Review article

Beneficial microbial diversity in the rhizosphere of *Casuarina equisetifolia* L. – A mini review

M. Manoranjitham[1*], T. Hemalatha[2] and A. Vinotha[3]

[1]Post Graduate and Research Department of Botany, Thanthai Periyar Government Arts and Science College(Autonomous), Khajamalai, Tiruchirappalli-620023, Tamil Nadu, India
[2]Department of Botany, Rani Anna Government College for Women, Palayapettai, Tirunelveli-627008, Tamil Nadu, India
[3]Post Graduate and Research Department of Botany, Thiagarajar College, Teppakulam,Madurai-625009, Tamil Nadu, India
**Email: drmanoranjithambotany@gmail.com*

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ABSTRACT

Numerous studies have demonstrated that diverse biological activities of microbial populations existing in rhizosphere have major impact on the growth and yield of plants significantly. The rhizosphere is the physical location in soil where plants and microbes interact. Casuarina equisetifolia L. is a multifunctional actinorhizal tree that grows quickly. Local people rely on Casuarina plants for non-wood forest products, small-scale timber, and fuel and are being used increasingly for rehabilitating deforested watersheds and other degraded landscapes. This brief review described the advantageous effects of several microorganisms found in the rhizosphere of C. equisetifolia, which contribute to the development, biomass, and enhancement of forest seedling quality by providing macro and micronutrients.

Keywords: AM fungi, *Casuarina equisetifolia*, *Frankia*, litter decomposers, rhizosphere

INTRODUCTION

The rhizosphere, that is, the tiny area surrounding and impacted by plant roots, is a hot spot for different kinds of microorganisms and is considered as one of the most intricate ecosystems on Earth. The rhizosphere is home to a variety of organisms including viruses, bacteria, fungi, oomycetes, nematodes, protozoa, algae, and arthropods.

Plants are invaded by an incredible number of microorganisms that can reach cell densities much higher than the number of plant cells. Additionally, there are many more microbial genes in the rhizosphere than that of plant genes. Numerous studies have shown that a wide range of microorganisms associated with plants may significantly impact plant growth and development, nutrition, illnesses, productivity, seed germination, and seedling vigor. In keeping with the nomenclature for microorganisms, the aggregate communities of microbes associated with plants are called the plant microbiome (Gevers *et al*., 2012).

Casuarina equisetifolia trees are frequently utilized to restore degraded areas. In India, China, Egypt, Tunisia and Senegal, they are utilized as windbreaks to repair sand dunes and safeguard nearby crops (Sayed, 2011). These trees are utilized in agroforestry systems to boost agricultural productivity and enhance soil fertility (e.g, intercropping with legumes) in India and China (Zhong *et al*., 2010). In

India, Casuarina trees are also utilized to produce hardwood for home building and paper pulp wood, as well as smokeless fuelwood with a high calorific value. In Asia, *C. equisetifolia* trees have created shelter belts that have been extremely protective during tsunami and typhoons (Zhong *et al*., 2010).

Numerous studies have examined the positive benefits of rhizosphere organisms on plant development and health and they include protozoa, mycorrhizal fungi, nitrogen-fixing bacteria, plant growth promoting rhizobacteria (PGPR), biocontrol microbes, and mycoparasitic fungi (Mendes *et al*., 2013). Majority of the farmers keep growing the *C. equisetifolia* trees continuously for three cycles lasting up to up to 9 years. Consequently the harvested biomass has a high rate of soil nutrient removal. Deficit illnesses and soil sickness are caused by this loss of nutrients. There are evidences that the rhizosphere of *C. equisetifolia* contains beneficial microorganisms like *Azospirillum*, *Azotobacter*, Phosphobacterium, AM fungi, and *Frankia*. These microbes are known to enhance the growth of Casuarina seedlings as well as trees planted in farm forestry (Rajendran, 2016). In this context, several studies have been carried out to explore the beneficial microorganisms existing in the rhizosphere of *C. equisetifolia* and evaluate their role in the growth and development in various parts of the world. The present work focused on the particular features of the advantageous microbes found in rhizosphere of *C. equisetifolia*.

Rhizosphere of *C. equisetifolia*

It is interesting to note that a number of pioneer actinorhizal plant species in the Casuarinaceae family may form extended rows of continuous rootlets in clusters throughout their root system (Arahou and Diem,1997).This special root development may therefore provide *C. equisetifolia* growing in nutrient-poor soils with an adaptive mechanism to maximize the uptake of insoluble nutrients needed for growth and nitrogen fixation.

Beneficial microbial populations of *C. equisetifolia*

There are many different microorganisms in the rhizosphere, or the zone of impact around plant roots. The rhizosphere soil of *C. equisetifolia* cultivated in several agroclimatic zones in Tamil Nadu, India, was examined by Rajendran *et al.* (1999) and the results showed that the population of *Azospirillum* (3.3×10^6 g/dry soil), Phosphobacteria (2.5×10^4 g/dry soil) and Actinomycetes (6.4×10^4 g/dry soil) were found in sandy clay loam soil. Panda (2010) estimated that 39.0×10^3 of bacterial and 43.8×10^2 of fungal populations were found in rhizosphere of *C. equisetifolia* in Ganjam district of Orissa, India. At species level, Vinod *et al*. (2014) identified *Streptomyces roseiscleroticus*, *S. flavochromogenes*, *S. vastus* and *S. praguaeneses* in rhizosphere of *C. equisetifolia* in Port Blair, Andaman, (Ojha and Arya, 2018) found *Aspergillus niger* and *Trichoderma viride* from Maharaja Sayajirao University of Baroda of Gujarat, India. Huang *et al*. (2020) noted *Bryometopus*, *Codonosigidae*, *Oligohymenophorea* and *Saccharomycetes* in Hainan Province, China and Lin *et al*. (2022a) reported *Acinetobacter nosocomialis*, *Bacillus cereus*, *Enterobacter cloacae*, *Enterobacter hormaechei*, *Enterobacter* sp., *Ochrobactrum* sp., *Pantoea* sp., *Pseudomonas* sp. in Hainan Province, China.

It was stated that the microenvironment has a significant impact on soil microbial populations, which are a crucial part of the soil ecological system (Mu *et al*., 2007). The activity and variety of the soil microbial population are influenced by soil characteristics, and soil microorganisms progressively enhance soil quality by breaking down organic materials (Chen *et al*., 2016). The various microbial populations found in the rhizosphere of *C. equisetifolia* have unique biological effects that contribute to the growth and development of *C. equisetifolia*.

It is widely known that the associative symbiotic diazotrophic bacterium *Azospirillum* can fix nitrogen from the atmosphere and generate compounds that promote growth. Recently, this bacterium has been shown to be an effective biofertilizer for a variety of agricultural crops by increasing the inorganic nitrogen requirements of the crops. Although these bacteria are widely distributed, their numbers are typically modest, with the exception of the rhizosphere where a two to three fold increase may occur. For their growth, they need available carbon molecules, sufficient quantity of inorganic nutrients including phosphorus, calcium, magnesium, molybdenum, and optional pH. Likewise, phosphobacteria are microbes that can dissolve insoluble phosphorus and provide it to plants in a form that they can use. Phosphobacteria include members of *Pseudomonas*, *Micrococcus*, *Bacillus* and *Flavobacterium* (Krishnamoorthy, 2002) .A class of filamentous bacteria known as actinomycetes contributes significantly to the encouragement of plant development by increasing the availability of nutrients, boosting plant defenses, and generating beneficial compounds. These are the major component of the rhizosphere and play a key role in soil nutrient cycling (Singh *et al*., 2018).

Several studies have proved that the above said microbial populations were significantly increased the growth and development of *C. equisetifolia* when applied individually or in combination with each other. For example, Rajendran *et al*. (2003) evaluated that the seedling of *C. equisetifolia* inoculated with combined application of *Azospirillum* + Phosphobacterium + AM + *Frankia* produced the maximum growth, biomass and quality. Likewise, Rajendran and Devaraj (2004) reported that combined inoculation of *Azospirillum* + Phosphobacterium + AM + *Frankia* and Phosphobacterium + AM + *Frankia* recorded maximum height, 20.56% and 17.53% increase over the control respectively at 24 months after planting in *C. equisetifolia*. Also, statistically there was no difference in the height between the triple inoculation of *Azospirillum* + Phosphobacterium + *Frankia* and *Azospirillum* + AM + *Frankia*.

Kumaravelu *et al*. (2004) studied the impact of combined inoculation of *Azospirillum*+ Phosphobacterium + AM fungi has significantly increased the growth and nutrient uptake by *C. equisetifolia* in field condition. They have found that the growth was 193% increase over control in inoculated plants. Saravanan *et al*. (2012) estimated that the highest total biomass was recorded in the seedlings inoculate with the combined application of *Azospirillum* + *Trichoderma* + *Pseudomonas* (54.87% increase over control), followed by *Azospirillum* + *Pseudomonas* inoculated seedlings (49.17% increase over control) and *Azospirillum* inoculated seedlings (47.34 increase over control) of *C. equisetifolia*. Gunasekara *et al*. (2016) noted that seedlings which were treated with *Frankia* sp. (one of Actinomycetes) showed the nodule formation ability in *C. equisetifolia*. Additionally, the plants treated with *Frankia* exhibited the highest levels of root and shoot growth.

Frankia as an endosymbiont

Frankia are symbiotic, nitrogen-fixing bacteria that are found in the root nodules of actinorhizal plants. They are members of the class Actinomycetales, which has over 200 species spread across 25 genera. Typically, actinorhizal plants are pioneer species that invade nitrogen-poor areas that are unsuitable for other plants for growth. Frankia is reported to exist in the majority of soils, even in areas where there have long been no appropriate host plants. It is interesting to note that non-host soils can occasionally support a higher nodulating *Frankia* population than host-containing soils (Diagne *et al*., 2014).

The N_2 fixing actinomycetes *Frankia*, which creates root nodulation, are associated with *C. equisetifolia*. Rajendran and Jothibasu (2006) observed that nodules were found at 0-30 cm soil depth in all the sites examined in various location of Tamil Nadu, India and root nodulation in trees (72.8 – 88%) was recorded in the sandy clay loam soil with nodular size at an average of 5 – 6.8 cm in diameter in three years old Casuarina plantation. Tani *et al*. (2003) identified a brown coloured *Frankia* nodule in sandy soil at Okayama in Japan with the nodular size about 1.5 cm size and 33 mg fresh weight. Karthikeyan (2016) isolated two different types of *Frankia* from two different study sites in Tamil Nadu, India: brown – pale yellow coloured nodules in rhizosphere of *C. equisetifolia* grown in Cuddalore and pink – light brown coloured nodules in Nagapatinam. They were 1.5 and 0.9 cm in diameter respectively.

It was reported that, *C. equisetifolia* is one of the non-leguminous trees that can fix nitrogen from the atmosphere by a symbiotic interaction that has been formed between the plant's roots and the soil microorganism *Frankia*. Within the nodules, the nitrogenase enzyme, which is produced by *Frankia*, catalyzes the conversion of atmospheric nitrogen to organic form. It was estimated that the Casuarina fixed 60 kg of nitrogen per hectare per year through *Frankia* (Karthikeyan *et al*., 2013).

Many studies have been conducted to enhance the growth and development for *C. equisetifolia* by using *Frankia.* Artificial treatments of *Frankia* nodule crush enhanced *C. equisetifolia* growth and biomass production by 30–40% and the increase was caused by *Frankia* which is closely linked to improved accumulation of nitrogen (Rajendran *et al*., 2003; Rajendran and Devaraj 2004; Rajendran and Jothibasu,2006; Saravanan *et al*., 2012; Karthikeyan 2016). Similarly, improved growth of C. *equisetifolia* rooted stem cuttings inoculum with *Frankia* was observed in both nursery and field conditions (Karthikeyan *et al*., 2013). Compared to uninoculated control seedlings, *C. equisetifolia* seedlings infected with Frankia and cultivated in degraded coir pith as a potting media in a root trainer exhibited more nodulation and nodular biomass and seedlings showed 35.33% increase in dry weight over the uninoculated control seedlings (Saravanan *et al*., 2012). It was also reported that rooted stem cuttings of *C. equisetifolia* infected with *Frankia* exhibited better growth and biomass under high CO_2 (Karthikeyan, 2017).

Mycorrhizae

A large number of actinorhizal plants may also establish mycorrhizal relationships and this tripartite symbiosis (host plant–*Frankia*–mycorrhiza) gives them a tendency to grow even in marginal and unfavourable soils (Dawson, 2008). Certain species have a high degree of adaptation to polluted soils, flooded terrain, desert areas, high salinity and severe p^H. Because of these characteristics, many actinorhizal trees are pioneer species that spread to damaged regions; they are often employed to re-vegetate various landscapes or prevent desertification, and they serve crucial ecological functions. The symbiotic relationship between fungus and root system of higher plants is called mycorrhiza, which literally means root fungus.

Arbuscular mycorrhizal fungus (AM) in rhizosphere of *C. equisetifolia*

The association of Arbuscular Mycorrhizae (AM) fungi is endotrophic, and has been called to as Vesicular-Arbuscular Mycorrhiza (VAM) in the past. This nomenclature has now been abandoned in favor of AM since not all fungus produce vesicles. The uptake of phosphorus and other micronutrients is significantly influenced by *Arbuscular mycorrhizae* (AM) in higher plants (Rajendran and Devaraj, 2004).

Rhizosphere of *C. equisetifolia* shows that the association of different species of AM fungi. A study carried out by Rajeswari *et al*. (1999) in various locations of Tamil Nadu, India revealed that 250/100 g of spore density with 53.88% of AM infection was found in Coimbatore, 301/100 g and 85.50% in Kanyakumari and, 340/100 g and 75% in Marakkanam respectively. The results of this study also stated that *Glomus* was found as dominant genus among AM fungi identified in all the study sites. There are several other studies also confirmed that *Glomus* was the dominant genus in rhizosphere of *C. equisetifolia* grown in different regions such as Chinnathachur in Villupuram, India (Senthilkumar *et al*., 2010), Western Morocco (Hibilik *et al*., 2021), Eastern coastline of Karaikal, India (Das and Sivashri, 2022) and North Sumatra, Indonesia (Delvian and Hartanto, 2022).

In the various age groups of *C. equisetifolia* plantations, the beneficial impacts of AM fungus was examined in Tamil Nadu, India (Rajendran, 2001;Munusamy *et al*., 2010) and AM fungi are effective used when this species is planted in deteriorated soils. Diagne *et al*. (2014) conducted a study to evaluate the impact of AM fungi to rehabilitate the salinized soil by growing *C. equisetifolia* and the results of this study revealed that *Glomus aggregatum* and *G. fasiculatum* inoculation enhanced the growth of *C. equisetifolia* in ten months after inoculation. Zhong *et al*. (2014) also discovered that mycorrhizal fungi enhanced *C. equisetifolia* development by increasing seedling nutrient intake, as well as abiotic stress tolerance and survival.

Litter decomposing microorganisms

Litter decomposition is influenced by environmental variables and also by physical-chemical characteristics of the parts such as stem wood, leaves, roots, etc. of the species studied and decomposing organisms present in the soil. The maximum degree of decomposition ascribed to the appropriate temperature, moisture, rainfall, micro-fungal population and improved soil aeration (Pande *et al*., 2002). Decomposers are creatures that consume, digest, and decompose dead living things. In the nutritional cycles, they are critically necessary. All living organisms, including humans, are essentially borrowing the components of their bodies. After they die, they must be recycled so that other plants and animals may make use of them. Significant amounts of nutrients are present in *C. equisetifolia* needle litter, and their release is impacted by the decomposition of the litter by a range of microorganisms that are active in different environments (Uma *et al*., 2014). Despite the litter of *C. equisetifolia* is colonized by a wide variety of microorganisms, although only a few number of species hold dominating positions. Panda *et al*. (2007) noted *Trichoderma*, *Aspergillus* and *Penicillium* were predominant occurrence in litter and have been found to be effective decomposers. Uma *et al*. (2014) reported eight dominant fungal species have been found in the rhizosphere of *C. equisetifolia* and they were *Alternaria alternata*, *Aspergillus niger*, *Penicillium* sp., *Fusarium* sp., *Rhizopus nigricans*, *Trichoderma viride* and *Curvularia eragrostidis*. They have also found two bacterial species viz. *Pseudomonas fluorescens and Azospirillum brasilense* were also present in the litter. Zhang *et al*. (2020) identified six bacteria (*Pseudomonas*, *Curtobacterium*, *Jatrophihabitans*, *Mycobacterium*, *Actinomyceto spora* and *Mucilagini bacter*) in Hainan Island, China. Lin *et al*. (2022b) reported two bacteria (*Pseudomonas* and *Sphingomonas*) and two fungi (*Phaeophleospora* and *Trichaptum*) from Guilinyang coastal area of Haikou, China.

Litter-decomposing microbes are essential to the growth of *C. equisetifolia* because they promote soil improvement and nutrient cycling. The organic matter in leaf litter is broken down by these microbes, returning vital nutrients like phosphorus and nitrogen to the soil for the tree to absorb. They also aid in improving the structure of the soil, which promotes healthier roots and trees in general (Ngom, 2020). The capacity of *C. equisetifolia* to fix atmospheric nitrogen through a symbiotic interaction with *Frankia* is well-known. However, other vital elements like potassium and phosphorus are released as microbes break down litter, and they are equally vital for the growth of the tree (Uma *et al*., 2014).

CONCLUSIONS

This review primarily presents the main conclusions from numerous studies carried out globally on the beneficial microorganisms found in the rhizosphere zone of *C. equisetifolia* and their useful function in enhancing growth and providing advantages for nutrient cycling. This review conclude that both small and large-scale farmers can increase plant productivity by using these advantageous rhizosphere microbiome, especially in dry and semiarid regions and the usage of chemical fertilizers in farm forestry may be reduced if these microorganisms are used to enhance growth. More research is required to assess the biological efficacy mechanism of this rhizosphere microbiota to provide theoretical evidence.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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