

Microbial Ecology of *Elaeagnus latifolia* L.

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By

Liza Handique and Vipin Parkash

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Dedicated
to
Our parents
and
Parents-in-Law



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ABBREVIATIONS

µg	Microgram
µl	Microliter
mm	Milimeter
nm	Nanometer
ng	Nanogram
u	Unit
g	Gram
mg	Miligram
rpm	Revolutions per minute
ppm	Parts per million
mM	Milimolar
Dia.	Diameter
cfu	Colony forming unit
Conc.	Concentration
w/v	Weight/volume
v/v	Volume /volume
°C	Degree celsius
SDW	Sterile distilled water
NB	Nutrient broth
PDA	Potato dextrose agar
PDB	Potato dextrose broth
PGPR	Plant growth-promoting rhizobacteria
VOCs	Volatile organic compounds
DMSO	Dimethyl sulphoxide
EDTA	Ethylene diamine tetra acetic acid
GPS	Global positioning system
BSA	Bovine serum albumin
FDM	Frankial defined minimal medium
BAP	Murry, Fontaine & Torrey's BAP Medium

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PREFACE

Microbial ecology, also referred to as environmental microbiology, studies the interactions between microorganisms and their surroundings. It involves viruses as well as the three primary domains of life, Archaea, Bacteria, and Eukaryota. Because they are everywhere, microorganisms have an effect on the entire ecosystem. Microbial life is a substantial carbon sink and is primarily responsible for controlling biogeochemical processes. Global biogeochemical cycling is governed by the major metabolic activities of microorganisms, which include carbon fixation, nitrogen fixation, sulfur metabolism, and methane metabolism, in addition to carbon fixation.

In each ecosystem, microorganisms play a crucial role, but this is particularly true in areas where photosynthesis is hindered by low light levels. Chemosynthetic microorganisms in these areas give other creatures energy and carbon. By utilizing alternative electron acceptors for respiration, these chemotrophic organisms can also survive in oxygen-deficient settings. Plant development and soil health are enhanced by the solubilization of phosphate by microorganisms.

The area of soil that is altered or impacted by plant roots, known as the "rhizosphere," is home to a wide variety of microorganisms. Certain microbes, known as "plant growth-promoting rhizobacteria" (PGPR) and including *Azospirillum*, *Agrobacterium*, *Pseudomonas*, various Gram+ *Bacillus*, and others, have favorable impacts on plant growth as well as PGPFs (Plant growth promoting fungi).

Although, their extent of contribution to nitrogen cycling is still poorly understood, PGPR are involved in free N₂ fixation and most likely create vitamins and phytohormones. Similar to mycorrhizae or nodules, several rhizospheric bacteria also aid in plant protection, despite their involvement being discovered relatively recently. Since genetic studies have been utilized extensively to find important genes for rhizospheric microorganisms, primarily bacteria, they offer valuable experimental models. Genetic investigations, however, are still not well developed for understanding processes of plant protection by mycorrhizal fungi.

Certain rhizospheric microorganisms, for instance, buffer abiotic stresses by changing the root environment. For instance, bacteria that produce exopolysaccharides can change the porosity and structure of the soil,

protecting against water stress (too much or too little), buffering temperature fluctuations, and facilitating better root penetration in the soil.

They can also degrade adverse compounds, such as xenobiotics and aromatic molecules, and constitute a major factor for life in extreme soil conditions.

The ecological activities of microorganisms in the plant species *Elaeagnus latifolia* L. are covered in this book, with a focus on the interactions between bacteria in their respective environments and communities. Students who want to learn the fundamentals of microorganisms in their natural habitats and the functions they perform are the target audience for this book. Since the activities of bacteria are what make microbial ecology such a significant study nowadays, the focus is on the biogeochemical and ecological processes of the target plant species. The richness of the microbial world and the issues being investigated by microbial ecologists studying various microorganisms and processes in various habitats and species will become more apparent to readers.

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CHAPTER I

INTRODUCTION

The area of soil that surrounds a plant root is known as the 'rhizosphere' (Subba Rao, 1993). This region experiences high levels of biological and chemical activity, which is influenced by substances released by plant roots and microorganisms like viruses, bacteria, actinomycetes, fungi, slime molds, and algae. Walker *et al.* (2003) defined the rhizosphere as the area of soil that is confined by plant roots and frequently extends a few millimeters (mm) beyond the root surface. Compared to the bulk soil nearby, this area of the soil has a significantly higher bacterial content (Hiltner, 1904). Microorganisms get their nourishment from the water-soluble substances that plant roots release as they grow, including sugars, amino acids, and organic acids. It is well known that the microbes give the plants nourishment as they grow. Moreover, rhizospheric microbes are recognized as plant growth regulators and signal molecules that can influence plant growth significantly (Bhattacharyya and Jha, 2012). Microbial secretions around the rhizosphere are also helpful in combining the soil particles into stable aggregates to retain soil moisture.

Soil microbiological diversity is highly intricate (Pang *et al.*, 2008). In soil, there are around 2.6×10^{29} prokaryotes. According to the most recent estimate, only about 5% of fungal species and 0.1% of total bacterial diversity are known, of which only a tiny portion have had their metabolic profiles studied. More than 4,000 microbial species have been identified per gram of soil in studies using molecular techniques. Compared to the plant, this microbial community has a far bigger collective genome. The composition and functions of microbial species in the rhizosphere are directly impacted by the chemistry and pH of the soil. The interactions between microbes in the rhizosphere can be either beneficial or pathogenic, causing the plant roots to be invaded or killed. They may be saprophytic that live on dead residues of plant bodies or neutral causing no visible effect to the plant. Thus, rhizosphere has appeared as a versatile and dynamic ecological environment of intense plant-microbe interactions (Mayak *et al.*, 2004).

However, other beneficial microbial interactions in the rhizosphere include the generation of antimicrobial chemicals, nodule formation, and mycorrhizal formation. Mycorrhizal symbioses can promote plant growth through a variety of mechanisms, including the improvement in nutrient acquisition pathway, producing plant growth regulators, improving rhizosphere soil conditions, altering the physiological and biochemical properties of the host, and protecting the plant roots from soil-borne pathogens (Hryniewicz and Baum, 2011; Harrier, 2001) which are some of the possible activities usually carried out by mycorrhizal symbioses for plant growth promotion (Bhattacharyya and Jha, 2015).

Microorganisms can enable symbiotic nitrogen fixation in plants through the production of nodules. Author also documented the emergence of legumes on arable land and illustrated how useful legume crops are for boosting soil fertility (Chew, 2002). The primary microbiological products of antimicrobial screening operations are antibiotics, without which the majority of microbial illnesses would be lethal. A significant source of many antibacterial metabolites is soil actinomycetes (Terkina *et al.*, 2006). In order to produce active metabolites, antagonistic actinobacteria of *Araucaria angustifolia* rhizosphere were isolated and tested by de Vasconcellos *et al.* (2010). Many complex and somewhat resistant organic molecules found in soil are reported to be broken down by metabolites, including chitinase and indoleacetic acid (IAA) which is responsible for the degradation of different complex and relatively recalcitrant organic compounds present in soil. Systemic disease resistance has been greatly enhanced by the microbial interaction with plants. Thus, even though microbial colonization of the rhizosphere is not a uniform process, it is thought to be an essential stage in applying microorganisms for advantageous objectives such as biofertilization, phytostimulation, biocontrol, and phytoremediation.

Endophytes

Microbes known as endophytes live inside the plant tissues and don't seem to affect their host (Petrini, 1991). Endophytism is the term for asymptomatic relationships between other living things and living plant tissues (Stone *et al.*, 2000). To date, fungi have been the most frequently researched endophytes (Stone *et al.*, 2000). It is now known that endophytes can colonize a large variety of plants. Endophytic fungi-colonized plants have the ability to develop more quickly than non-colonized ones (Cheplick *et al.*, 1989). In terms of both taxonomy and geographic distribution, the majority of endophytic fungi are restricted.

Nonetheless, a small number of fungi co-exist extensively with the host, suggesting a long-standing, tight, and advantageous relationship (Shukla *et al.*, 2012). Plants can experience a range of effects from detrimental to advantageous from endophytic interactions (Brundrett, 2004; Shukla *et al.*, 2012). In order to find an exceptional source of novel bioactive natural compounds with special qualities including anticancer, antidiabetic, insecticidal, and immunosuppressives, endophyte investigations are being conducted these days (Strobel *et al.*, 2004). Due to their great capacity to provide plants with stress tolerance and the ability to produce chemicals of therapeutic value, this group of fungus becomes an essential part of beneficial microflora. The absence of a localized interface of specialized hyphae and structures like arbuscules and vesicles—structures that are present in the majority of endomycorrhizas—the lack of synchronized plant-fungus development and the absence of plant benefits through nutrient transfer are the main ways that endophytic associations differ from Mycorrhizae. However, plants may benefit indirectly from endophytes by increased resistance to pathogens or stress, or by other unknown mechanisms (Saikkonen *et al.*, 1998).

Mycorrhiza

Mycorrhizae are the classical examples of mutualistic symbiosis between the roots of vascular plants and associated fungi (Usuki and Narisawa, 2007). German plant pathologist, A.B. Frank, was the first to note the existence of such a characteristic association in the roots of Cupulifereae in 1885 and coined the term ‘Mycorrhiza’. The term “mycorrhiza” simply describes the symbiotic association of plant roots and fungi. It is derived from Greek words mykos, “fungus” and riza, “roots” is a symbiotic (generally mutualistic but occasionally or weakly pathogenic) association between a fungus and the roots of a vascular plant. According to Sieverding (1991), mycorrhizas are the non-pathogenic association between soil-borne fungi with the roots of higher plants. With the development of more advanced molecular techniques, some 6000 species of Glomeromycotina, Ascomycotina, and Basidiomycotina have been identified as mycorrhizal. The precise taxonomic classification of the fungus partner and the host plant may determine the kinds of mycorrhiza that needs to be investigated (Bhattacharyya and Jha, 2015). Given that mycorrhizae are found on more than 90% of vascular plants, they are the most common symbiotic relationship between soil microbes and plant roots (Brundrett, 2009). On the other hand, several mycorrhizal associations have been documented to be greatly impacted by poisonous chemicals

such as phenolics, terpenoids, alkaloids, and tannins, which are primarily found concentrated in plant roots when present.

The benefits of mycorrhizae include: (i) the fungus gets nutrients from the plant's roots. As sucrose, the sugar that has been produced in the leaves, travels down the stem. The low sugar concentration in the fungus is caused by the conversion of sucrose into isomers like trehalose, which prevents sucrose from ever building up in the first place (Mohammadi *et al.*, 2011). In addition to providing the plant with nutrition, the fungal hyphae function as a massive root hair system, scavenging minerals from the soil and delivering them to the plant. In addition, the plant partner develops greater resistance to infections, drought resistance, and tolerance to pH and temperature extremes because of the phytoalexins released by the fungus (Morandi, 1996).

Classification of mycorrhizas started with Frank (1887) who distinguished two basic categories of mycorrhizas i.e., ectotrophic and endotrophic mycorrhizas (including ericoid and orchid mycorrhizas). Mycorrhizal interactions have also been classified by Bonfante (1984) into two basic categories like ecto and endomycorrhizas on the basis of their hyphal structures.

Ectomycorrhiza

The term “mantle of hyphae” refers to the sheath that the fungal hyphae in this association develop over the outside of the roots. A hyphal network known as the Hartig net grows from the mantle into the first few layers of the cortex, perhaps even deeper, and ultimately reaches the endodermis. Infected roots have decreased root hair creation, and their shape is altered by the recurrent development of small branches with blunt tips and restricted growth. The majority of ectomycorrhizal genera are found in the Basidiomycetes class, specifically under the Agaricales, which includes *Amantia*, *Tricholoma*, *Russula*, *Suillus*, *Leccinum*, and *Cortinarius*. However, there have also been reports of ectomycorrhizal Ascomycetes, such as truffles. They belong to the Basidiomycetes phylum and are more prevalent in temperate and boreal forest trees, with over 5000 species reported (Sieverding, 1991). Various growth-promoting chemicals, including auxins, cytokinin, and gibberellic acids, are secreted by the ectomycorrhizal fungi. However, they also create some antimicrobial compounds that shield the host plant from infections that are carried by the soil. Fungi obtain their carbon from their host in the form of glucose, fructose, or sucrose, which they then use to make glycogen, mannitol, and

trehalose. In poor to moderately fertile soils, these mycorrhizae are known to promote plant growth and nutrient uptake.

Endomycorrhiza

The mycorrhizae in which the fungal hyphae invade the root cells without forming any external sheath, mantle of hyphae, are called endotropic mycorrhizae or Endomycorrhizae (Harrier, 2001). Usually, some parts of invading fungal hyphae lie externally as a loose mass of hyphae but they do not form mantle. They are found in a wide range of habitats, usually in the roots of angiosperms, gymnosperms and pteridophytes. The occurrence of endomycorrhizal fungi in soil, their association with both forestation and agricultural crops are well documented (Parkash and Aggarwal, 2009; Singh, 2002). Inoculation with a suitable AM fungal strain to improve the growth and survival of plant seedlings in forestation is very essential. They also occur in the gametophytes of some mosses, lycopods and Psilotales which are all rootless (Mosse *et al.*, 1981). As of late, aquatic plants have also been found to have endomycorrhizas (Beck-Nielsen and Madsen, 2001; Radhika and Rodrigues, 2007). Nonetheless, some plant families, including Proteaceae, Cruciferae, Zygophyllaceae, Dipterocarpaceae, Betulaceae, Myrtaceae, and Fagaceae, have been demonstrated to be mycorrhiza-free (Brundrett, 2009). Functional vesicular arbuscular mycorrhizal (VAM) symbiosis is not established in nature by the majority of host plants of non-arbuscular mycorrhizal fungus. While it was previously believed that the Cactaceae, Chenopodiaceae, Cyperaceae, Amarantaceae, and Juncaceae were mycorrhiza-free, the majority of these plants were discovered to be infected in naturally disturbed grasslands. Although, the exact cause of some plants' inability to develop mycorrhizas is unknown, it could be due to the presence of fungitoxic substances in the cortical tissue or exudates of the roots. It may also be due to interactions between the fungus and the plant at the cell wall and (or) middle lamella level (Tester *et al.*, 1987). High concentrations of salicylic acid have been found to reduce mycorrhization (Medina *et al.*, 2003), meaning that plants with a genetic basis for high salicylic acid content have evolved to be mycorrhiza free.

Endomycorrhizas are of three different types *i.e.*, arbuscular mycorrhizae (AMs), ericoid mycorrhizas and orchid mycorrhizae (Mohammadi *et al.*, 2011), among which the AM fungi (AMF) are ubiquitous in diverse plant taxa. Arbuscular-mycorrhizal association is usually used to describe the associations of phycomycetes septate fungus belonging to Glomeromycota that corresponds to five different genera such as *Glomus*, *Gigaspora*,

Acaulospora and *Sclerocystis*, *Scutellospora*. The ericoid and orchid mycorrhizae are restricted only to the order Ericales and the family Orchidaceae respectively (Smith and Read, 2008). AMF play important role in maintaining the soil fertility and plant nutrition (Jeffries *et al.*, 2003) by enhancing the nutrient uptake and translocation of mineral nutrients like P, N, S, K, Ca, Fe, Cu and Zn from soil to the host plants. Significance of AMF in plant nutrition, especially for immobile elements such as phosphorus (P) is well known (Van der Heijden *et al.*, 2006). They generally do this by means of an extensive hyphal network system which spreads from the colonised roots into the associated soil environment and thereby helps in creating a skeletal structure that holds the soil particles together (Bhattacharyya and Jha, 2015; Parkash *et al.*, 2004). The external hyphae greatly increase the volume of soil and translocate the phosphorus to the roots. Plants are heavily infected with AMF in P-deficient soils and mycorrhizae are poorly developed when the P supply is adequate. It is, thus, a self-regulating system, increasing P uptake when the element is in short supply. The P, so absorbed, is converted into polyphosphate granules in the hyphae and passed to the arbuscles for ultimate transfer to host plant (Parkash and Aggarwal, 2009). Gianinazzi *et al.* (1991) have demonstrated that transfer of polyphosphate occurs in the presence of acid phosphatase during the life span or senescence of arbuscule. In addition to stimulation of phosphorus uptake, mycorrhizal fungi stimulate rooting, growth and survival of the transplanted seedlings (Parkash *et al.* 2004). It was reported that the AMF can enhance the nodulation in legumes; this decreases rots caused by fungal pathogens, root penetration and larval development of nematodes.

Through the movement of nutrients and water, AM fungi in most environments give plant communities the essential support they need (Smith and Read, 1997). AM fungi are known to increase plant uptake of water and nutrients from the soil, lessen the toxicity of heavy metals and other pollutants, and increase plant resilience to pathogenic invasion. Mycorrhizal symbiosis is, in fact, a crucial component of plant health in mature ecosystems. An increasing amount of research suggests that AM fungi can boost plant growth, particularly in infertile soils, and that this growth enhancement is caused by the infected roots' possible capacity to absorb nutrients.

AM fungi are especially important because of their widespread occurrence and association with agricultural crops. AM fungi are not of usual occurrence in continuously flooded sites. The role of AM fungi in improving the quality and survival of plant seedlings after plantation has been well recognized (Boureira *et al.* 2007). The AM fungi are also

known to maintain important ecological position among various microorganisms colonizing the rhizosphere of plants (Parkash *et al.*, 2015).

It is known that AM fungus can produce a variety of spore forms, including zygosporos, azygosporos and chlamydosporos. These spores are generated in an ectocarp manner in roots, soil, hypogeous media, or epigeous sporocarps. The fungi hyphae in AM produce vesicles and arbuscules, which are unique organs found inside the cortical cells of the roots. Vesicles are borne on the tip of the hyphae in the cortical cells of the root or in the intercellular gaps. They have thick walls and a spherical to oval form. The fungus uses these vesicles to store food. Vesicles are intracellular or intercellular globule bodies packed with lipid droplets that are created when an AM fungal hypha swells intercalary or terminally. These are the AM fungus' least explored structures. Various theories exist regarding the function of vesicles. Some authors have suggested that the vesicles are reproductive organs like sporangia. Others proposed that the vesicle function is to store the fat, which is then transferred to the host cell and digested by it.

Most significant structure in the AM association is arbuscules with their hyphal branching system like "small bushes". The arbuscules are tree-like dichotomously branched (extensively) haustoria which are developed within the cortical cells. Arbuscule is the site for fungus/plant metabolite exchanges. The arbuscular hyphae are filled with polyphosphate granules and rich in polyphosphatases. It is believed that the AM fungi can take up the phosphate from the soil, accumulate it as polyphosphate granules and transport them through the intercellular hyphae to the arbuscules. Here, the granules are degraded by polyphosphatases and phosphorus is released in the host cell.

Orchidaceous mycorrhizae are a separate kind of mycorrhizal association that differs greatly from AM. This form of plant has perfect phases that occur in Basidiomycetes and Ascomycetes, and the higher plant is either temporarily or permanently parasitic on fungi, primarily from the genera *Rhizoctonia* spp. The tiny (0.3–14 µg) orchid seeds have no appreciable food reserve. Certain seeds do not sprout at all unless they are infected with a fungus; others do germinate, but unless the seedling contracts the fungus, development quickly stops. The fungal hyphae make coils inside the cortical cells after penetrating them. These nutrient-rich hyphal coils, also referred to as "pelotons," eventually decompose and provide nourishment for the developing plant. The compound phytoalexin "orcinol" is implicated in this process.

The orchid receives its supply of vitamins, hormones, and carbohydrates from its decomposing pelotons, which are produced by a fungus that

grows outside of its roots. Most orchids eventually become green, at which point their relationship with the fungus may change from one of parasitism to mutualism. However, certain orchids, like the bird's nest orchid *Neottia nidus-avis*, continue to be achlorophyllous and parasites of the fungus throughout the whole of their lives. Ericaceous mycorrhizae are associated with three families *e.g.*, Ericaceae, Epacridaceae, and Empetraceae families, in which the fungus *Pezizeellaericae*, an Ascomycete, forms dense intracellular coils in the outer cortical cells. This fungus has tremendous capacity of mineralization and absorption of organic nitrogen; thus, it greatly stimulates nitrogen uptake and plant growth even in infertile peat soil. However, this mycorrhizal association results in no development of root hairs as well as the absence of epidermal cells of the root.

A third group of mycorrhizae exists in nature which is called ecto-endomycorrhiza which bears the characteristics of both ecto and endomycorrhiza. The fungal partner establishes mantle of hyphae on the surface of the roots as well as hyphal coils and haustoria within the invaded cortical cells of the root system.

Classification of Arbuscular Mycorrhizae

Old classification

According to Morton and Benny (1990) classification of VAM is based on the spore morphology, spore formation and spore wall structure. The order Glomales is divided into two sub-orders *viz.* Gigasporineae and Glomineae. The sub-order Gigasporineae is composed of a single family Gigasporaceae consisting of two genera *viz.* *Gigaspora* and *Scutellospora*. While the sub order Glomineae is comprised of two families *i.e.*, Glomaceae and Acaulosporaceae. The family Glomaceae is comprised of two genera *viz.* *Glomus* and *Sclerocystis* while the family Acaulosporaceae is comprised of two genera *i.e.* *Acaulospora* and *Entrophosphora* respectively.

New classification

Glomeromycota taxonomy was mostly morphologically based till the end of the last century. All glomeromycotean fungi, except one genus *e.g.*, *Geosiphon* are known to form arbuscular mycorrhiza (Oehl *et al.*, 2011). Their identification was based on spore morphology, spore formation and spore wall structure (Gerdemann and Trappe, 1974; Morton and Benny, 1990; Walker and Sanders, 1986) (Fig.1-1). However, as soon as molecular

phylogenetic tools became available, these were included in taxonomic analyses (Simon *et al.*, 1992) and soon became the foundation of a new taxonomy (Morton and Redecker, 2001). In 1990, the arbuscular mycorrhiza-forming fungi were organized in three families (Acaulosporaceae, Gigasporaceae and Glomeraceae) and six genera (*Acaulospora*, *Entrophospora*, *Gigaspora*, *Glomus*, *Sclerocystis*, and *Scutellospora*) within one order, *Glomerales* (Morton and Benny, 1990; Parkash, 2005) of the fungal phylum, Zygomycota. This classification was based on spore morphology and spore formation characteristics (Acaulosporoid, Entrophosporoid, Gigasporoid, Glomoid, radial-Glomoid, and Scutellosporoid) only. Differences in spore wall structure were used at the species level. Now-a-days, three classes are accepted (Archaeosporomycetes, Glomeromycetes and Paraglomeromycetes), five orders (Archaeosporales, Diversisporales, Gigasporales, Glomerales and Paraglomerales), 14 families, 29 genera and approximately 230 species (Morton and Redecker, 2001; Oehl and Sieverding, 2004; Oehl *et al.*, 2008; Palenzuela *et al.*, 2008; Spain *et al.*, 2006; Sieverding and Oehl, 2006) (Fig. 1-2).

