complex Functioning of Arbuscular Mycorrhizal (AM) Fungi in Improving Salt Stress in Plants

In AM relationship, fungus produces an appressorium (ap) on the root's surface, thereby gaining entry into its cortex by extension of its hyphae (h). The hyphae forms arbuscules (a) plus vesicles (v) in the cortex. Due to high salt content in the soil, plants are deprived of the essential supplies of water as well as nutrients, resulting in physiological drought besides reduction in osmotic potential followed by nutrient deficiency which renders the plant weak and also reduces its productivity. Arbuscular mycorrhiza is more efficient in salt-stressed plants in the presence of AM fungi by accelerating water plus nutrient intake: a reduction in osmotic potential by increase in the osmolyte buildup, water use efficacy, increase in photosynthetic activity, and antioxidant productivity (to scavenge ROS).

12.14 Conclusion

Extensive studies of halophilic bacteria for their beneficial use in salt-affected soils for increasing chance of survival and growth of agricultural crops in physiologically dry soils and their application in agriculture have been carried out all over the world. From the perusal of current available literature on the use of halophilic microbes for saline agriculture, the conclusion is that halophilic microbes are excellent choice for the reclamation of saline soils besides effective in increasing plant growth in salt stressed environments. Bacteria like *Bacillus* sp., *Pseudomonas* sp., PGPR, and AM fungi from saline environments can be used to reclaim saline soils. Microbes increase plant growth and productions in saline soils directly and are also used as indicators for water quality of wells in saline areas. Genetic engineering is a significant technique for enhancing plant resistance to saline soils. The development of salt-tolerant crops is presently in nascent phase. Thus, the single feasible substitute is using salt-tolerant bio-fertilizers for promoting growth and productivity.

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Chapter 13
Allelopathic Bacteria as an Alternate Weedicide: Progress and Future Standpoints

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Abstract The ever increasing population and global issue for food security have led us to use multiple approaches to overcome the weed problems that can reduce the crop productivity up to 70%. Chemical herbicides and mechanical and other biological approaches have overcome weed problem on one hand but also destroy the environment and caused some human health impacts on the other hand. Bioherbicides are biological control agents applied in similar ways to chemical herbicides to control weeds. There is a group of rhizobacteria that is being overlooked due to its non-parasitic nature towards plants; this group of rhizobacteria is known as allelopathic bacteria. It can excrete cyanide, phytohormones, and phytotoxins that can affect the metabolism of weeds negatively. Allelopathic bacteria emerge as an alternative and more effective weed control approach which not only eradicate the weed problem but also enhances the growth of the crops. This chapter will explain the general comparison between the different weed control approaches. The importance and impacts of the bioherbicides will also be explained also by elaborating the constraints which this approach is facing in its production and application.

Keywords Weedicides · Bacteria · Bioherbicides · Rhizobacteria · Phytotoxins

13.1 Introduction

Agricultural crops and pests and weeds have strong relations to each other. Farmers use different methods to control them to maximize their economic benefits. These methods are conventional and modern. Conventional methods include manual as well as mechanical. Manual and mechanical methods comprise manual weeding, sickling, mowing, tillage, and many others (Chauvel et al. 2012; Abbas et al. 2018). These methods have been used since crop cultivation began but has many disadvantages, including reduction in soil fertility, erosion, and destruction of roots (Birkás

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et al. 2004; Chauvel et al. 2012). Other restrictions include high costs and labor dependency. Because of their convenience, chemical herbicides are often preferred and have greatly reduced weed populations. Their use significantly increases crop yields. This method has limitation of weeds as well as destroying neighboring ecosystems. It destroys biodiversity and kills vulnerable animal and plant species (Liebman et al. 2001; Zhang and Chen 2017). These chemical herbicides also cause human health disruption like cancer and neurological and respiratory diseases (Alavanja et al. 2004; Alavanja 2009). For sustainable agriculture and the environment, biological methods are being studied as a possible weed control method. Biological weed control is the method which uses microbes and their exudates to control the growth, reproduction, multiplication, and control of weeds growing in crops without harming the crops (Harding and Raizada 2015). Previous biological control efforts have focused on pathogenic and herbivorous insects (Boyette and Hoagland 2013), and the efforts to control biological insects have been unsuccessful due to time-consuming process, broader host range, and the possibility of new pests (Denslow and D'Antonio 2005).

The need for suitable environmental conditions that lead to the development of infections and diseases limit the consistency and range of weed-controlled pathogens (Charudattan 2005). *Rhizobia*, which produces weed suppression phytotoxins, have long been ignored. These are called allelopathic bacteria. These bacteria have a higher selectivity in plants, reduce the ability to grow, eliminate potential drug resistance, lower costs, and offer the advantage of being an environmentally friendly approach (Kremer and Souissi 2001; Abbas et al. 2018). This chapter will help to gain a complete overview about the use of biological herbicides and their potential to increase soil and plant productivity compared to other conventional methods. Besides, this newest method has limitations in its application and development.

13.2 Chemical Herbicides and Their Impacts on the Environment

The chemicals used to control weeds are called weedicides. Chemical weedicides have been used as a pioneering method to eradicate weeds (Zimdahl 1999; Sodaeizadeh and Hosseini 2012). The control of weeds using chemical herbicides has a long history of success. They are used not only to inhibit weeds but also increase crop productivity (Ashiq and Aslam 2014). They inhibit weed growth by inhibiting acetyl-CoA carboxylase and acetolactate synthase and polymerize microtubules which play vital role in their growth and reproduction. This type of action is very extensive and relies on the chemicals used in the synthesis (Arteca 1996; Zimdahl 1999).

On the one hand, chemical herbicides are very resistant to weeds; on the other hand, they remain for prolonged time period in the environment and not only effects on its health but on human health as well. It will take 50 years for the environment to return to its original state when herbicide is applied in it. Certain other effects of herbicides use are also alarming, e.g., the extinction of birds, water, and vegetation. Also, the inflow of used herbicides into water bodies can increase the nutrient content of them and cause eutrophication problems (Sarwar 2015). Herbicides are detrimental to the human health and the environment. They also destroy the habitats of many species that play an important role in environmental processes. It causes cardiovascular diseases, cataracts, respiratory diseases, birth defects, and gene mutations in humans (Alavanja 2009).

In between 1980 and 2006, the average number of common birds decreased by around 10% and the number of farm birds by around 48%. In the United States, three species have been classified as at risk due to excessive herbicide use. Around 1211 bird species are susceptible to habitat loss and even extinction. They are also dangerous for beneficial organisms, like honeybees. In the United Kingdom, 95 major impact cases have been reported that have reduced the number of beneficial incest by habitat destruction or viral disease (Katayama et al. 2010; Edwards 1993; Mahmood et al. 2016; Lakhani 2015).

Chemical herbicides contaminate the groundwater by leaching. More than 6000 species of amphibians are also under threats due to water pollution by herbicides. The effects of atrazine on fish and frogs are serious. However, detailed and comprehensive studies are desired to assess the negative effects of chemical herbicides on aquatic organisms (Lakhani 2015).

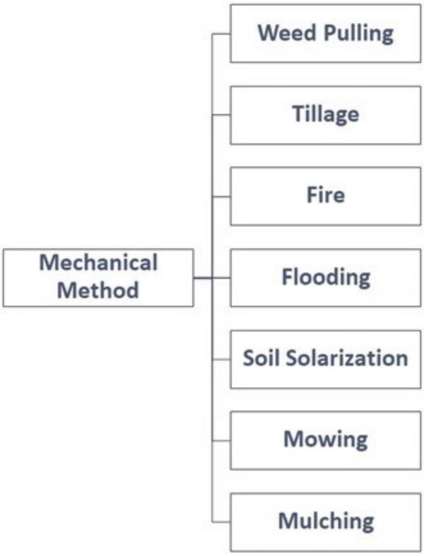
13.3 Allelopathic Bacteria as Alternate of Chemical Herbicides

Due to long-term harmful effects on environment as well as biodiversity, chemical herbicides are not recommended for use nowadays. Pesticides used to play a very active role in increasing yields and fighting weeds. To overcome the problems and effects of chemical herbicides, there was a need of alternatives that can be the source of sustainable agricultural system and that can protect the environment from further damage. Initially, weed pulling, tillage, fire, flooding of the fields, soil solarization, and mowing and mulching with crop residues were introduced to cope with weed problems (Fig. 13.1).

13.4 Mechanical Methods

Mechanical weeding is any physical action that hinders the growth of weeds. This method increases the chances of weed removal, kills, or makes the growing conditions unfavorable for weed growth. This method can directly damage weeds. There are two sub-types of this method, i.e., selective and non-selective. Selective attacks

Fig. 13.1 Mechanical methods of pest removal



can effectively control weed populations, but non-selective attacks can pose threats to crop too. Mechanical weed control methods can eliminate or control weed populations if used at the right time and the intensity (Li et al. 2006).

13.4.1 Weed Pulling

This method removes weeds by pulling them from the ground. This method is used for the control of shrubs, seedlings, and herbs. Annual plants and self-rooting weeds are often easy to remove. Many species can sprout from the roots that remain in the soil. Therefore, its effectiveness depends on the removal of as many weed plant roots as possible. Perennial weeds are difficult to control effectively because it is difficult to eradicate all root systems and must leave the plants for many years. Smaller weeds can be removed by hand, but bigger plants require a weed removal tool, such as a weed key or a root claw. This technique has little impacts on nearby non-target plants and crop plants. However, it requires labor and time which makes it harder for larger land holders to use it (Hussain et al. 2018; Smith et al. 2011).

13.4.2 Mechanical Mowing

This method works by cutting or shredding above-ground weeds and reduces seed germination and limits weed growth. It is done before weed maturation and onset of seeds because after weed maturation, seeds can be buried into the soil and can start its life cycle again. Some species can re-sprout from stem or root pieces that remain after mowing. The advantage of this method is it can be used in combination with different other methods (Tu and Robison 2013).

13.4.3 Crop Residues and Polythene Mulching

Mulches are relatively simple and inexpensive compared to other weed control methods. Covering weeds with crop residues or polythene sheets, etc. can eliminate them by blocking light to prevent weeds from growth. Many annual weeds have been successfully controlled, but some perennial weeds have not been affected (Tu and Robison 2013). Mulch consists of crop residues, vegetable leaves, wood chips, compost, and grass. Artificial coverings made from plastic or rubber materials can be used. The effectiveness of the cover mainly depends on the materials used. One or more synthetic materials can be used in combination to increase weed control (Gressel 2000).

13.4.4 Tillage Practices

Tillage is used more often for crops (Gressel 2000). It can be carried out in small fields with tools such as smaller manual motor hoeing or on a large scale with ploughs attached to the tractor. Tillage eliminates weeds from soil by disturbing soil; hence, vegetative parts of the weed are destroyed, and the roots are exposed, which leads to drying out (Saini and Singh 2019). In general, younger weeds are easier to control than the mature ones. Repeated tillage is required to control mature perennial weeds. If the soil is knocked over, the soil seed bank is also destroyed, which can lead to dormant weed seeds germinating without previous competitors. These new weeds can also be controlled by continuous tillage until the soil seed bank is exhausted (Saini and Singh 2019; Bajwa et al. 2019).

13.4.5 Soil Solarization

Soil exposure to the sun is a simple weed control method. It is achieved by using different soil covers. Soil covers are layers of transparent or black plastic sheet which are placed on the soils. Soil covered with black plastic captures the sun rays and increases the soil temperature. Many weed seeds and asexual propagation material cannot withstand the temperature and are killed. This method can be made more effective by keeping soil moist because weeds are more susceptible in the cool season than warm season. Covers do not contain a light that can be used to better control growing plants, and transparent plastic has been shown to lead to higher soil temperatures (Yaduraju and Mishra 2004).

13.4.6 Setting Fire in the Fields

If used carefully, burning the weed-affected fields can be a cost-effective method. In most plants, fire can cause a cell wall break if the temperature reaches 45–55 °C. Incineration can kill the unwanted vegetation by burning dry, mature plants and killing small emerging weeds. Buried weed seeds and plant propagation can also be destroyed by burning. Smaller flame uses include the use of propane burners with fan heads. This method can be used in areas where the soil is hard and humid to dig or plow. Burning works best on younger weeds, but repeated application can fight harder perennial weeds (Hynes 1995).

13.4.7 Flooding the Fields

Agricultural fields are flooded with water till saturation at a depth of 15–30 cm for 3–8 weeks. This creates anoxic conditions, thereby limiting weed growth or killing them. It is highly effective in limiting perennial weeds growth by reducing the weed seed germination (Hynes 1995; Gressel 2000).

13.5 Limitation of Mechanical Methods

Based on the overall efficiency of all above weed controlling methods, a global weed control methodology assessment was conducted to help farmers and technicians select more appropriate weed control methods. Herbicides alone or their combinations work effectively while reducing chemical pollution of the environment up to 50%.Weeding or weed pulling has some limitations and can only give satisfactory results when combined. Mechanical methods reduced weeds in rice crops by 75% compared to controls and increased grain yield by 25%, which was higher than the fields receiving herbicides (Van der Weide et al. 2008). Other studies have shown that mechanical weeds have a direct negative impact on probiotic arthropods (Hatcher and Melander 2003).

Mechanical plant treatment generally reduces the number of polyphage predators directly, for example, mechanical destruction and habitat disturbances (Upadhyaya and Blackshaw 2007). Thorbek and Bilde (2004) found negative effects on spiders but no effects on crustaceans or staphylococci. Effective weed control, whether mechanical or chemical, can adversely affect birds by reducing the amount of feed available, such as weed seeds and arthropods/pests. Besides, it is generally thought that mechanical weed control destroy a significant number of bird nests that breed in the field, such as the Lark (Li et al. 2006).

13.6 Biological Method

There are four strategies which are used in bio-control:

(I) Introduction: Useful foreign organism is introduced into a new area and fully establishes it. This strategy is usually used for the introduction of pests without local antagonists.
(II) Improvement: With this method, the laboratory inoculum is introduced to increase the ineffectiveness of current microbial preparations. The reason for the lack of control can be a small number of natural predators.
(III) Vaccination: Vaccination is released at the start of sowing if there is no native antagonist or the introduced antagonist cannot survive permanently.
(IV) Immersion: Large-scale cultivation of pathogens is urgently used in critical phases in which rapid suppression of the pest populations is required (Ghorbani et al. 2005; Charudattan 2001).

13.6.1 Insects as a Bio-control Agent

Selected herbivorous insects are used for this purpose based on their properties to remove specific hosts in the field. This technology uses genetically modified herbivore insects that feed on weeds and help to overcome weed problems. But, the success story of this method is very limited, but the results are very effective, a weed called *Opuntiaspp* is controlled by moths. After very careful laboratory tests, 3 million eggs were released onto the weed flora. It reduces the dense vegetation of the cactus and forms smaller spots. At the end of the nineteenth century, the vedalia beetle (*Rodolia cardinalis*) was imported from Australia to California to control the cotton seat cushion (Goeden 1988; Waage and Greathead 1988). The host range of classic biological control agents is usually broad. Once these insects are in the field, they can no longer be eliminated, which plays an irreversible role in weed control. The problem is that these insects may appear in the form of new pests and endanger the pests earlier. This genetic modification of insects takes time, and the way it works in the field is not as fast as our imagination. These limitations make the procedure unsuitable for further investigation (Abbas et al. 2018).

13.6.2 Fungi as a Bio-control Agent

It is in history that weed control was based on biological methods 200 years ago. However, these are eco-friendly approaches that take advantage of herbivore microorganisms. Microbial herbicides were investigated in the middle of the last century, which develop phytotoxins rapidly. In the past decade, some phytotoxins that have been picked from pathogenic weeds have shown a potential herbicidal activity. Some experts have suggested developing these phytotoxins as a new type of biological herbicide as an alternate chemical herbicide. Phytotoxins from many fungal species have herbicidal effects, such as AAL toxin, cornexistin, and tentoxin. The structure of the AAL toxins and their analogs inhibits ceramide synthase and leads to an accumulation of sphingosine and a breakdown of the membrane. Cornexitin is a metabolic inhibitor with a mechanism of action like that of amino acetate. Tenosin have two different mechanisms of action under different conditions. On the one hand, the formation of chloroplasts is to be interrupted by preventing the synthesis of encoded nucleoplasmic proteins, and on the other hand, it is an energy transfer inhibitor that controls photophosphorylated ATPase coupling factors (Duke 2012). The first microbial herbicide study in China was a suspension culture of *Clostridium cocci*. In the 1960s, the grass was called Lu Bao 1 (control of US silk). De Vine is the first company registered in the United States. Other biological herbicides like COLLEGO, Dr. BioSEDGE, BioMall, and Stumpout are also close behind (Charudattan and Dinoor 2000). Mycogen, of San Diego, California, plans to sell a variety of herbicides, including *Fusarium* species that control *Fusarium* and *Fusarium* species. Both types are major problems with weeds in soybean fields. It will require the collaboration of plant pathology, weed research, and fermentation research professionals to successfully carry out weed biological control (Charudattan 2001).

13.7 Bacteria as Alternate to Control Weed

Within the concept of sustainable agricultural ecosystems, weed control is accomplished through integrated weed management, in which all available strategies like cultural practices, herbicides, genetic manipulation, allelopathic effects, and biological control used to improve crops include competitiveness in crops. The development of integrated weed management considers all aspects of the planting system, each of which contributes to weed control, but is not necessarily fully controlled (Kennedy and Stubbs 2007). In this context, weed management shows how the biological and ecological aspects of weeds can be used as multiple control strategies to reduce weed seedlings in the soil, to prevent weeds from developing, and to preserve weeds and desired plants to minimize competition for growth (Charudattan and Dinoor 2000).

Factors that limit the scope, effectiveness, and reliability have questioned the development and acceptance of bio-control agents as applied management process in plant systems. As a single strategy approach, biological control is only effective in long-term weed control. The effectiveness of biological control can, therefore, best be demonstrated as a vital segment of weed control and combined with other weed control methods (Boyetchko 1997). Also, biological control methods can be used in biological weed control to reduce or prevent the growth and reproduction of weeds using living organisms or their products (Greaves et al. 1998). One or more organisms can be manipulated by increasing the inhibition of weed growth in one or more stages of the life cycle (Charudattan 2005).

One class of microorganisms that are widely overlooked as weed biological control agents include *Rhizobium*, which is characterized by non-parasitic bacteria (pathogenic bacteria) that colonize the surface of plant root and inhibits plant growth. It was first described on potato, beet, and wheat crop plants with beet (*Beta vulgaris L.*) inhibition (Abbas et al. 2018). Although the infestation is not parasitic, subtle nature, but rhizobia can be just as important as conventional bacterial pathogens in influencing plant growth. *Rhizobia* are plant-specific and, until recently, have not been studied in weeds. Their potential as a biological control agent has been described for the first time in downy mildew (*Bromus tectorum L.*) and several broad-leaved weed seedlings in winter wheat fields (Charudattan and Dinoor 2000).

13.7.1 Allelopathic Bacteria

The associated effects between soil microbes and plants do not only have a positive or negative effect on plant species. This interaction can reduce or increase plant diversity by creating positive and negative feedbacks (Caldwell et al. 2012; Ghorbani et al. 2005). This interaction takes place in the rhizosphere, the area of the soil that is directly affected by the plant root system. Rhizobacteria are found in the area, immediate to root surfaces. This area is suitable for settlement and can also take root in and around plant roots (Schroth and Hancock 1982; Haas and Défago 2005). The impacts of *Rhizobia* on plant growth depends on the ability of the rhizosphere bacteria to produce metabolites, the production capacity of root exudates, or their ability to compete with other soil microorganisms (Woltz 1978; Glinski 2018).

They can excrete hydrogen cyanide, different phytohormones, and phytotoxins which can reduce rates of plant metabolism. Cyanide is reported to be a basic inhibitory compound that can significantly reduce the growth of certain plant species such as alfalfa and barnyard grass (*Echinochloa crus-galli*) (Kremer 2006). They are also known as allelopathic bacteria, which can inhibit certain plant species by producing cyanide in the plant's rhizosphere, thereby inhibiting certain plant and weed species (Table 13.1). Allelopathic strains are very specific host plant selection. For example, *Pseudomonas* isolated from the rhizosphere can cause reduced growth of the pea variety (*Pisum sativum*) without affecting other varieties or wheat (Åström and Gerhardson 1988). Allelopathic bacteria have been used successfully to control the

Table 13.1 Allelopathic bacteria and their secreted toxins for weed control

Sr. No.	Bacterial species	Allelochemicals
1	*Achromobacter sp.*	Tannin
2	*Aspergillus flavus*, *A. niger*	Rutin
3	*Cephalosporium furcatum*	Ferulic acid
4	*Acinetobacter calcoaceticus*	DIMBOA, DIM2BOA
5	*Cephalosporium aphicola*	Cedrol.
6	*Pseudomonas*, *Cellulomonas*, *Achromobacter*	Vanillin
7	*Pseudomonas putida*	Gallate
8	*Pullularia fermentans*	Rutin
9	*Pseudomonas putida*	Juglone
10	*Penicillium adametzi*	Tannin
11	*Paxillus involutus*	Phenolic compounds
12	*Pseudomonas putida*, *P. nitroreducens*, *Rhodotorula glutinis*	*p*-Coumaric acid
13	*Phomopsis liquidambari*	4-Hdroxybenzoic acid
14	*Pseudomonas putida*	Phenolic acid, parthenin
15	*Rhodotorula rubra*, *Cephalosporium furcatum*, *Mortierella ramannians*	Ferulic acid
16	*Rhodococcus sp.*	Phloroglucinol
17	*Streptomyces setonii*	Cinnamic, *p*-coumaric, and ferulic acid
18	*Soil biota*	Thyme monoterpene
19	*Venturia inaequalis*	Phlorizin
20	*Cynara cardunculus*	Methanolic and ethanolic extracts
21	*Pseudomonas aeruginosa*	Hydrogen cyanide

Modified from (Mishra et al. 2013; Scavo et al. 2019; Tawfik et al. 2019)

growth of downy mildew (*Bromus tectorum L.*) and inhibit arthropods on wheat fields (Kennedy and Stubbs 2007; Kremer and Souissi 2013). The inhibition action of allelopathic bacteria depends on their ability to multiply in the root environment and on their ability to colonize the root surface. The reproduction and settlement of the population depend on the surrounding environmental factors (Charudattan 2005). The composition of the soil moisture, soil texture, and the root excretions of the host plants influences the colonization of the rhizosphere (Glinski 2018; Li and Kremer 2006; Abbas et al. 2018).

13.7.2 Cyanogenesis

It is the process of cyanide formation and occurs both in bacteria and plants. It has also been reported that some leguminous strains of *Rhizobium* produce cyanide. In most cases, it is reported that cyanide is produced from the amino acid glycine (Antoun et al. 1998). The adsorption and migration of cyanide, which is generated by such organisms, take place mainly through soil surface and solutions. It is known that cyanide formed in the soil is often combined with various metal ions, causing it to migrate quickly into the groundwater and then diffuse into the atmosphere (Rennert and Mansfeldt 2002). As previously mentioned, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* are the two most commonly studied bacteria used to produce cyanogen and are commonly found in the soil. *Pseudomonas aeruginosa* is an opportunistic pathogen which causes infections in humans, animals, and plants (Govan and Deretic 1996). In addition to various protein toxins, *Pseudomonas aeruginosa* also produces low-molecular-weight toxins such as cyanide, which promotes the overall toxicity of this possibility for many hosts (Walker et al. 2004).

Interestingly, certain *Pseudomonas* are known to be root colonizers of several crop plants that avoid pathogens (Bano and Musarrat 2003). The ability to produce iron carriers and cyanide in various *Pseudomonas* is related to their antagonistic and disease-inhibiting activities against several plant pathogens (De Vleesschauwer et al. 2006). The discovery that *P. aeruginosa* can infect plants has enabled various research groups to use the *Arabidopsis*-*Pseudomonas aeruginosa* infection model. The two opposing ecological effects of *Pseudomonas* sp. as a biological control agent and opportunistic plant pathogen indicate that the root colonization and pathogenesis caused by this bacterium are highly specific interactions. However, *Pseudomonas fluorescens* is combined with weed seedlings; toxic cyanide levels develop which significantly inhibit root growth (De Vleesschauwer et al. 2006). Although the potential for cyanation of rhizosphere bacteria from various strains (such as *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*) to inhibit weed growth or allelopathic effects has been investigated, few studies have attempted to investigate the cyanation of bacteria rhizotoxic effects (Åström and Gerhardson 1988).

13.8 Mechanism of Bacterial Phytotoxins

Phytotoxins are secreted by various plant growth-promoting rhizobacteria which inhibits germination and growth of weed seeds (Sindhu et al. 2018). These phytotoxins can be antibiotics, indole acetic acid (IAA) and its associated acids, amino levulinic acid (ALA), and hydrogen cyanide (HCN) (Radhakrishnan et al. 2018; Tawaha and Turk 2003). Different types of microbial allelopathic toxins are listed in Fig. 13.2.

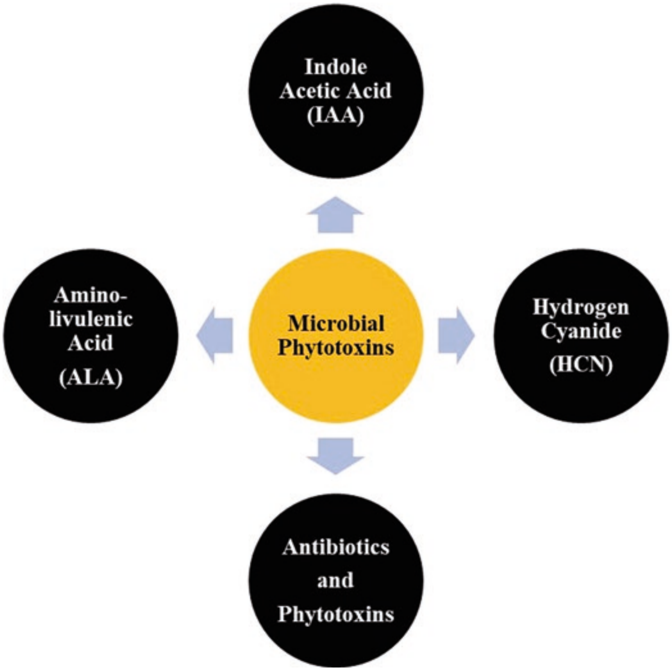


Fig. 13.2 Types of microbial allelopathic toxins

13.8.1 Indole Acetic Acid Production

The production of indoleacetic acid stimulates the plant growth at low concentrations and inhibits the same when there is higher concentration of it. It has been studied that IAA production by rhizobacteria contribute in reducing weed's root growth (Park et al. 2015) as indicated by a study in which bacterial isolates BWA18 and RWA52 with high IAA production ability ranged from 19.18 μg ml^{-1} to 53.80 μg ml^{-1} inhibited the growth of *Avena fatua* weed (Dahiya et al. 2019).

13.8.2 Amino-Levulinic Acid Production

The same mechanism as IAA production can be seen in this case. When fields with higher weed population are treated, there is increase in the accumulation of several chlorophyll intermediates, such as protochlorophyllide, protoporphyrin IX, and Mg-protoporphyrin IX which cause significant reduction (up to 92%) in root and shoot dry weights of *Lathyrus aphaca* weed by inoculation of ALA-producing *Bacillus flexus* strain JIM24 (Phour and Sindhu 2019).

13.8.3 Hydrogen Cyanide Production

Hydrogen cyanide production acts to inhibit weed root cell metabolism and cytochrome oxidase pathway (Del-Saz et al. 2016). It is also found that *Pseudomonas chlororaphis* produces HCN and secretes pyrrolnitrin and phenazine antibiotics which act as biocontrol agents (Nandi et al. 2017). Similarly, *Pseudomonas aeruginosa* (HM195190) strain KC1 isolated from the rhizosphere of castor plants (*Ricinus communis*) was found to produce cyanide (4.78 nmol L^{-1}), and seed bacterization with KC1 strain exhibited significant reduction in root length and shoot length of *Amaranthus spinosus* and *Portulaca oleracea* weed seedlings (Nandi et al. 2017).

13.8.4 Phytotoxin Production

A variety of phytotoxins are produced by plants which has the potential to be used as herbicides such as prehelminthosporal and dihydropore which kill the weeds, as per stated in a study that isolates from *Lasiodiplodia pseudotheobromae* growth inhibition to the *Poaceae* and *Valerianaceae* families. Another phytotoxin was obtained from *Pseudomonas aeruginosa* strain C1501 which has 2-(hydroxymethyl) phenol as toxin foe weeds (Adetunji et al. 2019).

13.8.5 Production of Antibiotics

Different antibiotics such as 2,4-diacetylphloroglucinol, pyrrolnitrin, phenazine-1-carboxyclic acid, 2-hydroxyphenazines, and phenazine-1-carboxamide are produced as a result of weed invasion. These antibiotics also trigger induced systemic resistance in the plant and contributes in disease suppression. Secondary metabolites isolated from *Pseudomonas syringae* strain 3366 were found inhibitory to downy brome, and these metabolites consisted of phenazine-1-carboxylic acid, 2-aminophenoxazone, and 2-aminophenol. Similarly, phenazine-type antibiotics produced by *Pseudomonas fluorescens* was found to inhibit the root growth of downy brome weed (Gealy et al. 1996).

Toxic metabolites produced by pathogenic bacteria destroy the tissue of the plant host and cause symptoms that lead to plant death (Boyetchko 1997; Kremer and Souissi 2013). Rhizosphere bacteria as one of the bacterial groups have been evaluated as weed control agents and their metabolites in various systems. It is necessary to characterize bacterial metabolites that inhibit weeds and to determine their mode of action to further improve the biological control formulations. Characterization of the mode of action can better ensure the targeted delivery of formulated products to these areas. Bacterial plant toxins use a multitude of mechanisms of action to inhibit plant growth (Li and Kremer 2006).

Most phytotoxic metabolites work by altering the metabolism of the host plant, and certain plant metabolites, once accumulated, are toxic to plant tissues (Li and Kremer 2006; Kennedy and Stubbs 2007). Syringomycin is a peptide plant toxin, made from *Pseudomonas syringae. P. syringae* induces beetroot protein-mediated plasma phosphorylation. This herbicide is toxic to many plants and fungi and is considered to cause certain important plant diseases. Syringomycin can destroy cell membranes, cause rapid K^+ release, and stimulate the ATPase of the plasma membrane. In fact, syringomycin can form pores in the plasma membrane that cause electrolyte leakage (Suzuki et al. 1992).

The ninhydrin reactivity of GAF indicates that it is a small peptide or amino acid analog and GAF inhibits the germination of *Poa annua L.* by affecting certain aspects of amino acid metabolism or amino acid function. Coronin works similarly to jasmonic acid, which is produced by plants under biological stress (Armstrong et al. 2009). Toxins and legumin have a strong antibacterial effect on glutamine synthetase or ornithine carbamoyl transferase. Genetic analysis shows the mechanisms responsible for toxin biosynthesis. The toxin comes from the lysine biosynthetic pathway. Activation of phytotoxin synthesis is controlled by a variety of environmental factors, including plant signaling molecules and temperature. *Xanthomonas campestris* strain (strain JT-P482) infects plants through wounds and multiplies in the vascular system, which prevents water transport through the production of polysaccharides and causes plants to wither and die (Shirdashtzadeh 2014).

Bacteria-secreted ethylene can also be considered a phytotoxin since pathogenic bacteria produce bacteria during the onset of the disease. Ethylene is involved in the virulence of *P. syringae*. Various studies have shown that there is a direct link between the ethylene production of diseased plants and the development of chlorosis and leaf degradation in different plant species (Shirdashtzadeh 2014). Plants react differently to ethylene than to chlorosis, senescence, and dandruff, which increases the susceptibility of plant tissues to disease. *Pseudomonas solani* and *Xanthomonas citri* are other examples of phytopathogenic bacteria that produce ethylene in plant tissues during disease development. Although ethylene as a plant hormone can influence many physiological processes in plant growth, the synthesis of microorganisms can lead to hormonal imbalances in infected plant tissues, which leads to an increased expression of the disease in various interactions between plants and pathogens (Weingart et al. 2001).

13.9 Bioherbicides Constraints

Despite the existence of enormous microbial species, only a few successful products have survived on the market due to the problems mentioned in the production of organic herbicides. The first challenge in using bacteria as a potential biological control method is the results of successful breeding laboratories and/or greenhouses in the field. Potential biological control bacteria and toxins that are responsible for weed control must survive in unpredictable environmental conditions that have a

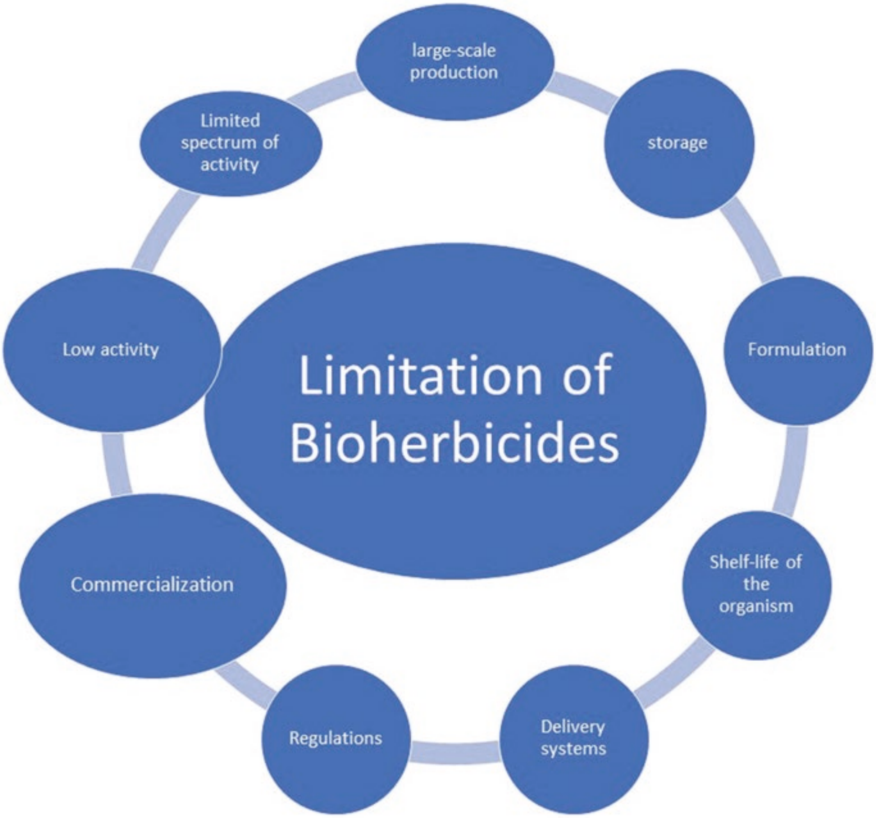


Fig. 13.3 Limitations of the bioherbicides

significant impact on the effectiveness of microbial herbicides. The inherent complexity of the rhizosphere represents an important ecosystem and an important interface in nature. It is made up of millions of genomes in 1 g of rhizosphere soil (Shirdashtzadeh 2014). Also, the diversity of bacterial species can lead to inconsistent results in biological control methods. Besides, weed absorption, age, and plant competition have changed the effectiveness of microbiological agents on weeds. The complexity of the interaction between bacteria and target weeds is another reason for the unpredictability and inconsistency of biological herbicides. For successful biological control interactions, both physiological and ecological properties should, therefore, be considered (Ghorbani et al. 2005). Some major limitation of the bioherbicides is presented in Fig. 13.3.

Biological herbicides act differently from chemical herbicides because the active ingredients of biological herbicides are living organisms. The mode of action of biological herbicides is therefore influenced by environmental conditions. Therefore, a more detailed understanding is required to increase the virulence of biological control agents. Before marketing, any negative effects must be reduced by examining the effects of active substances on non-target organisms. Potential microbial herbicides should only affect the target species without negative effects on non-target plants (Bailey 2004). An example shows the importance of host selectivity. Strain 3366 can be used as a soil root suppressant, but it is little or no selectivity between Katie rice and the weed tested, so future marketing of strain 3366 in rice is unlikely (Gealy et al. 1996).

Also, *Pseudomonas fluorescens D7* strains can suppress downy mildew, goat grass, and medusa (*Taeniatherum caput-medusae*) in bioassay, greenhouse, and field studies without having any negative effects on other plants (Kennedy et al. 2001). Extensive testing of other plant species is required because the lack of host testing often leads to the early extinction of potential biological control agents. As already mentioned, the marketing of bacterial preparations faces challenges such as the requirements for the preparations, the shelf life, and delivery systems. This can be limited by the sensitivity of the bacteria to ultraviolet light, the high humidity required for drying or infection. Formulations are necessary to protect bacteria, improve their growth or survival in the soil or in the leaves, and to provide viable active biological control agents. There are many factors to consider when formulating a suitable formulation, such as the use of aerosol, droplet size, and spray direction (Daigle and Cotty 1991; Boyetchko 1999; Byer et al. 2006; Mejri et al. 2013).

13.10 Upcoming Prospects

The discovery and development of biological control products are making great strides, but various factors limit the use of biological control methods in plant production systems. Many research projects have investigated various aspects of plant pathogens that are used for biological weed control. Examining the effects of individual environmental factors should be a first step towards understanding the limits of biological control success. Biological weed control methods are more dependent on specific environmental conditions than chemical methods. Knowing these factors can optimize the use of the time of biological control agents. The challenge is compounded by the fact that the wild environment consists of many factors that not only interact with each other but rarely remain constant over a longer period.

The chapter attempts to investigate the importance of environmental factors and the interaction of these factors in the activity of biological control agents, but compared to many biological control agents, more work, including modeling and molecular biology, would be advantageous. To weaken the link between the disease and the natural environment, it is necessary to provide potential biological control agents with a microenvironment suitable for their needs. This requires the use of formulation techniques in biological weed control, molecular biology, and new methods. It is proposed that improved strain selection, formulation, a better understanding of local soil and environmental conditions, and weed and herbicide interactions, in combination biological control methods with other non-chemical weed control strategies, should be invented to achieve more effective sustainability in sexual weed control.

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Chapter 14
Azotobacter as Biofertilizer for Sustainable Soil and Plant Health Under Saline Environmental Conditions

Shakeel Ahmad Dar, Rouf Ahmad Bhat, Moonisa Aslam Dervash, Zubair Ahmad Dar, and Gowhar Hamid Dar

Abstract *Azotobacter* belongs to the family Azotobacteraceae of kingdom bacteria which are gram-negative, motile, aerobic and free living in nature. *Azotobacter* shows peak growth within the temperature range of 20–30 °C and nurtures best in neutral to alkaline soil (pH of 6.5–7.5), but does not flourish when the pH is below 6. Conditions like pH, temperature, oxygen and inorganic salts disturb growth and nitrogen fixation capability of *Azotobacter*. It plays a vital role in the mineralization of plant nutrients, cycling of nitrogen (N) in nature and binding atmospheric nitrogen and its liberation in the form of ammonium ions into the saline soils (nitrogen fixation). The applications of *Azotobacter* as biofertilizer has shown positive outcome on germination of seeds, growth and increased proliferation of root and shoot length of plants and yield of different crops in isolation and in consonance with other bacterial biomass under saline conditions. It has been also proved beneficial with other phosphate-solubilizing microbes for improving the quality of compost. In short, *Azotobacter* as biofertilizer is beneficial to agriculture over chemical fertilizers/amendments, and due to its eco-friendly nature, it helps to address the menace of extensive agriculture and its negative impacts on the environment under saline condition.

Keywords *Azotobacter* · Saline soils · Biofertilizer · Nitrogen fixation · Growth promoter · Crop improvement

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14.1 Introduction

Azotobacter is typically motile bacteria that belong to the family Azotobacteraceae of kingdom bacteria with oval or spherical form. *Azotobacter* is described by its thick-walled cyst and can produce enormous amount of extracellular slime. *Azotobacter* is found as free-living microbe in the soil, and it plays an imperative role in the mineralization of nutrients, fixation of the atmospheric nitrogen (N) and its release in the form of ammonium ions into the soil environment (nitrogen fixation). Scientists described it as model organism for studying the diazotrophs and utilized it for the manufacture of biofertilizers, food additives and some active biopolymers. *Azotobacter* being gram-negative in nature survives well in neutral to alkaline soils (Gandora et al. 1998), in water (Martyniuk and Martyniuk 2003) and in association with some plants. After the discovery of *Azotobacter* sp., ample experimental work was initiated to utilize its ability to fix atmospheric nitrogen for crop yield improvement. *Azotobacter* promotes agricultural yield by fixing atmospheric N and by providing availability of essential plant nutrients and minerals, especially nitrogen (N) and phosphorous (P). After the application of *Azotobacter* as biofertilizer, its population increases rapidly in the rhizosphere. The existence of this bacterium has been reported from the rhizosphere of diverse types of crop plants including rice, maize, sugarcane, bajra, vegetables and plantation crops (Arun 2007).

14.2 Biological Characteristics of *Azotobacter*

14.2.1 Taxonomic Classification

Several types of *Azotobacter* are found in the soil and in the rhizosphere; some of them are *A. chroococcum*, *A. nigricans*, *A. paspali*, *A. armenicus*, *A. salinestris* and *A. vinelandii*. The genus *Azotobacter* includes six species, with *A. chroococcum* most commonly inhabiting many soils all over the world (Mahato et al. 2009). The taxonomic classification of *Azotobacter* is presented as follows:

Domain:	Bacteria
Kingdom:	Bacteria
Phylum:	Proteobacteria
Class:	Gammaproteobacteria
Order:	Pseudomonadales
Family:	Azotobacteraceae

According to Gaur (2010a), an *Azotobacter* group comprises several genera, viz. *Azotobacter*, *Azomonas*, *Azotococcus*, *Beijerinckia* and *Derxia* (Table 14.1).

Table 14.1 Vital features of the major species of *Azotobacter*

Parameters	*Azotobacter* species					
	A. chroococcum	*A. beijerinckii*	*A. vinelandii*	*A. agilis*	*A. indignii*	*A. paspali*
Habitat	Soil, water	Soil, water	Soil, water	Water	Water	Soil
Cell size (μ)	2.0–3.0 × 3.0–3.6	4.6× 2.4	3.4× 1.5	3.3 × 2.8	3.5 × 0.5	–
Cyst development	Present	Present	Present	Absent	Absent	Absent
Flagella type	Peritrichus	–	Peritrichus	Peritrichus	Polar	Peritrichus
Motility	Motile, especially in young culture	Non-motile	Motile (unseen in old cultures)	Motile	Motile	Motile
Pigment formation	With ageing	With ageing	In young cultures	Not formed or formed in young cultures	–	–
Pigment properties	Dark brown to black (water insoluble)	From yellow to pale brown (non-diffusible in water)	Yellowish-green, fluorescent (diffusible in water	–	–	–
Utilizes starch	Yes	No	No	No	No	No
Utilizes sodium benzoate	In some cases only	Yes (grows in a concentration of even 5%)	Yes (grows in concentration of 1%)	No	No	Yes
Utilizes mannitol benzoate	Yes	Yes	Yes	No	No	Yes
Utilizes rhamnose benzoate	Yes	Yes	Yes	No	No	Yes

14.2.2 Morphology

Morphologically *Azotobacter* is considered the highly variable microbe, and it also shows intricacy in the life cycle. It may be rod shaped or oval (Fig. 14.1). The early rod-shaped cell size may show variation from 2.0 to 7.0 to 1.0 to 2.5 μm, and rarely an adult cell may increase in size up to 10–12 μm (Sethi and Adhikary 2012). The cells may be disseminated or may form uneven bunch or occasionally form hackles of varying length. The new cells formed in a culture can move due to the presence of numerous flagella, but in more progressive stages, the cells drop their flexibility to move and develop capsules.

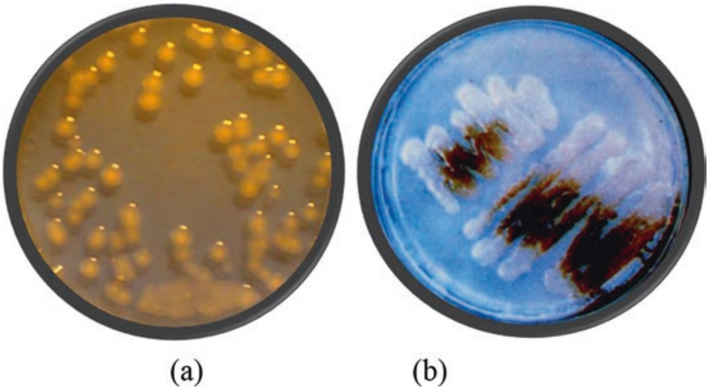

Fig. 14.1 Showing *Azotobacter* culture cells – (**a**) oval and (**b**) rod shaped

The main characteristic feature of the *Azotobacter* is its ability to form cysts. These cysts are resistant to most of the environmental factors like drying, ultrasound, gamma, and solar irradiations except heating (Fig. 14.2). In dry and water dehydrated soil climate, *Azotobacter* cysts have the ability to remain viable up to 24 years. The cysts of *Azotobacter* are spherical having central body of condensed vegetative cells bearing vacuoles embedded in a bilayer shell. Inner fibrous part of the shell is called as intine, and the external hexagonal crystalline portion is called as exine. The chief ingredients of exine are alkyl resorcinol. Cyst growth is stimulated by differences in the concentration of nutrients in liquid medium and through the addition of organic substances such as ethanol, n-butanol or βhydroxybutyrate. Bacterial polymers associated with the development of cysts are PHB, alginate, alkylresorcinols, 5-n-heneico-sylresorcinol, 5-n-tricosylresorcinol and their galactoside derivatives. Alkylresorcinols are lipids that substitute the membrane phospholipids at the time of encystment, and they also form important constituents of the external coat of the cyst (Pena et al. 2002; Segura et al. 2003a, b; Funa et al. 2006).

14.2.3 Distribution

Azotobacter species are universally occur in neutral to weakly basic soils, but never in soils of acidic. *Azotobacter* nurtures finely in the temperature range of 20–30 °C and propagates greatly in soils of pH in between 6.5 and 7.5, but is not able to flourish once the pH goes below 6, and therefore, this organism is not found in acidic soil. This bacterium has been isolated from the rhizosphere of various types of crop plants including rice, maize, sugarcane, bajra, vegetables and plantation crops (Arun 2007). Therefore, this bacterium has been given the name rhizobacteria, and it may also be found associated with different plants endophytically (Hecht 1998). These bacteria thrive well in the root region of crop non-symbiotically in case of presence of adequate amount of organic matter. They are also believed to thrive well in par-

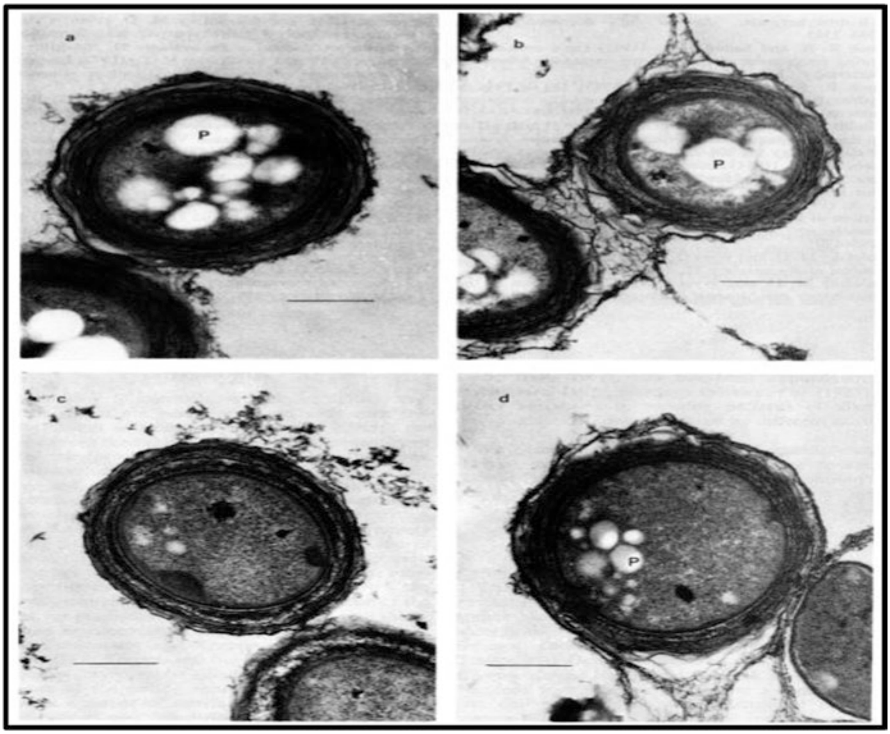

Fig. 14.2 Showing ultrathin units of mature cysts of *Azotobacter vinelandii* strain ATCC 12,837 cultured on Burk agar comprising of 0.2 mg n-butanol ml-i (**a**), 0.2 mg glucose ml-i (**b**), 0.2 mg BHB ml-i (**c**) or 0.2 mg glucose ml-a and 0.23 mM NH_4CI (**d**). Note PHB particles (P). The bar marker denote 0.5/m. (Source: Sillman and Casida Jr. 1986)

enchymatous cells of the root cortex and leaf sheath of plants. *Azotobacter* normally finds its use as biofertilizer in any type of the non-legume crops (Singh and Dutta 2006). In the soils of dry nature, *Azotobacter* can live in extreme conditions up to 24 years by forming drought tolerant cysts (Moreno et al. 1986).

14.3 Nitrogen Fixation by *Azotobacter*

14.3.1 Mechanism of Nitrogen Fixation

The N_2-fixing microorganisms can exist as self-regulating, free-living creatures or as associates of divergent grades of intricacy with other microorganisms, flora and fauna. This association may be slack, for example, associative symbiosis, or it may be intricate symbiotic associations in which the microbe and host plant share biological functions by communicating at molecular level (Sylvia et al. 1999). The mechanism of the conversion of N_2 to ammonia and the overall reactions involved there are shown in Fig. 14.3 (Brock et al. 1994).

The mechanism behind the fixation of N_2 into ammonia is briefly discussed as follows (Sylvia et al. 1999).

- Electrons released by a donor of low redox potential for example ferredoxin or flavodoxin are accepted by dinitrogenase reductase which in turn binds with two Mg-ATP molecules.
- The electrons (one at a time) are transferred to dinitrogenase.
- The complexation reaction between dinitrogenase reductase and dinitrogenase causes the transfer of electrons as a result of which two MgATP are hydrolysed to two MgADP+Pi.
- Dinitrogenase reductase and dinitrogenase detach, and the whole process is repeated.
- After the collection of sufficient electrons, dinitrogenase binds with a nitrogen molecule and reduces it into the ammonium molecule.
- Afterwards, dinitrogenase receives further electrons from dinitrogenase reductase, and the cycle is repeated.

14.3.2 Nitrogen-Fixing Capacity of Azotobacter

Nitrogen is an extremely important nutrient for the growth and development of plants as it is an imperative constituent of proteins, nucleic acids and plant pigments. The thought-provoking substitute to sidestep or cut the usage of chemically produced nitrogenous fertilizers and to safeguard the environment is the utilization of plant growth-promoting bacteria (PGPB) proficient of improving growth and yield of crop plants, with great agronomic and ecological significance. The family of microbes belonging to *Azotobacteraceae* bear tremendous potential of fixing nitrogen besides being obligate aerobes. *Azotobacter* species can fix up to 20 kg N/ha/per year approximately (Kizilkaya 2009).The isolated cultures of *Azotobacter* fix about 10 mg N/g^{-1} of carbon source under laboratory conditions. They are inexpensive and eco-friendly in nature. The nitrogen fixation abilities of innate 3-day old *Azotobacter chroococcum* strains inoculated in Ashby Media ranged from 3.50 to 29.35 μg N/ml with an average of 10.24. Besides this, *Azotobacter* sp. incubated with clayey soil, loam soil and sandy clay loam soil for 8 weeks recorded nitrogen fixation of 4.78–15.91 μ g N/g, 9.03–13.47 μ g N/g and 6.51–16.60 μ g N/g, respectively. This experiment proved that *Azotobacter* sp. show highest power of nitrogen fixation in sandy clay loam soils (Kizilkaya 2009).

Using of N-15 labelled urea has been in use to quantify the amount of atmospheric nitrogen that can be fixed by plants inoculated with *Azotobacter*. Data presented in Table 14.2 (Soliman and Momen 1994) show that nitrogen fixation contributed significant amount to total nitrogen content of corn and it ranges between 63 and 132 mg N/plant which represent the percentage between 13 and 20

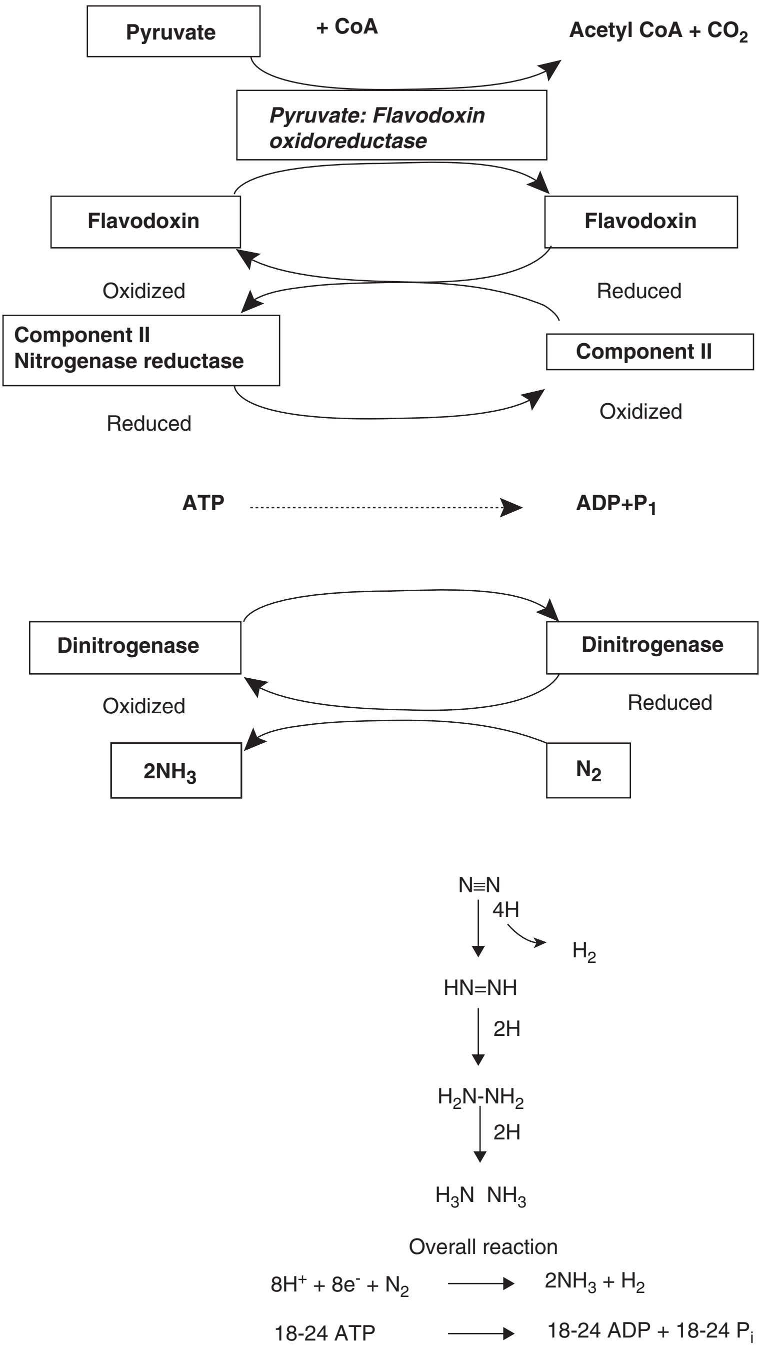

Fig. 14.3 Showing various phases involved in the nitrogen fixation process

Table 14.2 Nitrogen fixation (mg N/plant) by corn as inoculated by *Azotobacter* and fertilized with 60 kg N/acre (Soliman and Momen 1994)

	% wheat straw		
Treatment	0	1	2
Azotobacter + urea	63.0	87.0	92.9
Azotobacter + urea + DCD[a]	78.7	132.0	88.9
Azotobacter + urea + Serve[a]	71.0	99.5	91.3

[a] Nitrification inhibitors

of the total N in the corn plant (Soliman and Momen 1994). Ishac et al. (1993) reported that inoculating wheat with *Azotobacter* resulted an average of 10% nitrogen fixation when estimated using N-15 technique, and results of Table 14.1 indicate that the application of organic matter as wheat straw resulted in higher nitrogen fixation; this may be due to increase in nitrogenase activity due to the addition of wheat straw. Inoculating corn with *Azotobacter* not only reduced application of urea fertilizer (half dose) but also caused increase in fixation of nitrogen by corn. Application of organic matter and inhibitors had the additional effect on the amount of nitrogen fixation.

14.4 The Impact of Environmental Conditions on the Progress of *Azotobacter*

14.4.1 The Influence of pH on the Growth and Nitrogen Fixation Capacity of Azotobacter

The ideal pH for development and nitrogen fixation capacity of *Azotobacter* is 7.0–7.5; nevertheless, progress is constant in the pH range from 4.8 to 8.5 (George 2005). Additionally, growth is diminished in extremely acidic and alkaline conditions (Dhanasekar et al. 2003).

14.4.2 The Effect of Temperature on Azotobacter *Nitrogen Fixation*

The nitrogenase enzyme remains active over an impartially fine temperature range. At the lower limits of 5–10 °C, the activity of nitrogenase remains low. On the other hand, its activity reduces quickly at the upper limits (37–40 °C) due to the sensitivity of this enzyme to heat (Sylvia et al. 1999). The vegetative cells of the *Azotobacter* species are quite sensitive to heat, and these cells degenerate readily when kept at 45–48 °C temperature (Gul 2013).

14.4.3 The Effect of O_2 Level on Nitrogen Fixation Capacity of **Azotobacter**

Oxygen level inhibits nitrogen fixation due to the rapid and irreversible inactivation of dinitrogenase reductase. In the aerobic bacterial cell, nitrogen is fixed in presence of O_2. However, it is not needed in case of the preparation of purified enzyme such as nitrogenase. Therefore, purified enzyme is sheltered from inactivation by O_2 level through the elimination of O_2 by respiration, by the production of O_2-retarding slime coatings or by sorting nitrogenase in special type of cells called as heterocyst (Brock et al. 1994).

14.4.4 Oxygen Protection in Free Living **Azotobacter**

The fact that the supply of the nutrients, especially C, N and O_2 vary in environment and organism needs to develop some sort of protective mechanism to protect themselves from such fluctuations. *Azotobacter* has developed a well-organized system of conformational, respiratory and auto protection besides other physiological and morphological changes (Poole and Hill 1997). In *Azotobacter vinelandii* and *Azotobacter chroococcum*, a transient O_2 increase causes an activation (on) or deactivation (off) of *nitrogenase* (Poole and Hill 1997). During off mode, a safe but inactivated form of nitrogenase complex is formed called as conformation protection. This nitrogenase complex formation takes place through non-covalent bonding between Fe-S comprising redox protein (FeSII or Shethna) and Mo-Fe/Fe-nitrogenase proteins. The trigger mechanism for the creation of this complex is not known, but it is believed that the alteration in the redox state of FeSII protein or the dinitrogenase protein leads to the establishment of this complex (Moshiri et al. 1995). In case of certain O_2 shift, nitrogenase which is now in more oxidized state initiates the formation of this complex. In the switch-off mode, cells of *Azotobacter* adopt themselves to the higher ambient oxygen concentration by changing their electron flux. In high ambient oxygen environment, cyt-b oxidase is expressed which has a low apparent in vivo oxygen affinity (Marchal and Vanderleyden 2000). It is believed that cytb*d*oxidase performs in consonance with a disengaged NADH dehydrogenase (Bertsova et al. 1998). As a result of high electron transfer through this uncoupled chain, respiration rate increases, and intracellular oxygen is consumed fast without exhausting ATP and NADH pools. This process leads to the respiratory protection (Marchal and Vanderleyden 2000). Lowering of oxygen concentration by its consumption protects nitrogenase (auto protection).

14.4.5 *The Effect of Inorganic Salts on Nitrogen Fixation of* **Azotobacter**

Nitrogenase complexation requires Mg^{2+} ions for activation (Sylvia et al. 1999). Therefore, its necessity for nitrogen fixation is substantial. The occurrence of phosphorous in the medium may influence the rate of nitrogen fixation. It is the acknowledged fact that vanadium encourages N_2 fixation in various organisms as well as various species of *Azotobacter*, a few cyanobacteria, phototrophic bacteria and *Clostridium pasteurianum* (Brock et al. 1994). The reason for this is the capability of Vanadium to express vanadium-based nitrogenases in the molybdenum poor medium. Similarly combined nitrogen N_2 suppresses the nitrogen-fixing capacity of *Azotobacter* although small quantities of nitrogen enhance fixation, but higher rates of nitrogen doses drastically decrease the nitrogen fixation.

14.5 Use of *Azotobacter* for Crop Improvement

14.5.1 **Azotobacter** *as a Plant Growth-Promoting Rhizobacteria (PGPR)*

Plant growth-promoting rhizobacteria (PGPR) may be defined as ‘rhizosphere-colonizing bacteria that possess the ability to enhance plant growth when applied to seeds, roots or tubers are called plant growth-promoting rhizobacteria (Kukreja et al. 2004)’. Plant growth regulators are often defined as ‘non-nutrient organic compounds, either natural or synthetic, that affect the physiological processes of growth and development in plants when applied in low concentrations (Behl et al. 2006)’ . The term ‘plant hormone’ or ‘phytohormone’ is restricted to naturally found ingredients and comprises of four chief groups of compounds: auxins, cytokinins, gibberellins (GAs) and abscisic acid (ABA) (Behl et al. 2006). Recently, PGPR have been utilized to augment crop yield and increase agricultural sustainability. PGPR are openly involved in augmented uptake of nitrogen through biological nitrogen fixation, production of the phytohormones, mineral solubilization such as phosphorus and synthesis of siderophores that make iron available to the plant roots through chelation (Ahemad and Kibret 2014). The valuable influence of *Azotobacter* on vegetative growth and yield of maize when applied alone or in consonance with other PGPR strains was described by numerous authors (Biari et al. 2008; Gholami et al. 2009; Jarak et al. 2012). *Azotobacter* increases the yield through nitrogen fixation, synthesis of growth regulators and antibacterial and antifungal compounds (Mrkovački and Milić 2001; Wani et al. 2013). Ability of microbial inoculants can be improved by means of the notable mixture of valuable microorganisms, and it requires a vibrant characterization of helpful and vital characteristics of a microorganism selected for definite environmental circumstances. Narula et al. (2006) reported phytohormones produced by *Azotobacter chroococcum* strains (both identified and unidentified) and *Pantoea agglomerans*. They further observed increased nitrogen fixation capability of plants after inoculation with PGPR, undeviating increase of several growth parameters (increase in dry weight, growth and morphology of a root system, increased grain yield, protein content and mineral content), dislodgment of deleterious and pathogenic rhizosphere microbes, augmented phosphorus solubilization and increase in VA mycorrhiza population. Other proposed mechanisms may also be involved in crop growth, mainly enhancement of water and mineral uptake (Bashan and Levanony 1991; Bertrand et al. 2000) and production of biologically active substances (vitamins, amino acids, phytohormones, etc.) (Garcia de Salamone et al. 2001; Glick 1995; Persello-Cartieaux et al. 2003) and antibiotics (Giacomodonato et al. 2001). Verma et al. (2004) while conducting research on the relative performance of phytohormone production from the strains of Azotobacter chroococcum on wheat and suggested that the strains capable of making two phytohormones when applied with third phytohormone have synergistic effects on plant growth. He suggested that the strains capable of making two phytohormones when applied with third phytohormone have synergistic effects on plant growth. When all the three phytohormones were supplied exogenously with the non-producer strain, the non-producer strain could not compete with the strain that produced all three phytohormones. Hence, it was concluded that the cumulative effects of more than one factor are responsible for plant growth promotion. Other researchers also perceived similar results (Lippmann et al. 1995), although the specific mechanisms are not well understood yet (Glick 1995; Kloepper 1993). These results advocate the capability of *Azotobacter* strains to secrete enough quantities of phytohormones compulsory for healthier plant growth. The response of plant growth to *Azotobacter* inoculation cannot be credited to nitrogen fixation alone, but phytohormones also play noteworthy part in plant growth promotion. Since exogenously applied phytohormones deprived of *Azotobacter* inoculation could not mimic the growth effect, it is therefore recommended that other features present in *Azotobacter* have some role in plant growth encouragement. So, plant growth promotion by *Azotobacter* inoculation may be due to synergistic effects of several factors.

Kalaiarasi and Dinakar (2015) studied the application of diverse formulations of *Azotobacter* and *Paenibacillus* cells, viz. single strain inoculation, co-inoculation and co-aggregates application together with 75% suggested N and P level on the improvement of growth and yield parameters of maize under in vitro circumstances. They observed higher level of growth and yield of maize under the influence of each formulation of *Azotobacter* and *Paenibacillus* as compared to uninoculated control. Nevertheless, the application of *Azotobacter* and *Paenibacillus* cells as natural co-aggregates revealed the maximum performance followed by co-inoculation and single strain inoculation of PGPR cells. It became obvious that the use of PGPR cells, viz. *Azotobacter* and *Paenibacillus* as interbacterial co-aggregates, in collection with 75% suggested N and P level amplified the growth and yield attributes of maize to the maximum level after comparison with the control (100% recommended N and P level deprived of bio-inoculation), and thus the conservation of 25% suggested dose of N and P is probable after using co-aggregate formulations of PGPR cells. The use of co-aggregate formulations of *A. chroococcum* and *P. polymyxa* documented the maximum plant height, 68.25 cm, dry weight of root, 0.342 g/plant, dry weight of shoot, 1.713 g/plant, nitrogen concentration, 1.41% phosphorus concentration, 0.84% indoleaceticacid (IAA) synthesis, 16.31 mg/g organic carbon concentration, 0.714 per cent and chlorophyll pigment concentration and 1.54 mg/g of leaf during 45th DAS (days after sowing) (Table 14.3), respectively, after comparison with other formulations, and the maximum value of grain yield (2.84), stalk yield (3.310) and cob yield (3.18) was also recorded (Table 14.4) (Kalaiarasi and Dinakar 2015).

Bjelić et al. (2015) isolated 50 bacterial isolates from rhizosphere of maize plants through morphological and biochemical classification, which included 13 representative isolates from genus *Azotobacter*. They evaluated *Azotobacter* isolates for plant growth-promoting (pgp) properties and antifungal activity. All segregates synthesized IAA in the medium without L-tryptophan and the quantity of IAA synthesized improved with concentration of precursor in the medium. Isolate Azt10 was considered as the top IAA synthesizer in the medium deprived of precursor (26.16 μg ml^{-1}), whereas isolates Azt4 (37.69 and 45.86 μg ml^{-1}) and Azt5 (29.44 and 50.38 μg ml^{-1}) formed the principal quantities in medium supplemented with 2.5 and 5 mM L-tryptophan (Table.14.5).

Azotobacter isolates present the maximum antifungal properties against the fungus *Helminthosporium* sp. The minimum hostile impact on the *Macrophomina* sp. strain RGI, attained by conflict of isolates with verified pathogens, ranged from 10% to 48%. *Macrophomina* sp. and *Helminthosporium* sp. show significant decreasing trend in the growth attributes due to the antagonistic effect of Azt1 (19.61% and 46.76%) and Azt7 (21.96% and 48.25%) isolates. *Fusarium* sp. manifest maximum antifungal activity against Azt1 and Azt2 (38.43% and 39.21%) (Table. 14.5). Other isolates reveal notable aggressive effect against the tested pathogens. This confirms the antifungal (bio-protection) activity of *Azotobacter* inoculants. The likelihood of application of *Azotobacter* inoculants as biocontrol agents were acquired in many experiments. SubbaRao (2001) demonstrated that isolates of *Azotobacter chroococcum* formed an antibiotic which suppressed the growth of numerous pathogenic fungi. Examining the consequence of *Azotobacter* isolates against *Aspergillus flavus*, *Cercospora* sp., and *Fusarium oxysporum*, Ponmurugan et al. (2012) observed the bigger inhibition zone at advanced suspension of culture. Suresh et al. (2010) recommended the use of isolates obtained from maize rhizosphere (possessing PGPR characteristics) as potential bio-fertilizers.

14.5.2 *Effect of* Azotobacter *on Seed Germination*

Azotobacter inoculation enhanced seed germination of soybean, rice and cotton. *Azotobacter* is commonly employed in any non-legume crop. It helps in the growth and development of several cereal crops (Singh 2006). *Azotobacter chroococcum* application boosts the germination of rice seeds, maize seeds and wheat. Seeds of *basmati* rice variety, when treated with *A. chroococcum* showed 44% germination after 24 h in comparison with 3% in untreated. Further, it was observed that per cent

Table 14.3 The outcome of diverse preparations of PGPR cells on the augmentation of growth attributes of maize (Kalaiarasi and Dinakar 2015)

Treatment	Sampling after 45th DAS							
	Plant height (cm)	Dry weight of root (g/plant)	Dry weight of shoot (g/plant)	Nitrogen concentration (%)	Phosphorus concentration (%)	IAA synthesis (mg/g)	Organic carbon concentration (%)	Chlorophyll concentration (mg/g of leaf)
Control*	48.20^{e}	0.286^{e}	0.981^{e}	1.12^{e}	0.62^{e}	15.44^{e}	0.398^{e}	0.90^{e}
*Azotobacter*** *alone*	58.65^{c}	0.311^{c}	1.380^{c}	0.31^{c}	0.75^{c}	15.82^{c}	0.581^{c}	1.15^{c}
*Paenibacillus*** *alone*	53.80^{d}	0.296^{d}	1.200^{d}	1.14^{d}	0.68^{d}	15.67^{d}	0.413^{d}	1.00^{d}
Azotobacter + *Paenibacillus* CO-I**	64.20^{b}	0.320^{b}	1.561^{b}	1.34^{b}	0.78^{b}	16.24^{b}	0.631^{b}	1.31^{b}
Azotobacter + *Paenibacillus* CO-A**	68.25^{a}	0.342^{a}	1.713^{a}	1.41^{a}	0.84^{a}	16.31^{a}	0.714^{a}	1.54^{a}
LSD (P = 0.05)	0.037	0.99	0.36	0.01	0.005	0.05	0.43	0.04

a – Mean of three replication ±SD; b, values marked with different letters are significantly varied at 5% level according to student's t 'test'. * – at 100% suggested level 'N' and 'P'

**– At 75% suggested level 'N' and 'P'; CO-I, co-inoculation (1×10^7 CFU/mL inoculum level); CO-A, co-aggregation (1×10^7 CFU/mL inoculum level); DAS. days after sowing

Table 14.4 The outcome of diverse preparations of PGPR cells on the augmentation of crop parameters in maize (Kalaiarasi and Dinakar 2015)

Treatment	Grain yield^{ab} (t ha^{-1})	Proportion over control (%)	Stalk yield^{ab} (t ha^{-1})	Proportion over control (%)	Cob yield (no. of cobs/plant)	Percentage over control
Control*	2.27 ± 0.63^{e}	–	2.421 ± 0.75^{e}	–	2.96 ± 0.72^{e}	–
*Azotobacter***alone	2.54 ± 0.32^{c}	11.89	2.896 ± 0.64^{c}	19.61	3.46 ± 0.62^{c}	16.89
*Paenibacillus***alone	2.34 ± 0.22^{d}	3.08	2.842 ± 0.63^{d}	17.38	3.24 ± 0.56^{d}	9.45
Azotobacter + *Paenibacillus* CO-I**	2.70 ± 0.62^{b}	18.94	3.101 ± 0.71^{b}	28.08	3.76 ± 0.67^{b}	27.0
Azotobacter + *Paenibacillus* CO-A**	2.84 ± 0.35^{a}	25.11	3.310 ± 0.67^{a}	36.78	3.81 ± 0.62^{a}	28.71
LSD (P = 0.05)	0.015	–	0.03	–	0.07	–

a – Mean of three replications, ±SD. b – Values marked with different letters are significantly varied at 5% level as per student's t 'test'.* – At 100% suggested level 'N' and 'P'; **, at 75% suggested level 'N' and 'P'; CO-I, co-inoculation (1 × 10^7 CFU/mL inoculum level); CO-A, co-aggregation (1 × 10^7 CFU/mL inoculum level)

Table 14.5 Screening of *Azotobacter* isolates for plant growth promotion and antifungal properties (Bjelić et al. 2015)

	P-Sol					IAA Production (μg ml^{-1})			Radial growth inhibition (%)		
Isolates	PVK	NBRIP	Siderophore	HCN	EPS	0	2.5	5	Macrophomina	Helminhosporium	Fusarium
Azt1	*	*	*	–	*	4.57^{d}	28.71^{c}	36.20^{c}	19.61^{a}	46.76^{ab}	38.43^{ab}
Azt2	*	*	*	–	*	2.96^{e}	23.73^{f}	24.38^{f}	18.03^{a}	42.28^{ab}	39.21^{a}
Azt4	*	*	*	–	*	7.26^{b}	37.69^{a}	45.86^{b}	10.59^{a}	29.36^{b}	35.29^{bc}
Azt5	*	*	**	*	*	5.26^{c}	29.44^{b}	50.38^{a}	10.98^{a}	40.79^{b}	27.06^{bc}
Azt7	*	**	–	–	–	4.13^{d}	24.66^{e}	28.79^{e}	21.96^{a}	48.25^{a}	34.50^{bc}
Azt10	*	*	**	*	*	26.16^{a}	27.09^{d}	30.83^{d}	18.82^{a}	42.79^{ab}	30.58^{c}

“P-sol: 1–4 mm of halo diameter; (**) 4–7 mm of halo diameter; Siderophore: (-) no colour change (*) 1–5 mm wide of orange zone; (**) 5–15 mm wide of orange zone; HCN, EPS: (*) positive reaction; (–) negative reaction; IAA: the numbers marked with letters express a significant difference at 0.05 level of significance ($P< 0.05$)”

seed germination of wheat cv. *Sonalika* and *Pennisetum typhoides* (*P. americanum*) cv. HB-10 was 99 and 56%, respectively, when treated with the bacterium after 24 h, whereas the per cent of seed germination reached 100% after 48 h in wheat and 72 h in sorghum. Germination of rice seeds treated with *Azotobacter* was quicker with a length of shoots much more than those of control rice seeds (Singh 2006). It was further observed that nine different rice cultivars responded to *Azotobacter* differently in terms of variation in the root and shoot length. The biomass production in each cultivar was more than that of control (Singh and Singh 1996).

14.5.3 *The Effect of* Azotobacter *on Growth and Yield of Different Crops*

The use of *Azotobacter* as biofertilizer has shown positive results on growth attributes of different crops in isolation and in consonance with other bacterial biomass. Siddiqui et al. (1993) described the result of *Azotobacter*-treated mulberry cultivation after 1 month of its inoculation. The vegetative growth (plant height, the number of leaves and their size) increased significantly with *A. chroococcum.* Verma and Shinde (1993) reported that vegetable crops in general and potato, onion and brinjal in particular responded well to *Azotobacter* treatment. Baral and Adhikari (2013) studied that the *Azotobacter* has significant influence on the growth attributes of maize. Their results revealed the significant effect on the growth attributes of treatments. The inoculation of *Azotobacter* alone augmented 15–35% grain yield as compared to control. The advantage of *Azotobacter* use was greater in the lack of chemical fertilizer use. *Azotobacter* inoculation in agriculture has been evaluated for the increase in grain yield of many crops. Table 14.6 shows the response of the grain yield of different crop types by *Azotobacter* inoculation. Grain yield of pea increased by 36–60% after inoculation by *Azotobacter* over control, finger millet by 37–39%, cabbage by 33.5%, chickpea by 19–42% and similarly other crops also

Table 14.6 Enhancement in crop productivity due to *Azotobacter* inoculation

S. No.	Crop	Percent increase in grain yield
1.	Cotton	15–23
2.	Wheat	6–17
3.	Maize	15–20
4.	Sorghum	8–35
5.	Potato	6–14
6.	Pea	36–60
7.	Cabbage	33.5
8.	Rice	17.7
9.	Onion	10–17
10.	Chickpea	19–42
11.	Finger millet	37–39
12.	Pearl millet	10–12

Table. 14.7 Gain of biofertilizers (*Azotobacter* and *Azospirillum*) on yield, yield contributing characters and oil content of seeds of canola (*Brassica napus L.*) comparing to control (Yasari and Patwardhan 2007)

Treatment	Yield of Control treatment	Yield of biofertilizer inoculated treatment	Percentage increase over the control
Yield (Kgha^{-1})	2086.22	2527.99	21.7
Pods/plant	149.26	173.22	16.05
Seeds/pod (of a main stem)	24.53	24.47	−0.24
Number of branches	3.48	3.89	11.78
1000 grain weight (g)	4.143	4.264	2.92
Seed oil content (%)	44.44	45.21	1.73

showed positive response to percent grain yield against *Azotobacter* inoculation over control.

Singh et al. (1999) stated eight methods for *Azotobacter* application on rice cultivation. Out of them, inoculation of *Azotobacter* on seed and root and two top dressings (one during maximum tillering and other during the booting state) was found the best and most effective method for getting maximum grain yield of 7.73 t/ha followed by seed, root and top dressing during maximum tillering state with grain yield of 7.56 t/ha and seed, root and soil application with grain yield of 7.18 t/ha (Singh et al. 1999). Out of seven dissimilar water depths, 0.0 (100% moisture content) water depth revealed the effective results in terms of tillers per hill (2.83), panicle length (22.01 cm), number of filled grains per panicle (97.33), 1000 grain weight (27.10 g) and grain yield (3.73 t/ha). The maximum plant height was observed at 15.0 cm water depth (83.43 cm) when rice variety, *Leimaphou*, was inoculated with *Azotobacter chroococcum* (Singh et al. 2000). Grain yield of rice (cv. Sona) grown with 50:30: 30 kg NPK/ha was less than that of an unfertilized crop inoculated with *Azotobacter*. For the Integrated Nutrient Management (INM), joint effect of biofertilizer inoculation and chemical fertilizers on yield of crops has been studied extensively. The use of *Azotobacter* and *Azospirillum* increases the yield of canola crop. Yasari and Patwardhan (2007) investigated the mutual consequence of *Azotobacter*, *Azospirillum* and chemical fertilizers on the productivity of canola (*Brassica napus L.*). The yield increased by 21.17% as compared to control, elevated the quantity of pods per plant (16.05%), the number of branches (11.78%), grain weight (1000 grain) (2.92%) and the quantity of oil in the seeds (1.73%) (Table 14.7).

14.6 The Use of *Azotobacter* and Phosphate Solubilizer Inoculant in Improving Compost Quality

Azotobacter biofertilizer can be applied in combination with other phosphate solubilizers for improving the quality of compost. After thermophilic phase of composting is over, an efficient strain of *Azotobacter chroococcum* and a phosphate solubilizer (*Aspergillus awamori*) can be inoculated in a compost mass where

Table 14.8 Enrichment of the composts prepared from agricultural residues with *Azotobacter* and phosphate-solubilizing microorganisms as amendments (Gaur 2010b)

Location	Substrates	Organic carbon (%)		Total nitrogen (%)		C/N ratio	
		Without Inc.	With Inc.	Without Inc.	With Inc.	Without Inc.	With Inc.
New Delhi	Sorghum stalk + Wheat straw	28.5	21.9	1.38	1.82	20.6	12.0
New Delhi	Paddy straw	23.6	21.2	1.52	1.82	15.6	11.6
New Delhi	Paddy straw + *Leucaena* (4:1)	25.6	22.6	1.16	1.85	22.2	12.2
New Delhi	Banana leaf	36.6	32.5	1.90	2.80	19.3	11.6
Kanpur	Dairy farm waste	12.6	10.4	0.58	0.64	20.1	15.5
Hisar	Mixed crop residues	34.2	32.7	1.30	1.40	26.0	23.0
Pune	Sugarcane trash	36.0	34.5	1.08	1.34	33.0	26.0

organic matter, reaction rate and phosphorus content are optimum (Gaur 2010b). Experiments were conducted at New Delhi and other locations to prepare enriched compost. Rock phosphate was added @1–2% to narrow the C/P ratio and stimulate the process of composting, whereas the small amount of mineral fertilizer was added to lower down the C/N ration to 60:1. *Aspergillus awamori* and *Azotobacter chroococcum* were inoculated after 4 weeks of initial composting because mesophilic microbes are sensitive to the initial high temperature (Gaur and Mathur 1990). The results (Table 14.8) showed that paddy straw alone or mixed with organic residues, amended with rock phosphate and inoculated with the above-mentioned biofertilizers, not only lowered inorganic carbon and reduced the bulkiness of compost but also increased the nitrogen content of the final product from 1.3% in the control sample to 1.8% in the inoculated compost. This resulted in a total gain of 50 kgN/10 ton of the compost as compared to the control. Similarly banana leaf compost contained 2.8% N and C/N ration of 11.6: 1 as compared to control (1.9% N and C/N ration of 19.3:1). Dairy farm waste was also improved. Sugarcane trash compost contained 1.34% nitrogen with C/N ration 26:1 against 1.08% nitrogen and C/N ratio 33:1.

Enriched compost was produced from different organic residues and tested for nutrients and effects on crop plants. Enriched compost possessed better manorial properties than compost obtained from the conventional method. The yield of green gram (*Vigna radiata*) increases after the use of enriched compost. Residues (60 kg N) corrected with rock phosphate, *Azotobacter* and *phosphate solubilizers* increased overall yield up to 830kg/ha (Gaur 2010b). Highest fruit and seed yield was achieved through the application of vermicompost combined with *Azotobacter* (Thakur et al. 2012). The application of the vermicompost along with biofertilizers (*Azotobacter* and *phosphate-solubilizing bacteria*) considerably enhances crop yield attributes (Nag and Singha Roy 2008).

14.7 Degradation of Pesticides by *Azotobacter*

The capability of different species of *Azotobacter* to degrade aromatic compounds is well known. It has capability to degrade benzene and its derivatives ('*p*-hydroxy benzoate (PHB), Lindane, 2,4- D, etc.') and thus is used as bioremediation agent. *Azotobacter* has the capability to exploit many aromatic compounds, including phenolic compounds and chlorinated phenols (mono, di- and trichlorophenols). The degradation of numerous chlorinated phenols, viz. '2-chlorophenol, 4-chlorophenol, 2,6-dichlorophenol and 2,4-6-trichlorophenol', by *Azotobacter* has also been reported (Gaofeng et al. 2004).

Anupama and Paul (2010) investigated the potential of *Azotobacter chroococcum* for degrading lindane. From the ten isolates cultured during the experiment, the best strain, viz. *A. chroococcum* JL 102 was selected for further studies. This strain was exposed to a lindane concentration of 10 and 100 ppm. The bacterium was grown in two dissimilar media, viz. Jensen's broth and soil extract broth. Further, ex situ deprivation of lindane was studied for 6 days. The highest degradation of lindane was noted at 10 ppm concentration (Fig. 14.4). The degradation was greater in Jensen's medium, compared to the soil extract broth. The performance of *Azotobacter* in pot culture experiment comprising both sterile and non-sterile soils and amended with 10 ppm of lindane concentration revealed temporal increase in the degradation potential of these bacteria. The maximum degradation is being witnessed at the end of the 8th week of cultivation (Fig.14.5). Approximately 95% of lindane used in the

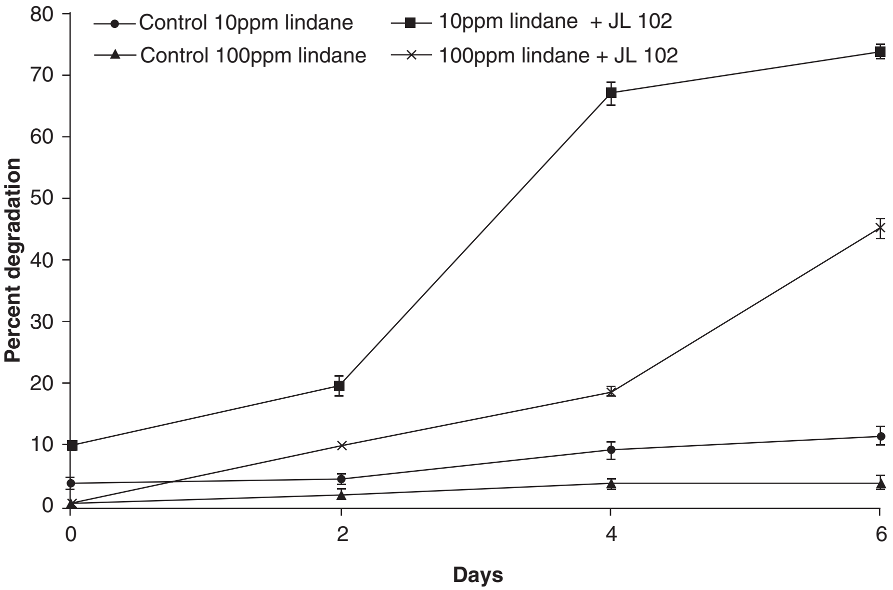

Fig. 14.4 Showing lindane degradation potential of *Azotobacter chroococcum* strain JL 102 in Jensen's broth (Anupama and Paul 2010)

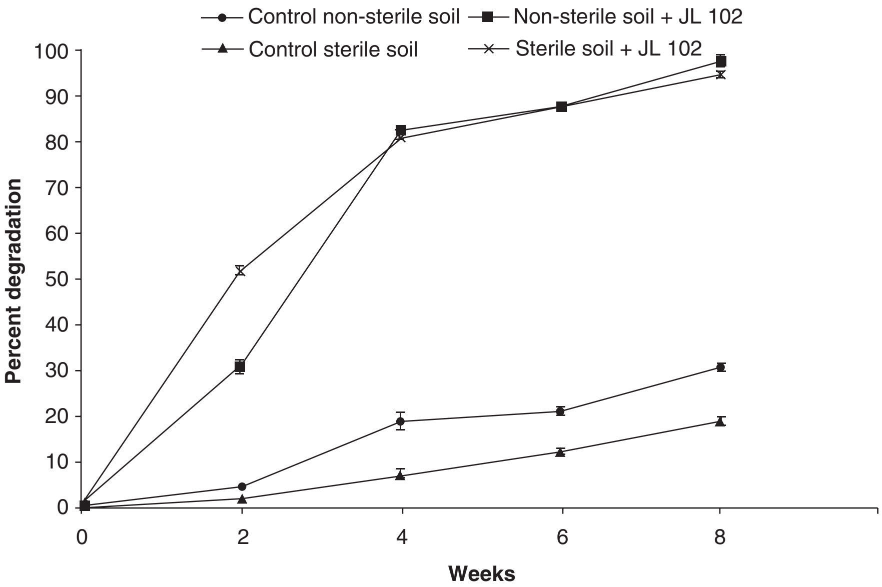

Fig. 14.5 Lindane degradation potential of *Azotobacter chroococcum* strain JL 102 in soil (Anupama and Paul 2010)

experiment was degraded at the end of 8th week. The outcomes from the above-mentioned results confirm the ex situ and in situ lindane-degrading ability of *A. chroococcum* strain JL 102. Thus, this strain can be used for bioremediation of lindane contaminated sites.

14.8 Advantages and Limitations of Using *Azotobacter* as Biofertilizer

Azotobacter is beneficial in many perspectives for agriculture over usually applied chemical fertilizers. Application of *Azotobacter* strains as biofertilizer inoculants to the crop fields raises the proportion of seed propagation and seedling growth. For example, to confirm the combined effect of biofertilizer and inorganic fertilizer on the germination and growth of the tomato plant, the combined mixture of inorganic fertilizer (N) and biofertilizer (*Azotobacter*) was used. The results obtained showed overall increase in the growth of the tomato crop (Mahato et al. 2009). Application of *Azotobacter* inoculants also increases root and shoot length of plants. Singh (2006) observed that germination of rice seeds treated with *Azotobacter* was quicker with the length of shoots much more than those of control rice seeds. Improved nitrogen uptake and nutrition is another important benefit of using *Azotobacter* as biofertilizer without creating environmental problems. *Azotobacter* is free-living heterotrophic microorganism, proficient enough to fix approximately 20 kg N per hectare per year. Besides, it also synthesizes plant growth-promoting ingredients and is also considered as the antagonistic agent to the disease causing microbes (Kizilkaya 2009). Application of *Azotobacter* biofertilizer in rhizosphere or its foliar use reduces the incidence of diseases by inhibiting the growth of many pathogenic microorganism organisms. *Azotobacter* species also have the potential to produce antifungal precursors to fight against many plant pathogens (Chen 2006). One of the species of the *Azotobacteraceae* family, namely, *A. nigricans*, has been in use as potential biocontrol agent. This species bears antifungal properties besides its role in plants as PGPR. It can be effectively exploited for the control of the potential mycotoxigenic *Fusarium* sp. linked with the cereal crops (Nagaraja et al. 2016). *A. chroococcum* is considered as the potential inhibiter of the egg hatching process of insects such as *Spodoptera litura* (Fab.), *Spilarctiaobliqua* (Walker) and *Corcyra cephalonica.* It also drastically reduces the egg laying capacity, per cent pupation and the development of adults from pupae. It prevents harmful microbes by generating siderophores and antifungal amalgams and by encouraging defence enzymes. *Azotobacter* application escalates the post-harvest seed quality in relation to germination. Further, it also increases the grain yield, when applied as biofertilizer.

In terms of disadvantages, *Azotobacter* biofertilizer has several selling constraints at this stage of development. This biofertilizer has short shelf life. It must be used within prescribed time. Good storage facilities are lacking particularly in developing countries. Literally consumers are generally not fully aware about the use of *Azotobacter* biofertilizers due to fewer efforts for promotion and production of biofertilizers. Environmental limitations are soil pH, temperature conditions and improper and excess use of chemical amendments. Draught, high temperature, water logging conditions, antagonism from other microbes and incompatibility with other pesticides are big constraints in the way of use of *Azotobacter* biofertilizer. Mass production of contaminated *Azotobacter* biofertilizers by unskilled staff and technical constraints in the beginning has created problems in utilization of biofertilizers by farmers. Thus there is a desperate need of awareness among the consumer community to regain their faith about the benefits of using different types of biofertilizers for the sustainable production of crops.

14.9 Conclusion

Azotobacter has been extensively evaluated for its use as biofertilizer. This species has the potential to survive in basic to alkaline environmental conditions but not in acidic conditions. *Azotobacter* can fix nitrogen, solubilize nutrients or produce growth-promoting precursors on its application in the root zone (rhizosphere) of the crops. It can perform nicely both in isolation and in combination with other bacterial consortia. This species is able to increase plant yield and protect crops from pathogens and has the potential to degrade recalcitrant biocides. Therefore, its use as biofertilizer is recommended chiefly in harsh environmental conditions.

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Chapter 15

Role of Microbiota in Composting

Lone Rafiya Majeed, Sumaira Rashid, Heena Nisar Pahalvi, Bisma Nisar, and B. A. Ganai

Abstract Waste management in developing countries is so hard to achieve owing to limited resources; composting paves the way due to its adaptability for long-range situations and environmentally sound method as it reduces pollution to a larger extent and also has less potential for environmental degradation comparatively. Decomposition needs direct interaction between the substrates with decomposing substance and the exterior layer of different microbial species. As chemical decomposers, microbiota such as microbes, fungi and actinomycetes break down organic matter to carbon dioxide, water, heat, humus and relatively stable organic end product. Knowledge on waste management demonstrates that composting is an environmentally and economically sound waste treatment process. One of the benefits is that organic waste is converted to a mineral and generates organic fertilizer by application of microorganisms. Composting reduces waste to be dumped in landfill, recycles humus and nutrients in soil, protects and improves the microbial diversity of the cultivated soils and thus reduces the overall pollution. Composting has main disadvantage of odour nuisance, proliferation of potent pathogens and groundwater pollution when carried out onsite.

Keywords Decomposition · Actinomycetes · Anaerobes · Nuisance

15.1 Introduction

Generation of wastes worldwide is the expected and accepted artefact of urban sprawl, unsustainable development and population growth. A worldwide Review of Management of Solid Waste estimated global waste, in its 2012 edition of What a Waste: world's annual waste production is 1.3 billon tonnes (Fig. 15.1) (Hoornweg

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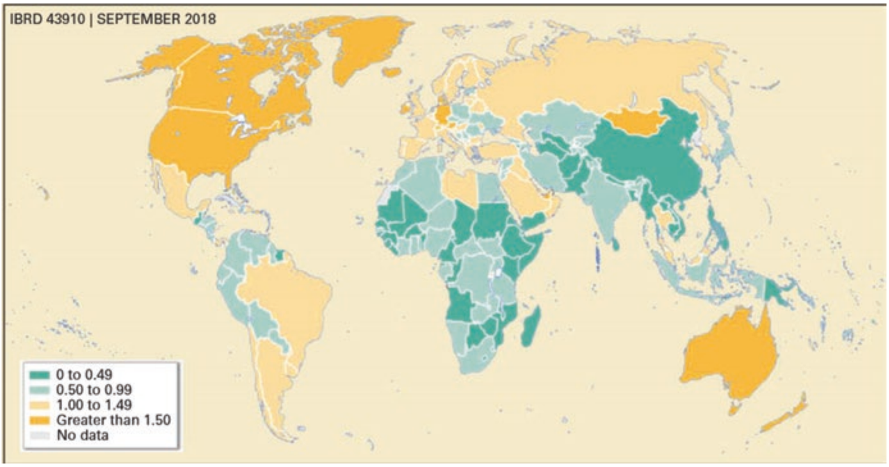

Fig. 15.1 Worldwide waste generation. (Source: IBRD 43910 September 2018)

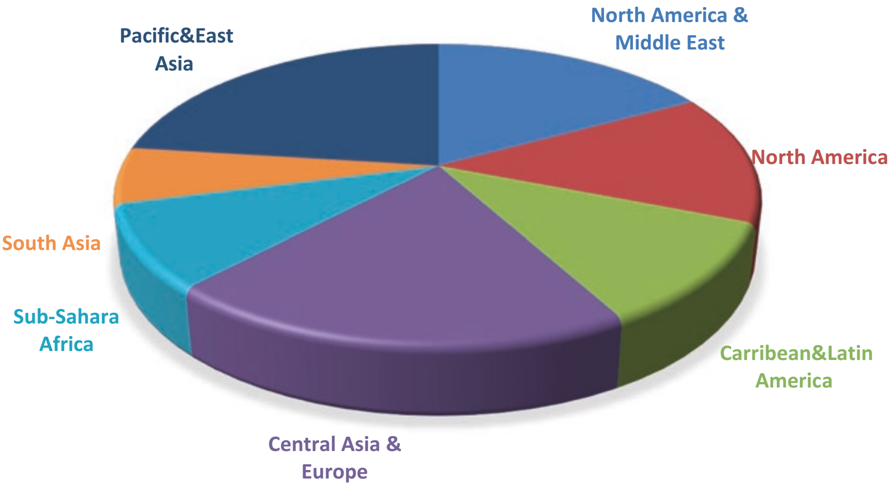

Fig. 15.2 World share in waste production

and Bhada-Tata 2012). Economic prosperity and population growth of every nation offers more goods and services to its citizens that contributes to high waste production. In recent eons, waste generation has escalated at levels constant with preliminary prognoses, and data tracking and reportage have upgraded considerably. The latest data available shows that during 2016 waste generation reached to 2.01 lakh tonnes. Countries of Central and East Asia and Pacific and Europe account for about 43% of global waste by magnitude and account for about 468 million tonnes annually in 2016, with an average per capita of 2,21 kilograms a day, and the top countries with highest per capita waste generation are Canada, Bermuda and the USA (Fig. 15.2). Waste production in Sub Saharan and African areas is least and makes up 15% of the global waste with less than 129 million tons a year, a total of 0.52 kg per day in South Asia, 0.46 kg a day in Sub-Saharan Africa and 0.56 kg a day in Eastern Asia. Overall, the global waste production estimated for 2016 is approximately 20,52 billion tons per capita (World Food Programme 2017). Economic development and waste generation has a positive correlation. Rising shifts in wages usually show that waste production rises at a slightly lower rate of income. Waste composition is the classification of kinds of constituents in municipal solid waste. Food loss and waste (FLW) is an ubiquitous issue (Fig. 15.3) and a serious challenge for, food safety and security, and environmental sustainability. Magnitude of food loss and wastage has no accurate assessment, but research indicates it has a share of 30% of all global waste production that sums about 1.3 billion tonnes per year (FAO 2015). FLW represents not only loss of Food but it represents losses through labour, land, water, and energy used to produce food (McClellan 2017). Food and Loss wastage has its contribution to global climate change also as its cultivation, production, processing and decay when wasted produce considerable levels of greenhouse gases like methane (African Union Commission 2014). Waste audits at different levels and places around the world showed that about 44% of the discarded waste is food and greens (Hoornweg and Bhada-Tata 2012) which in various countries is subjected to composting with purpose of manure and biofertilizer (SAVE FOOD 2018).

Mixed organic substrate is prerequisite for every composting facility. As per its Etymology, the word composting has been taken from Latin word ‘Compostium’ which means mixture that refers to a mixture of substrates biodegradation done by a microbial group consisting of different populaces in strong and oxygen rich conditions under the name of fermentation or organic oxidation, but not composting, microbial transformation of pure substrates. The process of compositing involves numerous aerobic microorganisms like yeasts, bacteria, fungi and other organisms who putrefy raw organic material into humus, CO_2 and water vapour with the release of energy in

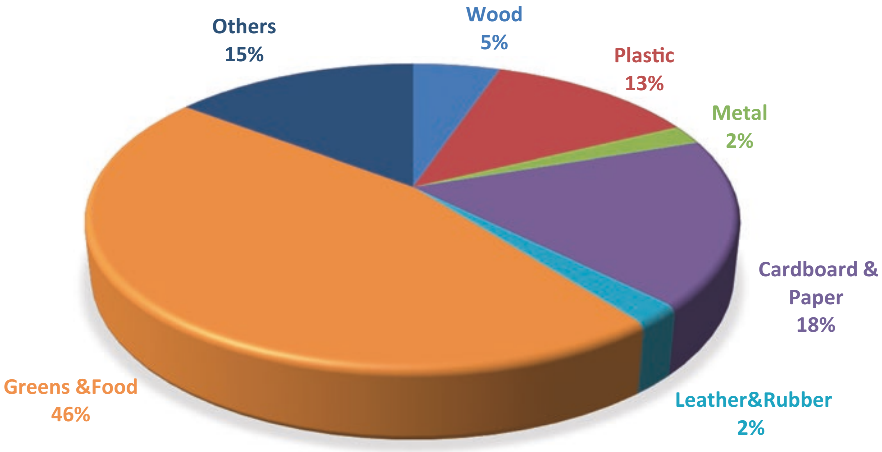

Fig. 15.3 Composition of solid waste produced

Fig. 15.4 Process of compost formation

the form of heat (Ryckeboer et al. 2003) to gain material and energy required for evolution and replication (Fig. 15.4). The by-products of this process is called compost which is due to disintegration of biomass of both living and dead organisms and the undegradable parts of raw material (McClellan 2017).

Composting involves the microbiota that needs special environmental settings of nutrition micro- and macronutrients in passable amounts with oxygen and water. Microbiota of composting only flourishes within the limited range of pH and temperature. Most of the microorganisms for composting are readily available in the municipal solid waste itself, and they tend to multiply rapidly in favourable conditions (Amner et al. 1988; Faure and Deschamps 1991; Finstein and Morris 1975; Strom 1985; Beffa et al. 1996). The area normally required for composting is approximately 25m^2/tonne of municipal solid waste with additional area needed for machinery, packing and storage. Composting facilities also need to have leachate treatment, sanitary landfill and facilities area to have recycling mechanisms without harming ecology (Advisory on On-Site and Decentralized Composting of Municipal Organic Waste June, 2018). Treating organic waste by using class of higher organisms (Oligochaeta) of Annelida, i.e. earthworms *Perionyx excavatusa*, *E. eugeniae*, *E. fetida*, etc. This process is odourless, very much natural and aerobic and different from the traditional composting (Fig. 15.5). Earthworms in their alimentary canal change the consumed waste into fertilizer and lay that as oil droppings called as castings (Khan et al. 2017). Maintaining higher levels of aeration in pile of composting is very much challenging as meeting this issue creates many other issues like loss of moisture content (Sahoo and Gupta 2017).

15.2 Methods of Composting

Various composting methods are used for the farming operations. Many factors govern the method to be chosen like land availability, labour investment, capital investment, time investment and quality of raw material. In comprehensive compositing, the procedures utilized are the passive piles, aerated static piles, windrows and then the in-vessel systems (Central Environmental Authority 2013). In developed countries, municipal-scale compositing is very common and is carried out to progress waste management for the sake of environment and financial purposes. The various levels of mechanization involved in composting are high-tech forced aeration, biofilters and low-tech manually turning of piles (Herder and Larsson 2012). High technology is not inherently an asset, although towns can choose a lower cost, manual method for aerated wind composting using a mechanical in-vessel composter

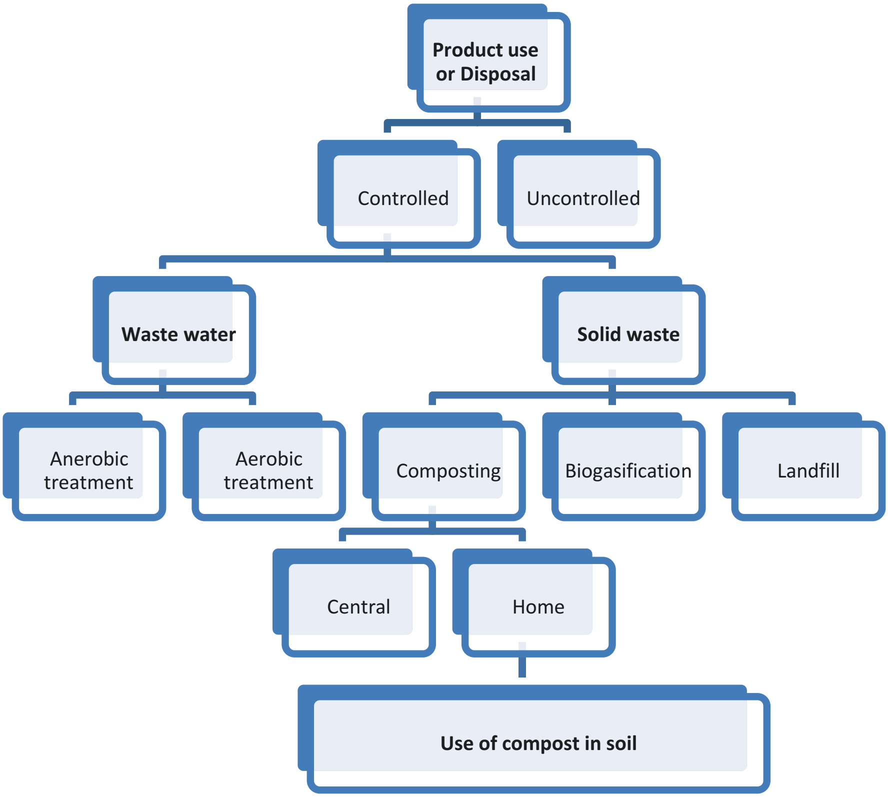

Fig. 15.5 Flow chart showing the process of composting

depending on job, organic waste feed and land availability (Fig. 15.6). For example, in case of MSW, the organic waste used should be pure; only then in-vessel composting is applicable; otherwise aerated windrow composting can be carried out (Tables 15.1 and 15.2). Worldwide different operators are involved in municipal-scale composting like farmers, private companies, non-governmental organizations and municipalities themselves (Sourav 2015).

15.3 Microbiota in Composting

Browne (1933) has been the first person to show how biological activity is responsible for self-heating of composts. In the 1930s, Waksman published numerous articles on composting microbiology and was the first person to highlight research on dynamics of population (Waksman 1932). Since then, isolation and cultivation methods have been the base for the study of compost microbial populations for five decades (Finstein and Morris 1975). Soils and sediments demonstrate that with the

Fig. 15.6 Depicting various field composting methods

techniques that are currently available, it is only possible to grow a small fraction (< 1%) of microorganisms (Torsvik 1980). DNA and RNA methods recently developed indicate that multiple unknown microorganism species still exist (e.g. Beffa et al. 1996) in composts. Production of green manure is so precarious part of organic waste handling, and use of compost in soil management is increasing exponentially. However we lack thorough knowledge about microbial community involved in composting. So high quality techniques to evaluate the composition and diversity of micro biota of composting facility are used to produce quality compost at commercial scale.

15.3.1 Bacteria

Bacteria are most abundant biotic elements surpassing 1 billion per gram of soil; they stabilize aggregates of soil particles of smaller size by defecating the compounds that fix soil particles and organic matter together (Mansfieldct.org). The role of bacteria in composting was unkempt owing to the microscopic nature of bacteria that was over shadowed by visibility of actinomycetes and fungi. Type of composting depicts the role of particular microbe; like in composting of sewage sludge, bacteria have more prominence over others (Strom 1985). Other factors that determine role of bacteria are time and temperature of composting. When temperature is

Table 15.1 Type of composting

Composting types	Scale	Associated problems	Required resources
On-site composting Composting on grounds using bin or a pit in the soil	Small-scale	Odour	Pit or bin
Vermicomposting Composting is done bins where earthworms degrade organic materials	Small-scale	Temperature sensitivity	Worm, worm bins
Aerated windrow composting Composting is done in open in which organic materials are structured in rows and repeatedly turned/aerated	Large-scale	Area, zoning, controlling implementation	Plot, apparatus, continuous labour
Aerated static pile composting Composting done in stationary piles in which organic materials that are internally aerated with blowers	Large-scale	Area, zoning, controlling implementation	Land, substantial fiscal capitals, apparatus like fans blowers, pipes and sensors
In-vessel composting Composting is done through mechanized machine that processes substrate and then needs compost to settle outside the machine for some weeks	Medium-scale	Reliable power supply, financially exhaustive, technical expertise required	Electricity, expert employment, continuous financial resources, land facility

Source: United States Environmental Protection Agency

kept under 60 °C during first 7 days, 40% of waste is decomposed by bacteria. Temperature varying from 50 to 65 °C shelters the bacteria of *Bacillus* genus generally. In temperatures that are exceeding 65 °C, species like *B. stearothermophilus* thrives. Moisture content, temperature and acidity are limiting factors for bacterial growth. Moisture exceeding 5% and a given range of temperature is basic to keep bacteria in composting pile alive (Cornell.edu). Bacteria compost waste very efficiently excretes vital plant nutrients NPK (Abu-Bakar and Ibrahim 2013). Compost piles are dominated by bacteria mostly that of psychrophiles (13 °C), mesophiles (21–32 °C), and thermophiles(45–71 °C). Psychrophilic bacteria thrive even in freezing temperatures, and their activity shoots up around the temperature of 13 °C. Contrarily, mesophiles flourish at warm temperature, i.e. 22 °C–32 °C. With the rise in temperature of pile, mesophiles are gradually replaced by thermophiles, i.e. temperature ranging between 45 and 71 °C. Thermophiles raise the temperature of pile continuously for 5 days and consume maximum of the degradable waste available in the pile. As the available bioproducts start to decline, the thermophiles get replaced by the mesophiles which consume the leftover and aid other organisms of higher order.

Table 15.2 Various microorganisms composting

Genus species	Ecological relevance (B)	Succession stage	References
Bacteria			
Pseudomònas putida strain *ATCC 11172*	Potent pathogenic		Alfreider et al. (2002)
Pseudomònas sp.			Miller (1996)
Methylòsinus trichòsporium	Methanotrophic		Murrell et al. (1998)
Caulòbacter spp.		Early	Michel Jr et al. (2002)
Erythròbacter lòngus		Early	Michel Jr et al. (2002)
Nitrosòspira briensis	Nitrifier		Murrell et al. (1998), Kowalchuk et al. (1999)
Nitrosòmonas europaea	Nitrifier		Murrell et al. (1998), Kowalchuk et al. (1999)
Nitrosòlobus multiformis	Nitrifier	Middle	Michel Jr et al. (2002)
Escherichia coli	Potential pathogen		Lott fischer (1998)
Methylòmonas methanica	Methanotroph		Murrell et al. (1998)
Azotòbacter chròococcum	N-fixer	Late	Bess (1999)
Salmonella sp.	Pathogenic		Lott Fischer (1998)
Streptomyces rectus			Miller (1996)
S. thermòfusus			Miller (1996)
S. Violoaceus- ruber			Miller (1996)
S. thermòviolaceus			Miller (1996)
Streptòmyces sp.			Miller (1996)
Nòcardia sp.			Miller (1996)
Microbispora bispora		Thermophilic	Miller (1996)
Actinomadura sp.		Thermophilic	Degli-Innocenti et al. (2002)
Bacillus stearòthermophilus	Classic thermophilic bacterium in composts		Various
B. thermòdenitrificans	Thermophilic denitrifier		Miller (1996)
B. brevis			
B. circulans			
B. sphaericus			
B. subtilis			
B. lichenifòrmis			
Bacillus sp.	Potential pathogen		Lott Fischer (1998)
Clòstridium thermocellum			
Clòstridium spp.	Some are N-fixers	Anaerobic	de Bertoldi et al. (1983)

(continued)

Table 15.2 (continued)

Genus species	Ecological relevance (B)	Succession stage	References
Klebsiella sp.	N-fixation		de Bertoldi et al. (1983)
Actinomycetes			
Saccharòmonospora viridis	Pathogenic		Lott Fischer (1998)
Streptòmyces thermovulgaris	Pathogenic	Thermophilic	
Actinòbifida chròmogena			Miller (1996)
Thermòactinomyces vulgaris		Thermophilic	Miller (1996)
Micròpolyspora faeni			Miller (1996)
Pseudònocardia thermophile			Miller (1996)
Thermomonòspora curvata			Miller (1996)
T. viridis			
T. sacchari			
Thermus sp.		Thermophilic	Beffa et al. (1996)
Hydrògenobacter			
Fungi (Zygomycetes)			
Mòrtierella turficola	Decomposers		Miller (1996)
Mucor miehei	Zymogenous	Early	Miller (1996), de Bertoldi et al. (1983)
M. pusillus		Thermophilic	
Rhizòmucor pusillus	20–25 °C, typical early colonizer		
Rhizòmucor sp.			Miller (1996)
Fungi (Ascomycetes)			
Chaetòmium elatum	Soil inhabitant	Early and late	Ivors et al. (2002)
Chaetòmium thermophilum	Decomposer	Thermophile	Miller (1996)
Dactylomyces crustaceus			Miller (1996)
Aporòthielavia leptòderma			Ivors et al. (2002)
Thielavia thermòphila			Miller (1996)
Thermòascus aurantiacus		Thermophile	Miller (1996)
Fungi (Basidiomycetes)			
Armillaria mellea	Cellulolytic and ligninolytic	Mesophilic	de Bertoldi et al. (1983)
Clitòpilus insitus			
Pleuròtus ostreatus			
Fomes sp.			
Còprinus sp.	Coprophilous	Early	Miller (1996), de Bertoldi et al. (1983)
C. Cinereus			
Lenzites sp. L trabea			
Aspergillus fumigates	Wood putrefying	Mesophilic and thermophilic	Miller (1996), de Bertoldi et al. (1983)

(continued)

Table 15.2 (continued)

Genus species	Ecological relevance (B)	Succession stage	References
Humicòla insolens	Potential allergenic pathogenic, heterotrophic	Early and late, thermophilic	Lott fischer (1998), de Bertoldi et al. (1983)
Thermòmyces lanuginosus		Thermophilic	http://helios.bto.ed.ac.uk/bto/microbes
Paecilòmyces sp.	Cellulolytic		de Bertoldi et al. (1983)
Scopulariòpsis brevicaulis	Cellulolytic		de Bertoldi et al. (1983)

Source: The Humanure Handbook Third Edition (2005)

15.3.2 Actinomycetes

Actinomycetes are higher forms of bacteria that choose slightly alkaline or neutral pH to disintegrate complex substrates. Actinomycetes prefer moderate heat range of pile. Their routine is affected by the bacterial presence due to antibiotic they produce, so actinomycetes stay away and live in greyish clusters. Actinomycetes have the ability of decomposing more complex materials like proteins and starches and so they discharge carbon, nitrogen and ammonia that creates earthy smell in the pile (Shukla 2014). Actinomycetes decay the most sturdy complexes of the last stage of pile. Enzymes that chemically produced by various actinomycetes help in the break down of difficult waste, such as woody stalks, leaves, and newspapers (Moset et al. 2015). Actinobacterial growth is especially strong in the second phase. *Actinobacteria* can be seen in deep blisters with the naked eye (the temperature is around 45 ° C and humidity is relatively low).This process is called a firefang time. This actinobacterial process is essential for the cultivation of *Agaricus*. The regulated moisture and air supply must attempt to maintain a temperature of 48 ° C across the whole substratum (not only within a certain zone). The main purpose is to replenish ammonium by microbial activity. Compost overheating (> 70 °C) will cause an irreversible material alteration and release of ammonia.

15.3.3 Fungi

Fungi are very significant among the microbiota detected throughout composting owing to their capability to decay dry intractable substrates that are acidic and less nitrogen containing as compared to bacteria. Fungi get nutrients from dead plants and break down debris in compost, allowing bacteria to continue decomposing without cellulose. Like single cells, they form elongated filaments into long hyphae, which are bigger compared to actinomycetes (Ivors et al. 2002). Composting materials which include both chemical and mechanically obstinate organic matter like lignin and cellulose can be penetrated by hyphae (Nutongkaew 2014). Fungi are prevailing in both thermophilic and mesophilic stages of composting that is totally anaerobic and so provide ventilation and drainage to the compost to allow it to get stabilized (Kowalik and Sadurska 1972). During foremost phase of composting, fungus race with bacteria for the existing substrate still gets outpaced by bacteria owing to their maximum specific growth (Griffin 1985). Good oxygen supply is also more critical in fungi than in bacteria, and transient anoxia conditions may also occur in force-aerated systems. This is why fungi play a trifling function through the thermophilic phase but also because of the lower thermotolerances. One exception is the composting of cellulose- and lignin-rich substrates. Fungi are the most critical during the whole cycle (Franke-Whittle et al. 2006). The need for water reduces, which is an advantage for fungi, in subsequent composting stages.

15.3.4 Higher Animals

Higher organisms continue to enter the pile when the stack cools up to acceptable temperatures. Protozoa, rotifers and nematodes include these species. They eat biomass from bacteria and fungi and help kill lignin and pectin. These species tend to prevent the compost's infection (Fig. 15.7).

15.4 Factors that Effect Composting

15.4.1 Moisture Content

An essential element in the microbial putrefaction of organic waste is the compost's optimal humidity content (MC). For an appropriate composting, the initial MC of around 60% is adequate (Elcik et al. 2016). It has a significant role in dissolving and transporting nutrients, vital for the microbiota of compost pile (Liang et al. 2003; Cabanas-Vargas et al. 2005; Kumar et al. 2010). Below 40% of MC the microbiota of pile becomes inactive and above 60% decomposition slows down, and anaerobes release objectionable odour .Optimum moisture requirement is given in Table 15.3.

15.4.2 Temperature

Composting is a heat-generating exothermic process because of the oxidative aerobically proceeded composting reactions of materials. The compost's operation generates heat and decomposes (oxidizes) the organic material through activity of

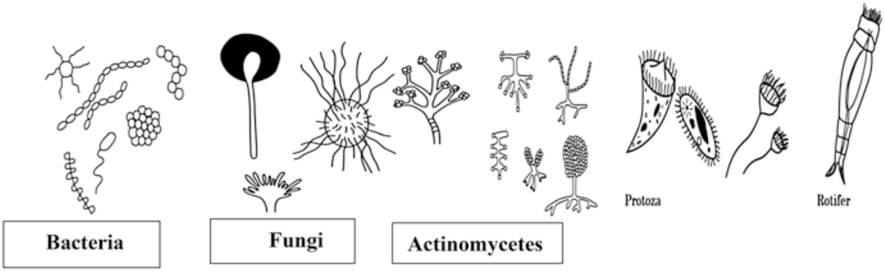

Fig. 15.7 Organisms used in composting

Table 15.3 Optimum moisture content for compost facility

Optimal MC	Raw material used	References
69%	Wheat straw with pullet manure	Petrica et al. (2009)
<80%	Sludge and sewage	Liang et al. (2003)
65 –70%	Corncob sludge manure	Zhu (2006)
50–60%	Pig manure with sawdust	Tiquia et al. (1996)
60–70%	Solid portion pullet and straw	Kalyuzhnyi et al. (1999)

bacteria and other microorganisms. The optimum temperature range is from 32 to 60 °C for its compost production. The behaviour, if the temperature is outside this range, is slowing or could be killed. The rise in compost temperature beyond 55 ° C kills plant's bacteria, like *Shigella* and *Salmonella*, thereby reducing the risk of the transmission of diseases from contaminated soil. Outside the composting process the temperature has an influence. In spring and summer process exhibits peak, the compost process is slower in winter comparatively.

15.4.3 Carbon to Nitrogen Ratio

Microbiota responsible for the organic matter decomposition require C:N to expand and replicate as a nutrient. When the C:N ratio is 30:1, the microbes function vigorously. If the C:N ratio surpasses 30, the composting rate will decrease (Michel et al. 1996). The organic waste material decomposition will get dawdling down if the C:N ratios come down to 10:1 or surpass 50:1 (Table 15.4).

15.4.4 Amount of Lignin

Lignin forms the main component of plant cell wall owing to its complex chemical structure; it is highly sturdy to microbial degradation (Richard 1996). There are two implications for this lignin type. Lignin declines the bioavailability of additional

Table 15.4 Ideal C: N ratio for compost pile

Carbon/nitrogen ratio	Raw material used	References
19.6	Food waste	Kumar et al. (2010)
20	Saw dust and chicken manure	Ogunwande et al. (2008)
20	Rice straw and pig manure	Zhu (2007)
15	Swine compost and saw dust	Huang et al. (2004)

cell wall constituents, and the true C: N ratio is reduced in contrast with the normal ratio of biodegradable C and N. Moreover, lignin acts as a permeability intensifier and provides favourable aerobic composting conditions. The extra lignite-disintegrating fungi sometimes upsurge the availability of C in compost and decrease N loss, but C:N ratio and low porosity may be greater in other situations.

15.4.5 Polyphenols

Polyphenols contain water-soluble and reduced tannins (Schorth 2003). Tannins that are insoluble fix proteins and cell wall to reduce their physical or chemical availability to decomposers. Protein interacts with soluble tannins that weaken the microbes and thereby decreases N. Lignin and polyphenols are more appropriate as inhibiting factors. Palm et al. (2001) recommended the use for more productive usage of the natural resources on farm with organic materials, like composting, in separating the content of these two posts.

15.4.6 Oxygen (Aeration)

The supply of continuous oxygen is required for aerobic fermentation. In order to achieve maximum performance, aeration must be maintained in a controlled way. For aerobic composting, in particular during the starting process, large amounts of O_2 are needed (Diaz et al. 2002). Ventilation is the oxygen source and so is essential for aerobic composting (Cabanas-Vargas and Stentiford 2006). If O_2 is not adequately given, the growth of aerobic microorganisms is reduced and the breakdown is slower. An excessive breath, vapour and other pillowed gases are prevented by aeration. The chance of overheating and fire is higher in warm climates. There is a higher risk of overheating and burning. For warmer climates, heat control is particularly important. Good aeration is therefore necessary for efficient composting. The material consistency, pile size, ventilation and the related rotation regularity can be tracked (Table 15.5).

Table 15.5 Optimum aeration rate for composting pile

Ideal aeration rate	Raw material used	Aeration method	References
0.4 L/min/kg OM	Grass, tomato, pepper and eggplant wastes	Alternating aeration of 15 min on/45 min off	Kulcu and Yaldiz (2004)
0.25 L/min/kgVS	Dairy manure with rice straw	Continuous aeration	Li et al. (2008)
0.6 L/min/kgin active phase, 0.4 L /min/kg in curing phase	Active municipal solid waste	Continuous aeration	Rasapoor et al. (2009)
0.5 L/min/kgOM	Chicken manure with sawdust	Continuous and intermittent aeration	Gao et al. (2010)
0.1 m^3/ min/m^3	Chicken manure with straw and dry Grasses	Continuous and intermittent aeration of 30 min on/30 min off	Shen et al. (2011)

15.5 Physical Characteristics

The physical properties of the pile must be taken into description when mounting a compost pile. Physical properties control the aeration, decomposition and also the aerobic conditions of pile. Physical properties that effect the pile mostly are porosity, structure and texture (Table 15.6).

15.5.1 Porosity

The ratio between the airspace within the pile of compost is known as porosity. It offers air flow through the pile. If the pore space gets filled with water in presence of high moisture content, then there is an increase in air space resistance, which results in oxygen deficit in pile, and anaerobes started dominating aerobes. Porosity is improved with a more complex combination of quantifiable matter which produces sufficient moisture, air space consistency and larger particles, which increase the size of the pore and lower the resistance to airflow. Larger particles would promote airflow but also reduce the surface area of the particles. Because of the preponderance of microbial activity within a thin, liquid layer on the surface of the compost particles, the more exposed the air, the higher is the degradation.

15.5.2 Texture

The texture represents the comparative proportion of different sizes of particles and the quantity of area that the microorganisms have access to. The finer the material, the more microbial activity is present in the region. The reduction of particle size using methods such as sorting and grinding often increases the total surface area of the pile material left to microbial decomposition.

Table 15.6 Factors effecting composting process (CPHEEO 2018 www.mohua.gov.in)

S. no.	Factors	Ideal range during different phases			Remarks
		Active composting	Curing	Product storage	
1	Concentration of oxygen	13–18%			Maintaining higher oxygen concentration in a compost pile is challenging and may in fact lead to other difficulties, like low moisture content
2	Air space	40–60%			There should be enough void space in the compost pile for oxygen convenience
3	Size of particle	Mixture of particles should be between 3 and 50 mm		90% of material must pass through 4.0 mm IS sieve	Smaller particles have a more surface area compared to their volume, which means more of the material is out for microorganisms. However, too smaller particles adversely affect the FAS within the material
4	Structure	Particles in the composting pile maintain their structural properties throughout the process			If particles have less structural characteristics like cardboard, the FAS within the composting pile is abridged
5	Moisture content	55–65%	45–55%	15–25%	If moisture levels are too low, the size and activity level of the microorganism populations is inhibited, resulting in slower composting and/or curing. When moisture levels are too high, there is a risk that too much of the pore space between individual particles fills with water, which can lead to anaerobic conditions and unpleasant odours
6	Temperature	55–60 °C	Less than 50 °C	Ambient	Too high temperature for continual periods (>65 °C) rapidly deteriorates the population of advantageous microorganisms. Too low temperatures can allow less efficient microorganisms to thrive and make over all composting slow.

(continued)

Table 15.6 (continued)

S. no.	Factors	Ideal range during different phases			Remarks
		Active composting	Curing	Product storage	
7	C:N ratio	25:1–30:1	18:1–23:1	15:1–20:1	If C:N ratio is <, the available carbon may be fully consumed before all the nitrogen is stabilized, and the balance nitrogen can be transformed to ammonia and lost as a gaseous emission. If the C:N ratio is higher, the composting process proceeds at a slower pace, since the microorganism's population size is limited by the lack of nitrogen
8	pH	6.5–8		6.5–7.5	Microorganisms cannot subsist in environments that are too acidic or alkaline. Also, when the pH is more than 9, nitrogen is more readily transformed to ammonia and becomes biologically inaccessible, increasing the C:N ratio and slowing the process

15.5.3 Structure

The structure refers to a particle's ability to resist compaction and settlement. The assessment and the preservation of porosity in the composting process is an important factor. Structure is essential as composting cannot be carried out quickly even with all the components needed. When the pile starts to settle down and shut down air spaces, the compost process slows down. It appears that the highly absorbing content has a better structure than the less absorbing material. Therefore a compromise between optimization of porosity, surface optimization, and increasing structure must be the ideal particle size of the compost content.

15.6 The Process of Composting

Like any other degradation process, composting too follows the usual biochemical pathway. Usually the substrates fed to the composting facility are biogenic that is the products originated from the biological activity of photosynthesis or consumer biomass. This means that all available substrate for composting is of microbial, plant or animal origin. Quantity wise the plant materials make up highest amounts followed by animal tissue and microbial products. Major natural compounds found in compost substrate are listed in Table 15.7.

Table 15.7 Substrate for compost pile

Compound	Composition	Function	Degradability
Lignin	Polymerisates of phenylpropane derivatives, e.g. coniferyl alcohol	Structural compound	Very resistant, mainly by fungi
Cellulose	β-1,4 bonds	Structural compound (plants leaves, stem)	Easily, mainly by fungi, but also bacteria, actinomycetes
Starch	Amylose: linear α-1,4 bonds; Amylopectin; branched α-1,4 bonds	Storage compound in seeds and roots	Good; aerobically and anaerobically (*Clostridium*)
Glycogen	α-1,4 and α-1,6 bonds	In animal muscles	Good
Laminarin	β-1,3 bonds	Marine algae (Phaeophyta)	Fair
Paramylon	β-1,3 bonds	Algae (Euglenophyta and Xanthophyta)	Fair
Dextran	1,6 bonds	Capsules or slime layers of bacteria	Fair
Agar	Polymer of galactose and galacturonic acid	Marine algae (Rhodophyta)	Resistance
Suberin, cutin	High polymeric esters of saturated and unsaturated fatty acids	Structural compound	Poor
Pectin	Polymer of galacturonic acid (3×10)	Dissolved and in the cell wall, in seeds fruits and in young wood parts	Easy, by most microorganisms, among them often pathogens
Sucrose	Glucose- fructose disaccharide	Vacuoles	Very easy by most microorganisms
Lactose	Glucose- galactose disaccharide	Milk	Easy by lactic acid
Hyaluronic acid	Polysaccharide of glucuronic acid and N-acetyl glucosamine	Connective tissue	Easy
Chlorophyll and pigments		Plastids	Easy
Alkaloids, tannins	Sugars, mainly alpha-D-glucose	Vacuoles	Variable
Fats, waxes	Glycerine and fatty acids	Storage compound	Variable
Poly-β-hydroxybutyric acid		Vacuoles, storage compounds	Easy
Murein	Peptidoglycan	Cell wall of bacteria	Easy
Chitin	Poly-N-acetyl glucosamine	Cell wall of fungi; crustacean, insects	Fairly
DNS, RNS	Nucleic acid	Mitochondria, nuclei	Easy

15.6.1 Lignin

Lignin is an essential plant structural component that is most gradually degraded. The wood's lignin content ranges from 18% to 30%. The monomer unit numbers are not large, but the degradation is very complex due to their extraordinary diversity between their basic monomer compounds (phenylpropane derivatives, particularly coniferyl alcohol). Lignin degradation is quite often co-metabolized since it is marginal to produce energy from the degradation of lignin. Lignin degradation is primarily caused by fungi, which also grow on live plants as pathogens. The white-rot fungi, including turkey tail (This Turkish Tail) and *Stereum hirsutum* (the false turkey tail), are known as lignin-degrading fungi. The lignin is degraded and the light cellulose sections are left off. Many fungi like *Pleurotus ostreatus* simultaneously break down cellulose and lignin.

15.6.2 Cellulose

The most abundant plant part is cellulose. Almost all forms of organic waste contain cellulose. The waste generated by remains of plants with a high proportion of structural elements (wood industry waste, agricultural waste and household waste) is the most influential. Cellulose molecules are β-d-glucose chains of 40,000 grams of polymerization. Glycosidic bonding blends the glucose molecules with β-2,4. The action of three enzymes results in enzymatic cleavage:

1. Endo-β-1,4-glucanases break β-1,4 bond within the molecule, which leads to long free end chains.
2. The disaccharide cellobiotic is isolated from the free ends by exo-β-1,4-glucanases.
3. Cellobiosis is hydrolysed by beta-glucosidases, and the microorganisms eat up the excess glucose. The catalytic effect (mechanical destruction of large structural components of micronucleus) is deemed significant. Most micro-fauna species are degraded under aerobic conditions. In general, fungi are more important for the degradation of cellulose than bacteria, which are particularly important where the lignin (e.g. wood or straw) encrusts cellulose. Since cellulose is rich in C but it does not contain N or other important components, it provides a competitive advantage to the mycelial structure of the fungi. *Chaetomium*, *Fusarium* and *Aspergillus* are a few fungi to note. Among the bacteria are mainly Myxomycetes (*Cytophagus*, *Polyangium*, *Sorangium*) or associated community taxonomies. In addition, cellulose degradation is known to pseudomonas and the related generation, although a few actinomycetes are involved. Cellulose is degraded primarily by mesophilic and thermophilic *Clostridia* in anaerobic environments.

15.6.3 Hemicelluloses

The most common of hemicelluloses is Xylan, present in grass, bagasse (up to 30%) and wood (2%–25%). Pentoses (xylose, arabinose) or hexose (glucose, mannose, galactose) are composed of Xylan. The polymerization degree is 30–100. The principal degrading enzymes are xylanase (constructive in some cases) produced by many bacteria and fungi. Pectin (polygalacturonides) consists of polygalacturonic acid ramified chains. Pectinase, which is most commonly present in fungi and bacteria, is degraded. Most herbal pathogens create pectinases. Amyloses are a series of d-glucose chains (as opposed to cellulose, amyloses is helical, because of 1.4-position β-glycosidic bond). In addition to this, amylopectin is branched to 1,6 and contains residues of phosphate and Ca and mg ions. There are two different forms of enzyme starch degradation:

Phosphorolysis by phosphorylases, starting at the free, non-reducing end of the amylose chain, releasing single glucose-1-phosphate molecules. At the 1,6 branches, it comes to a halt and only continues after action of amylo-1,6-glucosidase.

Hydrolysis: α-amylase cleaves the α-1,4 bonds within the molecule.

15.6.4 Murein

A branched N-acetyl glucosamine and N-acetyl muramic acid chains consist of murein. Muramic acid is related to variable amino acids by lactyl groups. The key component of most bacteria's cell wall is murein.

15.6.5 Chitin

Chitin is less essential than cellulose, considering the masses. The chemistry is very similar to cellulose and chitin. Thus glucose is the monomer of cellulose; N-acetyl glucosamine is the monomer of chitins. High nitrogen levels in chitin are the main difference for degraders (around 7% N, the C/N of chitin is approximately 5). A large number of fungi and bacteria, e.g. *Aspergillus*, may use chitin for both nitrogen and carbon. Chitin can also be used as a source of nitrogen and carbon. Chitin is degraded to the resorption, fructose-6-P transformation of N-acetyl glucosamine, and thus incorporated into the metabolism of carbohydrate by exoenzymes. Chitin is the most essential structural compound in fungal cell walls and forms the exoskeleton of insects and crustaceans. Chitin is an essential waste product in areas with shellfish industries.

The composting process is conceded out by a miscellaneous population of principally aerobic microorganisms that decompose organic material in order to propagate and reproduce. The activity of these microorganisms is reinvigorated through the controlling of the compost pile's carbon-to-nitrogen ratio (C:N), moisture content, oxygen supply, temperature and pH. Appropriately accomplished composting increases the rate of natural decomposition and provides enough heat to kill seeds of weeds, pests and larvae of fly (Alfreider et al. 2002). Microbial transformation of substrate is an exothermic process that produces heat enough to raise the temperature of pile enough high that process of composting can be differentiated into three phases based on the temperature of that very phase**.** The activity of these microorganisms is reinvigorated through the controlling of the compost pile's carbon-to-nitrogen ratio (C:N), moisture content, oxygen supply, temperature and pH. Appropriately accomplished composting increases the rate of natural decomposition and provides enough heat to kill seeds of weeds, pests and larvae of fly. The process of composting may be divided into two main periods:

1. Active composting
2. Curing

Effective composting is the duration of penetrating activity of microbia during which readily decomposable material and some of the more decay-resistant material, such as cellulose, are decomposed.

Curing follows active composting and is characterized by lower microbial activity levels and further decomposition of active composting phase products.

The compost is said to be stabilized when healing has reached its final stage.

Over the active composting cycle, the compost pile goes through a wide range of temperatures. As the temperature varies, certain microorganisms become unsuitable for conditions while at the same time being perfect for others. The active composting time consists of three temperature ranges. These areas are identified as microorganisms that dominate the pile at such temperatures as the psychrophilic, mesophilic and thermophilic types (The Town of Patterson 2014).

Typically, the psychrophilic temperatures have been described as below 50 ° F, between 50 and 105 ° F and below 105 ° F. The definition of these temperature ranges does not mean that during the psychrophilic phase, there are no microorganisms found in the pile.

Alternatively, these ranges are described in order to create a rough line of temperatures at peak growth rates for certain groups of microorganisms and efficiencies. For instance, mesophilic species, which occupy the pile, but not control the microbial population because they are not functioning to the optimum levels in thermophilic or psychrophilic temperatures, depending on the ambient environment and the content mixture temperatures (Anastasia 2015).

The initial composting stage is defined by either psychrophilic or mesophilic models. At the start of the composting cycle, a short time period is common before the temperature rises rapidly. The time required for the microbial population to develop is this lag period.

Through the auto-isolating composite material, the temperature produced from the microbial activities is absorbed as the microbe group degrades the easiest content, and the population is rising. When the heat in the pile accumulates, the compost pile temperature begins to rise. The temperature continues to rise gradually as the microbial population increases and diversifies through the ranges of psychrophilic and mesophilic temperature. The compost stack usually takes about 2–3 days, depending on the process, to rise above mesophilic temperatures and enter the thermophilic stages of composting. As the pile temperatures go up in the thermophilic range, a diverse group of micro-organisms occupy the pile with a high level of growth and efficiency. This intense microbial activity enhances the heat required to kill bacteria, fly larvae and weeds. A wide range of materials from basic, degradable materials into more complex, decay-resistant subjects such as cellulose can also be decomposed by the variety of the microbial community. At around 130–160 degrees Fahrenheit, temperatures continue to rise. When this threshold is reached, microbial activity starts to fall because of a lack of readily decreased oxygen and materials or because the temperature at which their function is adversely affected is exceedingly high. Microorganisms degrade material by transferring soluble materials across their body walls or using extracellular enzymes to break down the substance before it is brought into the cell body. If the temperature is too high, the enzymes that cause the breakdown denature are not working so that the microorganisms are unable to obtain the nutrients they need to survive. High temperatures may not be lethal to all microorganisms but may affect their efficiency and may lead to a decline in microbial activity.

Some microorganisms often form spores in response to excessive heating. Spores are the inactive form of certain microorganisms, such as heat and moisture, which protect them against adverse survival conditions. These spores germinate once again in favourable conditions. If the microbial activity decreases, the pile loses more heat than it generates and the pile cools down. Diverse microorganisms rehabilitate the stack by migrating from cooler spots when sports come in cool temperatures. The spores germinate as survival conditions are more suitable. The process of decomposition is further supported by such microorganisms. The compost stack stays in the thermophile range between 10 and 60 days according to the operation. When the temperature drops below 105 degrees Fahrenheit, it is possible to begin a healing cycle or aerate the stack to reactivate active composting.

At any set point, no active composting is established. When the pile conditions cannot be increased enough to reheat the battery, microbial activity is generally considered to be complete. It is normally if the temperature dropped below 105° F (Trautmann 2015). While not as extreme microbial activity and the bulk of the organic material has already been degraded, curing forms a major component of the process. The cure has a lower microbial activity level and stabilizes production material composting.

The stability of acids and compounds resistant to degradation, the formation of humic compounds and the formation of nitrate-nitrogen involves further decomposition. Another advantage of treatment is that certain fungi begin to live in the pole and add to the suppressive qualities of the compost. When microbial activity declines and works on a lower level, a small heat volume is produced, and the pile temperature is still rising or weak. Proper moisture and oxygen control are still needed for microbial activity during the healing cycle (Neklyudov et al. 2008). During the treatment process, the pile should also be handled to ensure that weed seed is not repolluted.

The cure piles can be covered or relocated to reduce the potential for recontamination. During the treatment process, the reactions are relatively slow and therefore take enough time. Type of operation, the duration of the active composting cycle and the intended final application of the compost will vary. For the decomposition and stabilization sufficient, long active compost periods require extended healing periods. Various organisms involved in various stages of composting are depicted (table given).

Susceptible end-use compost, for example, on sensitive plants or in potting media, requires a prolonged treatment period. When the pile returns to ambient temperature after repeated mixing, treatment is generally considered complete. It is important that cooling caused by adequate cure and cooling due to an inadequate amount of oxygen and/or humidity is distinguished. It usually lasts from 1 to 6 months. In the compost pile, three types of organisms are inhabiting: bacteria, fungi and actinomycetes, a higher type of bacteria. They may be optionally anaerobic, aerobic or anaerobic (Calrecycle.ca.gov). Strict anaerobic organisms do not use oxygen and die from oxygen deprivation.

15.7 Chemical Transformation

During the compost, microorganisms degrade the compost mix's raw material to synthesize and obtain the energy for their cellular processes. Various chemical transformations occur when complex compounds get divided into simpler ones and thus result in the formation of new compounds (Nakasaki et al. 1994.) Microbiota requires enough energy so that new cellular material can be synthesized. The most reliable source of energy for microbiota in compost pile is either respiration or fermentation (Chen and Hadar 1986).

15.8 Respiration

Respiration takes place either in aerobic or anaerobic form. In aerobic form, microorganisms use molecular oxygen to release bulk of energy with carbon source producing CO_2 and H_2O.

$$\{\mathrm{C,O,4H}\} + \mathrm{O_2} \rightarrow \mathrm{CO_2} + 2\mathrm{H_2O} + \mathrm{ENERGY}$$

This conversion is not accomplished through a single reaction but through a series of reactions. These reactions not only liberate energy but also result in formation of organic intermediates that serves as the source for other various synthetic reactions. Aerobic respiration outpaces anaerobic respiration and fermentation during composting because it is more proficient, generates more energy, works at higher temperatures and does not produce the much quantity of odorous compounds. Aerobes can also use a greater variety of organic compounds as a source of energy that results in more complete degradation and stabilization of the compost material. In anaerobic respiration, the microorganisms consume electron acceptors other than O_2, such as nitrates (NO^{3-}), sulphates (SO_4^{2}-) and carbonates (CO_3^{2}-) to obtain energy. This use of alternate electron acceptor sources in the energy producing metabolism results in odorous and objectionable compounds like hydrogen sulphide and methane.

Respiration of anaerobes also leads to the formation of intermediates of the organic acid that tend to accrue and are disadvantageous to aerobic microorganisms. Aerobic respiration also results in formation of organic acid intermediates, but these intermediates are freely consumed by consequent reactions so that they do not stance as significant impending for odours as in anaerobic respiration.

15.9 Fermentation

Fermentation is the simplest means of energy generation. It doesn't need oxygen and is quite inefficient. Most of the carbon decomposed through fermentation is converted to end products, not cell substituents, while liberating only a small amount of energy. Unassimilated protein as nitrogenous organic residue is broken down to obtain the nitrogen essential for the synthesis of cellular material in heterotrophic microorganisms. Nitrogenous organic residues, or proteins, undergo enzymatic oxidation (digestion) to form complex amino compounds through a process called aminization. Carbon dioxide (CO_2), energy and other by-products are also produced.

$$\text{Proteins} + O_2 \rightarrow \text{complex amino compounds } + CO_{2+} \text{ other products} + \text{Energy}$$

The complex amino compounds formed can then be synthesized into the microorganisms or undergo additional decomposition into simpler products. The general reduction in complexity of the amino compounds proceeds from proteases to peptones to amino acids and acid amides (Hansen et al. 1990). The products of the digestion of the proteins and complex amino acids can only be used in the production of new cellular material if sufficient carbon is available. If not enough carbon or energy to incorporate these amino compounds into the cells is available, unstable nitrogen forms and accumulates through the process of ammonification. Because the ammonia group is characteristic of amino acids, ammonia (NH3) or ammonium ions (NH_4+) will accumulate.

$$RNH_3 - HOH \rightarrow R - OH + NH_3 + ENERGY$$

The ammonium compound that is formed interconverts between two forms depending on the pH and temperature of the pile. This interconversion between NH_3 and NH_4+ is described by reaction shown in the equation.

$$2NH_3 + H2CO_3 \rightarrow (NH_4)2CO3 \leftrightarrow 2NH_4 + +CO_{2^-}$$

Acidic conditions (pH < 7) promote the formation of NH_4+, while basic conditions promote the formation of NH3. Elevated temperature also favours the formation of NH_3, and, because of the low vapour pressure of NH_3, it generally results in gaseous NH_3 emissions from the pile (Martins and Dewes 1992). Another key chemical transformation of the process of composting is nitrification, the process by which ammonia or ammonium ions are oxidized to nitrates. Nitrification process involves two steps. In the first step, NH^4 + –N is oxidized to form nitrites (NO_2–) through the action of autotrophic bacteria that use the energy produced by this conversion. The nitrites are then promptly converted to nitrates (NO_3–) by a different group of microorganisms called nitrifying bacteria.

$$NH_{4^+} + 1\frac{1}{2}O_2 \overset{\rightarrow}{} NO_{2^-} + H_2O + 2H^+ + \text{energy}$$

$$NO_{2^-} + \frac{1}{2}O_2 \overset{\rightarrow}{} NO_{3^-} + \text{energy}$$

Nitrification occurs during the curing period. Since nitrites (NO^{2-}) are toxic to plants and nitrates (NO_3–) are the forms of nitrogen most usable in plant metabolism, enough time must be allowed for the curing period so nitrates are the final nitrogen product in the compost. In addition, proper aeration of the compost pile must be maintained during curing because nitrification requires oxygen. Denitrification occurs in oxygen-depleted environments. It can be carried out by either aerobic or anaerobic bacteria. If denitrification is carried out by aerobic bacteria, nitrate is being used in place of oxygen as a hydrogen acceptor resulting in the following progression of nitrogen:

$$NO_{3^-} \rightarrow NO_2 - (\text{nitrous oxide}) \rightarrow N_2\,(\text{Gas})$$

If denitrification is carried out by anaerobic bacteria, the general reaction is

$$HNO_{3^-} + H_2 \rightarrow NH_2 + N_2O$$

Because nitrous oxide is an odorous compound and results in the loss of beneficial nitrate-nitrogen, denitrification is not desired and can be avoided by maintaining aerobic pile conditions. This, of course, is accomplished with proper aeration (Hansen et al. 1990).

Basic conditions and elevated temperatures in compost pile promote formation of NH_3 in gaseous form while as acidic conditions tend to form NH_4+/ The nitrification, a process by which ammonia or ammonium ions are oxidized to nitrates. Nitrification process has two steps. In the step first, NH^4 + –N is oxidized to form nitrites (NO_2–) under action of autotrophic bacteria that use the energy formed by this conversion. The nitrites are then promptly changed to nitrates (NO_3–) by a diverse group of microorganisms known as nitrifying bacteria.

$$NH_4^{\ +} + 1\frac{1}{2}O_2 \rightarrow NO_2^{\ -} + H_2O + 2H^+ + \text{energy}$$

$$NO_2^{\ -} + \frac{1}{2}O_2 \rightarrow NO_3^{\ -} + \text{energy}$$

Nitrification takes place during the curing period. Nitrites (NO_2–) are lethal to plants, while nitrates (NO_3–) are most usable in plant metabolism (Rymshaw et al. 1992). Curing phase needs enough time to produce nitrates as final products of compost. Curing also requires proper and maintained oxygen supply as nitrification requires oxygen.

Denitrification is the another chemical transformation during composting which takes place anoxic conditions and can be carried out by both aerobes and anaerobes.

If this is carried out by aerobic bacteria, nitrate is utilized in place of oxygen as a hydrogen acceptor resulting in the following progression of nitrogen:

$$NO_{3^-} \rightarrow NO_2 - (\text{nitrous oxide}) + N_2\,(\text{Gas})$$

If it is carried out by anaerobic bacteria, the general reaction is

$$HNO_{3^-} + H_2 \rightarrow NH_2 + N_2O$$

As nitrous oxide is an odorous compound and is a result of the loss of advantageous nitrate-nitrogen, denitrification is not preferred and can be avoided by maintaining aerobic pile conditions (Fig. 15.8). This, of course, is accomplished with proper oxygen supply.

15.10 Applications of Compost

15.10.1 Land Application

The wide and frequently elusive impacts that compost can have on soil and plant development make it hard to decide absolutely what the application rates should be for land-applied compost (Chen and Hadar 1986). The differed characteristics and attributes of the completed compost and the feed stocks used to generate the compost make it hard to propose application rates. On-going exploration is broad on the use of compost to farming and plant crops. This exploration assesses the impacts of compost on soil nutrient content, soil-nourishing properties and disease control. Compost showcased based on its nutrient content requires permitting by most state divisions of horticulture.

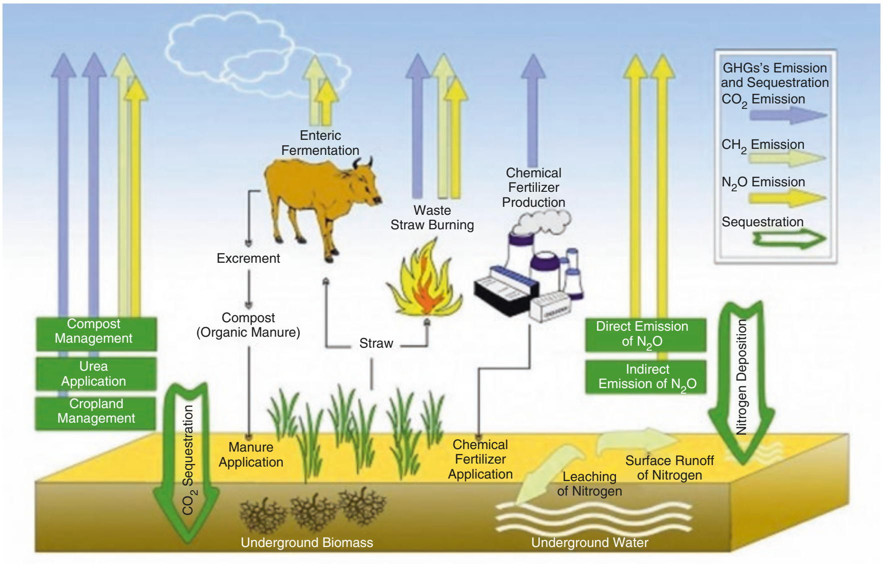

Fig. 15.8 Depicting composting process (Liu et al. 2015)

Compost performs its principal purpose as a soil conditioner by applying humus and organic matter to the soil. Humus is the dark, carbon-rich and relatively stable residue which is a result of organic matter decomposition (Brinton, Jr., William F). Adding humus and organic matter increases the soil's retaining ability for water and nutrients, reduces soil bulk density and enhances soil aeration and pore structure. Compost material has direct effect on environment and the indirect effects caused by encouraging soil microbial activity and earthworms that cause nuisence (Gajalakshmi et al. 2002).

The soil changes caused by adding compost stimulate root growth. The improved root system makes a plant more resistant to drought, because it can get more water from the soil. The improved root system also helps the plant to increase their absorption of nutrients. Improved soil retention ability of water and nutrients due to increased organic matter produced by the compost also reduces the leaching.

Although compost's main value lies in improving soil structure and water holding capacity, compost does contain many nutrients. Nevertheless, these nutrients are not available in the same amounts per volume unit as inorganic fertilizer and will require higher levels of application (Brodie and Herbert 1993). The advantage of using compost as a fertilizer is that it gradually releases nutrients, usually under the same dry, moist soil conditions needed for plant growth, so that the release of nutrients is balanced with plant consumption. It results in a more effective use of nitrogen and a decreased potential for nitrogen leaching. Leaching potential also exists when nutrient release conditions from the compost are appropriate, but there are no plants available to use the nitrogen. For example, this can happen in early fall after crops have been harvested, but soil moisture and temperature are still sufficient for plant growth and release of nutrients.

A compost nutrient analysis reveals the amount of nutrients in the compost. The study does not, however, indicate the amount of nutrients available to the plants immediately or how much will be produced in the following seasons. Different studies found a broad range of values for the amount of nitrogen that was available during the first growing season. Such variability is due to the compost's nitrogen content and its highly variable mineralization levels. Depending on the raw material and process used to produce the compost, the amount of nitrogen available during the first growing season varies from 8% to 35% of the total nitrogen. It is commonly thought that in the first growing season, 10–25% of the nutrients are available. Compost produced from manure has a higher nitrogen content than other composts generally. Of the manures, compost from poultry manure has the greatest fertilizer value. Although compost applied at fair rates does not provide sufficient nutrients to substitute commercial fertilizers in their entirety, it may reduce the amount normally used.

Certain other nutritional benefits of compost include ensuring a stable supply of ammonium, increased cation exchange capacity to hold nutrients in the soil and buffering capability to avoid plant-damaging acidic conditions.

Compost acts as a suppressant of the disease by increasing the microbial activity in soil. The increased number and abundance of soil microorganisms provides a competitive edge to beneficial organisms over pathogens. Research for the use of compost as a disease suppressant was concentrated primarily in the field of composted bark and sphagnum peat due to the need for strict quality control. That is not a characteristic of agricultural composting operations. Therefore, the use of compost in container media and nurseries has been emphasized for its disease suppressive nature (Chen and Hadar 1986). The composted manure was found to be effective as a peat replacement in container media and was suppressive to soil-borne pathogens like *Fusarium*, *Pythium*, and *Rhizoctonia*.

The application rate of compost varies depending on the mineralization rate and whether the compost is used as a soil inoculate or as a primary source of nutrients. Compost used as a soil inoculate to boost soil tilth and high-quality organic matter content should be added at a rate of 2–3 tonnes per acre. If compost is used as a primary source of nutrients, the rate of application rises to over 100 wet tonnes per acre. The application density is more than 2 inches thick, at 100 tonnes per acre. Usage levels should not be greater than 50 dry tonnes per acre (4 yd3/1000 ft2). Compost is usually distributed at 1–2 inches thick on land. If used at a rate greater than this, it becomes difficult to get incorporate into the soil (Reider et al. 1991).

One major concern when applying compost to land is the presence of viable weed seeds in the compost. Weed seeds found in the compost can of course lead to problems of weeds. The compost stack must maintain thermophilic temperatures to kill any weed seeds which may be in the raw material. However, finished compost pile can also be recontaminated with weed seeds. This is particularly a problem when the pile is stored outdoors. Either position the pile under protective cover or in areas where exposure to weed seeds is minimized to avoid any recontamination.

When used in combination with commercial fertilizer, compost typically has the best effects on plant growth. Compost improves soil when used alone, and it has been shown to increase crop yields and crop height, particularly during the initial growth stages and during drought. The use of immature compost or one that includes weed seeds, pests or soluble salts can have a negative effect and can encourage disease. Plus, when spreading, potash and phosphorus should not be over-applied.

15.11 Marketing Considerations

Marketing puts additional managerial demands that may outweigh possible revenues on the composting project. Nevertheless, when a waste is to be used off-farm, it will be less difficult to market it as a compost than to market the raw material (Logsdon 1993). Marketing compost's main challenges are to create a market and then consistently satisfy the market's quality demands. The successful sale of compost depends on creating an appropriate customer base and then regularly meeting both volume and quality standards. Most retail outlets sell compost generated by big, commercial operations that can generate compost of high quality at a lower cost than smaller agricultural operations. Even nurseries and landscapers are starting to branch out into composting yard waste and trimmings. Mostly other landscapers and nurseries are the compost market for their products (Laliberty 1987). The agricultural compost market is usually home gardeners. In general, this market is local with the compost sold on site, through local stores, or to some buyers in bulk. Selling the compost on-site or to selected parties often saves on packaging, marketing and advertisement. The selling of compost generated on the farm gives an opportunity for another source of income. However, the advisability of selling compost must be carefully measured because the additional demands it imposes on the farm operation may not result in income if all costs are taken into account. However, regulations may require that compost meet certain requirements, especially if it is to be sold as a fertilizer.

Nitrogen may in some cases be applied to the final compost to increase its fertilizer value.

15.12 Health Risks of Composting Operations

15.12.1 Odour Generation

The primary concern of composting facility is generation of odour. Ecological conditions need to be manipulated the way odour generating ways could be restricted without impairing the treatment facility (Allen 2013). Nature of material used in composting at the beginning has maximum role in odour generation. As the process proceeds, the odour generation diminishes accordingly (Buckner 2002). Odour generation also finds its source in microbial respiration and chemical reactions that take place in compost pile. Volatile fatty acids (intermediates in carbohydrate metabolism), sulphur compounds (cysteine, methionine), nitrogen compounds (decomposition of proteins) and the dust are the main sources of odour generation in compost pile (Brinton 1998). Amount and type of odour are also influenced by ecological factors. The preliminary chemical composition of the pile, concentration of oxygen, diffusion rates of oxygen, particle size, moisture content and temperature has a considerable impact on creation of odour. Increased vapour pressure due to progress in temperature levels aids higher odour levels and also chemical reactions taking place in the pile produce odour by producing compounds with the decrease in aerobic decomposition (Edelmann et al. 1999). Maximum odour-producing compounds are metabolic intermediates that are further to be decomposed and utilized by other microorganisms readily. Biofilters are effective in removing odour of compost pile. Soil filters also control odours, particularly those caused by gaseous products, such as ammonia and volatile organic acids (Reinhardt 2002). Soil is an effective medium for removing odours through chemical absorption, oxidation, filtration and aerobic biodegradation of organic gases.

15.12.2 Pathogens

Pathogen destruction is the key aspect that makes composting a preferable alternative over untreated manure. Pathogen content of compost is of significance because if improperly treated and subjected to environment as such can infect new host and thus pose a threat to animals and humans. Quantity and type of pathogen in the initial stage of composting depend on the waste fed. Pathogenic biota in compost includes bacteria, viruses, fungi and parasites. Although parasites and viruses cannot reproduce apart from their host, they can often survive for extended periods. Fungi and bacteria doesn't need host to survive so if in pile their population is reduced, they still exhibit the property to recover. So reducing number isn't enough pathogens needs to be killed in the pile (Itävaara et al. 1997). Conditions that restrict to pathogenic growth are lack of assimilable organic matter and moisture content less than 30% as such pathogens can be destroyed by heat, competition, destruction of nutrients, antibiosis and time (Hoitin et al. 1991). Antibiosis is the process by which a microorganism releases a substance that, in low concentrations, either interferes with the growth of another microbe or kills it. Pathogens can also be destroyed as a result of competition with the indigenous microbial population for nutrients and space.

15.12.3 Bioaerosols

Compost facility operation has a huge health concern of bioaerosols (Petruzzelli 1996; Strauch 1996). Bioaerosols are the biological agents or organisms, that are transported through air and cause serious allergies when inhaled in specific quantity (Biocycle 1992). Bioaerosols include mycotoxins, pathogens, glucans, microbial enzymes, endotoxins, fungi, bacteria and actinomycetes. Abundance of bioaerosols also matters in generation of infection. So bioaerosols need to be enough sufficient in quantity to create infection. Lowered immunity because of disease and some medications can render an individual vulnerable to infection (Amner et al. 1988; Faure and Deschamps 1991; Finstein and Morris 1975; Strom 1985; Beffa et al. 1996). *Aspergillus fumigatus* and endotoxins are the bioaerosols of main concern at the composting facility (Beffa et al. 1996). *Aspergillus fumigatus* is a secondary pathogen that interferes with the patients of low immunity disorders, and bioaerosol infection makes such patients more susceptible (Strauch 1996). Additional health-wise apprehension of composting facility is presence of endotoxins. Endotoxins are metabolic products of gram-negative bacteria embedded in the cell wall and exist even after the death of bacteria. Endotoxins are not known to be toxic through air-borne transmission but can cause such symptoms as nausea, headache, and diarrhoea.

15.13 Waste Management in the South Asia Region

South Asian region has only eight countries but a large population. The three population hubs of India, Pakistan and Bangladesh together have a population of 1.68 billion people; Afghanistan, Nepal, and Sri Lanka are home to nearly 85 million people; and the smaller states of Bhutan and Maldives have about 1.2 million people (Li and Judy 2015). The South Asian countries are diverse not only in population but also in economic development and geography. Almost all cities in the South Asian region practice some open dumping, but cities are increasingly developing sanitary landfills and pursuing recycling. Most cities hire private contractors or non-governmental organizations to collect waste from neighbourhoods and institutions and pay collectors based on the amount of waste transported to disposal sites (World Bank Open Data). Although rules and regulations have been developed at national and state levels, these criteria are still being translated into practice and accountability structures at the city level. Implementation of policies is challenging because of a lack of enforcement mechanisms. In addition to improving legal enforcement, strengthening the technical and institutional capacity of administrators at all levels of solid waste management systems, from municipal staff to the regulators and operators, is a common priority. The South Asian region generated 334 million tonnes of waste in 2016, at an average of 0.52 kilogram per person each day. Rural waste generation is significantly lower than urban waste generation and reduces the average amount generated in the region. The islands of Maldives generate the most amount of waste per capita. In cities in South Asia, waste generation rates vary widely, with cities such as Kabul, Afghanistan, generating about 1.5 kilograms per capita per day, and cities such as Butwal, Nepal, generating only about 0 0.2 kilogram per capita per day (Asian Development Bank 2013; World Bank 2017b). Most waste in the South Asia region is organic. A large proportion of waste is not classified, though it is assumed that most of this waste is inert. Municipalities mostly mix waste cleaned from drains and silt with their solid waste disposed off. Demolition and construction waste is also included in the data reported for South Asia though it will progressively be managed separately as a result of new rules in India established in 2016. Waste collection excluding Maldives, Sri Lanka and Afghanistan, where data were not available, urban waste collection handling in South Asia is about 77%, although coverage varies considerably by country and city. Rural areas have lower collection coverage rates of about 40% waste collection services, where they exist in cities, typically occur door to door. In some cities, such as Kota, India, and Butwal, Nepal, residents discard off waste at a primary collection point, from which collected waste is transported to the final disposal site. This practice is extremely common, and designated primary collection sites or open plots of land often eventually become unofficial sites for dumping. In Indian city Navi Mumbai, a waste collector calls the residents of that area to bring waste to the collection vehicle (India, Ministry of Urban Development 2016; Karnataka Compost Development Corporation Ltd. 2016). Unceremonious waste collections with materials retrieval activities are prolific in South Asia. Cities studied reported between 100 and 1, 200 lively waste pickers. The large cities of Delhi, India, and Dhaka, Bangladesh, reported 90,000–120,000 active waste pickers, respectively. At landfills, waste pickers are typically organized or are part of a cooperative (Enayetullah and Hashmi 2006). Waste disposal open dumping is common in South Asia, and most existing landfills lack leachate collection and treatment, landfill gas collection and sometimes even liners. However, the remediation of dumpsites and construction of formal landfills are actively taking place, and official and well-functioning facilities tend to be privately operated. For example, Maldives is mitigating dumping of waste by improving waste collection systems and constructing sustainable disposal sites that can serve multiple islands (World Bank 2017a). Out of the eight countries, only four recycle between 1% and 13% of waste, and out of the eight, seven countries have begun composting for organic waste management. Waste to energy incineration potential has gained interest, but substantial results are yet to be proven. Initiatives to improve waste disposal began in India in 2014, and interest in other South Asian countries is growing. Many cities are establishing central authorities to increase capacity to plan and operate the waste management sector. The focus is on developing waste disposal strategies that include locally tailored and cohesive approaches (Croitoru and Sarraf 2017). Depending on the locality, cities are navigating varied constraints related to land, capacity, availability of local operators, financing and alignment of waste technology and waste composition, and more than one solution is needed. Waste management is increasingly recognized as not only a social, health and environmental issue, but an economic one, in which waste recovered and land used wisely, can generate financial savings. Indian cities can access funds, mainly from the Swachh Bharat Mission (India, Ministry of Urban Development. 2016) to improve waste management programmes.

15.14 Conclusion

Given the growing world population, especially in developing and emerging countries, it is very urgent to have a creative solid waste management that may contribute to urban development. Composting is an important approach to converting biodegradable wastes through the transformation of future organic waste into a useful product, for example, compost. Adequate educational knowledge is necessary, in a participatory manner, to improve the understanding and increase the use of compost, in order to enhance this organic waste management approach to the community. In organic waste management, composting may offer an endless opportunity. Higher education knowledge, therefore, is important to understand certain key barriers to the use of compost to enhance the learning of the use of composting in order to achieve sustainable management of organic waste. The achievement of sustainably managed waste requires an environmentally friendly option. Such a technique must be reliable, successful and cheaper than many alternatives. Efficient disposal methods such as incineration, settlement, pyrolysis and gasification, however, have negative environmental effects as well as public health threats. Sustainable composting with various advantages, such as biofertilizer production, relatively low air and water pollution, low operational costs and the generation of income. In many developed countries around the world, the use of composting to bioremediate polluted soils has increased significantly. Compostage could, however, lead to methane production, smelling and the accumulation of heavy metals in the final product when improperly designed. The application of compost contributes to the sustainable agriculture of the ecosystem by various processes, e.g. green manure, leaf manure, mulching and composting. The process of microbial discomposure is therefore influenced and balanced as a result of the community dynamics of different microbial genera by the microbial population. Factors such as temperature, pH, oxygen, etc need to be pre considered as these factors affect the organic waste decomposition directly. These factors are central to the problem of sustainable waste management practices, which are based on high-value soil recycling compost prepared by improved technologies, and found to be productive for agricultural production. The Panorama Composting Plant contributes positively to sustainable development by discharging waste from sites. Mechanisms must be investigated for increasing the diversion rate. Moreover, the scale of the plant is limited, although it contributes to the social and economic dimensions of sustainable development.

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