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Research Article

Gmelina arborea Rhizomicrobiome as Potential Plant Growth Promoter and Antagonistic Agent against *Fusarium oxysporum*

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Abstract

An attempt was made to screen and identify the potential Plant Growth Promoting (PGP) isolates and their antagonistic efficacy against the plant pathogen, *Fusarium oxysporum* under *in vitro*. Total of 24 isolates (5 Actinomycetes; 10 Bacteria and 9 Fungi) with different morphology were isolated from the rhizosphere soil of *Gmelina arborea* plants in Tamil Nadu. All these isolates were screened and tested for their PGP traits, such as IAA production and phosphate solubilization efficacy. It was found that some of the Actinomycetes isolates produce significant amount of IAA production and phosphate solubilization efficacy and this is followed by few bacterial isolates and one fungal isolate. All these short listed isolates were further screened to record their bio-control efficacy against selected plant pathogenic fungus, *Fusarium oxysporum* and it was found that the Actinomycetes isolate exhibited maximum inhibitory effect (A3-76%), followed by fungal isolate (F9-70%) and bacterial isolate (B3-51%). The potential isolates were identified as (A3)-*Streptomycetes* sp. (F9)–*Pencillum* sp. and (B3)–*Rhizobium* sp. based on morphological and biochemical characters. Application of these potential isolates as microbial bio-fertilizers will protect the crops from soil and root-borne fungal pathogens and also improve the growth of the plants.

Keywords: *Gmelina arborea, Fusarium oxysporum*, antagonistic, *Pencillum* sp., *Rhizobium* sp., *Streptomycetes* sp.

Introduction

Gmelina arborea is a fast-growing, deciduous and economically important tree species. The roots of this plant are one of the ingredients of "Dashmuladikwath" and "Bhrahatpanchamool" of Ayurveda, which constitutes a number of Ayurvedic preparations, used as tonics (Sharma *et al*., 2001). The roots and bark materials of this plant are useful in hallucination, piles, abdominal pains, burning sensations, fevers, 'tridosha' and urinary discharge (Nadkarni, 1976; Kirtika, 1984). Plant rhizosphere soil has a unique biological niches with a diverse microorganisms consist of bacteria, fungi, actinomycetes, protozoa and algae. This community gets support from the high nutritional input of organic materials derived from the plant roots and root exudates that are essential for microbial growth (Lynch, 1990). Depending on plant species the composition and quantity of root exudates differs (Smith, 1976) and the physical environment such as humidity and temperature also affects the niche of microbes (Martin and Kemp, 1980). Modification in root exudation by plants favored different root associated microbial population. Many rhizosphere beneficial microbes restrain the growth of plant pathogens through competition for nutrients, the production of inhibitory metabolites, and various other unknown mechanisms, naturally reduce the spread of plant disease in the eco-system (Hartmann *et al.,* 2008). Diseases and insect pests constitute major biological determinants of forest productivity, particularly in nurseries and plantations. They cause heavy damage to seedlings and hence reduce both quantity and quality of planting stock. Thus, the economic loss resulting from nursery diseases are considerable. Therefore, raising disease free, healthy tree seedlings including medicinally valuable plants is not only important for maintaining a good nursery stock but also essential in establishing a healthy stand in the field for better productivity (Solaiman and Anawar, 2015). Different disease problems like damping-off, root rot, stem rot, seedling wilt, collar-rot etc. caused by various soil and root-borne pathogens and resulting heavy loss of quality seedlings in tree nurseries in India (Bakshi, 1971; Mehrotra, 1990; Sharma *et al.,* 1985; Sankaran *et al.,* 1986; Mohanan and Sharma, 1993; Mohan and Manokaran, 2001; Mohan *et al*., 2002; Mohanan, 2008; Mohan and Manokaran, 2013; Mohan, 2016). One of the soil borne pathogens, *Fusarium oxysporum* has been reported to cause damage on different tree species including *G. arborea* in nursery (Bagchee, 1953; Bloomberg, 1971; Sharma *et al.,* 1985; Florence and Sankaran, 1987; Jamaluddin *et al*., 1988).

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Application of chemical fungicides was adopted to control the said pathogen in nurseries and they are not safe to the environment. Integrating suitable control methods like use of biological control agents and bio-fertilizers should be adopted in tree nurseries to produce high quality and healthy plants (Kean *et al.,* 2010). Hence, in the present study, an attempt was made to study the status of different beneficial microbes associated with the rhizosphere of *G. arborea* and also to determine their potential for plant growth promotion and bio-control ability against the soil borne fungal pathogen, *Fusarium oxysporum* under *in vitro* conditions.

Materials and methods

Collection of rhizosphere soil samples: Rhizosphere soil samples were collected from the root zone of *G. arborea* plantation at Coimbatore, Tamil Nadu. Three set of soil samples were collected up to 15 cm depth by random sampling method. Samples were brought to the laboratory in zip lock poly bags, sealed tightly and immediately transported to laboratory. The samples were kept in refrigerator at 4°C until further use.

Isolation and enumeration of different microbes from rhizosphere soil samples: All the collected samples were analyzed for isolation of different rhizosphere microbes such as bacteria, fungi and actinomycetes by adopting standard techniques (Parkinson *et al,* 1971; Subba Rao, 1993; Oskay *et al*., 2004). One gram soil was dispensed in 100 ml distilled water, the 10 fold dilution was prepared and 0.1 ml was spread plated on to Nutrient Agar for isolation of Bacteria, Starch Casein Agar for actinomycetes and Potato Dextrose Agar for fungi. Plates were kept for incubation at appropriate temperature for 2-7 d and then based on colony morphology different colonies were pure cultured and stored in 4°C for further study.

Determination of Indole Acetic Acid (IAA) Production: The test tubes containing nutrient broth with tryptophan (2 mg/mL) was sterilized and inoculated with 1 mL of bacterial isolates. Then the test tubes were incubated for 7-8 d. After incubation, the culture broth was centrifuged at 10,000 rpm for 30 min and the pellet was discarded. About 1 mL of the supernatant was taken in a clean test tube and 2mL of freshly prepared Salkowski's reagent (50 mL 35% $HClO_4$+ 1 mL 0.5 M $FeCl_3$) was added. The test tubes were incubated in dark for 30 min for the development of pink colored complex. After 30 min, the absorbance was measured at 530 nm. Various concentrations of tryptophan were used as the standard. Standard graph of Indole Acetic Acid was prepared using standard values and the concentration of IAA produced by each isolates was determined by extrapolating the absorbance value in the Y- axis of the standard graph and determining the corresponding value of IAA (µg/mL) in the X axis (Bent *et al*., 2001).

Determination of Phosphate Solubilisation: All bacterial isolates obtained were re-tested by plate assay for phosphate solubilization in Pikovskaya's agar medium. These bacteria were stabbed in triplicate using sterile toothpicks. The halo zone around the colony was presumptive confirmation of phosphate solubilization and was measured after 7 d of incubation at 30°C. Halo size was calculated by subtracting colony diameter from the Total zone of colony and halo zone (Sharma *et al.,* 2007). Solubilization efficiency (SE) was calculated by the formula as described by below.

$$\text{Solubilisation Efficiency (SE)} = \frac{\text{Solubilisation diameter}}{\text{Growth diameter}} \times 100$$

Determination of antagonistic activity: The short listed better plant growth promoting isolates were further screened and tested their bio-controlling ability against the selected plant pathogenic fungus, *Fusarium oxysporum* causing damping-off, root rot, collar rot, stem rot and seedling wilt of many plant species including *G. arborea* in nurseries. The pure culture of plant pathogenic fungus was brought from Forest Pathology Laboratory, Forest Protection Division, IFGTB, Coimbatore. Dual culture method described by Estrell *et al.* (2003) was adopted to determine the antagonistic efficacy of all the short listed microbial isolates under *in vitro*. The selected microbial isolates such as Bacteria and Actinomycetes were streaked on opposite sides of PDA plates and five day old fungal culture plug (6 mm) was kept at the center of the plate, incubated at 25°C for 5 to 7 d. The PDA plates along with fungal culture without antagonistic microbes were kept as control. Percentage of inhibition by antagonistic microbes was recorded by measuring the reduction of fungal mycelial growth and compared it with control plate. Percentage of inhibition was calculated by using the following formula:

$$\text{Percent of inhibition} = \frac{C - T}{T} \times 100$$

Where, C is the mycelial growth of pathogenic fungi in control plate and T is the mycelial growth of pathogenic fungi in treatment.

Characterization and identification of selected microbes: Characterization of the best isolate was carried out by following standard procedures. Taxonomic study of the selected bacteria was carried out according to Buchanan and Gibbons, (1994).

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All Bacteria and Actinomycetes isolates were characterized by adopting standard methods described in the International *Streptomyces* project (Shirling and Gottlieb, 1966) and Bergey's Manual of Systematic Bacteriology (Locci, 1989). The selected fungal isolates were also subjected to cultural characteristic and microscopic analysis (microstructure) (Bissett, 1991).

Results and discussion

The population density of rhizosphere microbes enumerated from different samples collected under the root zone of *G. arborea* and the data is presented in Table 1. The occurrence and distribution of bacterial population (25×10^6 CFU/g soil) was found to be maximum, followed by actinomycetes (20×10^4 CFU/g soil) and fungi (12×10^3 CFU/g soil). Based upon morphological variations, difference isolates were selected for further study. Total of 10 bacterial, 9 actinomycetes and 5 fungal isolates were pure cultured and used for screening of PGP and antagonistic potential under *in vitro* condition. The findings of the study are in accordance with the findings made by other researchers in India and elsewhere. Germida *et al.* (1998) reported that the diversity of microbial community in a specific environment was dependent on the plant species. Many of the environmental factors such as temperature, light and atmospheric CO_2 influences the diversity and population of microbes (Rovira, 1959). Koeberl *et al.* (2013) suggested that the plant species were vital drivers in structural and functional diversity of microorganisms in soil. Karthikeyan *et al.* (2008) reported that the microbial population was more in the rhizosphere soil as compared to non-rhizosphere soil of the medicinal plants such as *Ocimum sanctum, Coleus forskohlii*, *Catharanthus roseus* and *Aloe vera* screened by them. Diversity status of beneficial microbes from saline soils in Tamil Nadu and Puducherry was reported by Mohan and Sangeetha (2015) and they found that Total of 51 PGPR isolates (Phosphate Solubilizing Bacteria-18; *Azotobacter* spp.-16 and *Azospirillum* spp.-17). Recently, Srinivasan and Mohan (2016) reported the status of microbial diversity in agro-forestry systems in Tamil Nadu and recorded bacterial population (64%), followed by Actinomycetes (23%) and fungi (13%) in different rhizosphere soils screened.

Determination of phosphate solublization efficacy: Experiment was conducted to screen the efficacy of phosphate solubilization efficacy of different beneficial microbes isolated from the rhizosphere of *G. arborea* under *in vitro*. It was found that 2 bacterial isolates *viz.*, B7 (75%) and B3 (60%), 2 Actinomycetes isolates such as A3 (73%) and A4 (80%) and 1 fungal isolate *viz.*, F9 (100%) showed maximum phosphate solubilization potential as compared to remaining other isolates. The study is in accordance with the findings made by other earlier researchers.

Most of the microorganisms in the rhizosphere are related to plant species that can efficiently solubilize poorly soluble inorganic P and mineralize organic P sources (unaccessible to plants) and markedly increase plant growth in soils with low P availability (Solaiman and Anawar 2015). Mohan and Sangeetha (2015) found that phosphate solubilizing bacterial isolates showed a high rate of phosphate solubilization efficiency (SE) with *Bacillus megaterium* indicating a SE of 140% and *Bacillus subtilis*, a SE of 120%, making both the isolates as strong phosphate solubilizers.

Determination of IAA production: All the 24 isolates of different microbes were screened for phytohormone production and details are presented in Fig. 1, 2 and 3. It was recorded that isolates of Actinomycetes like A3 (OD_{530} 0.6213) showed maximum production of IAA, this is followed by the fungal isolate F9 (OD_{530} 0.5123) and bacterial isolate B3 (OD_{530} 0.5). The Plant Growth Promoting Rhizobacteria (PGPR) help in the production of siderophores, fixation of atmospheric nitrogen, solublization of mineral like phosphorus, synthesis of phytohormone such as IAA was reported by various authors. There are many earlier reports about production ability of phytohormones by diazotrophic PGPR organism including bacteria *viz., Azotobacter* (Wendo *et al.,* 2002), *Azospirillum* (Yasmin *et al.,* 2007) and also Rhizobial bacteria (Arshad and Frankenberger, 1998). Merckx *et al.* (1987) reported that the production of IAA and siderophore by the actinomycetes and they act as plant growth promoter. Narayana *et al.* (2007) reported that among actinomycetes, the IAA production was found to be maximum in *Streptomycetes* sp. In the present study, the promising isolates which showed both IAA production and P solublization efficacy were selected for conducting antagonistic experiment under *in vitro.*

Fig. 1. IAA production by Actinomycetes isolates obtained from rhizosphere of *G. arborea.*

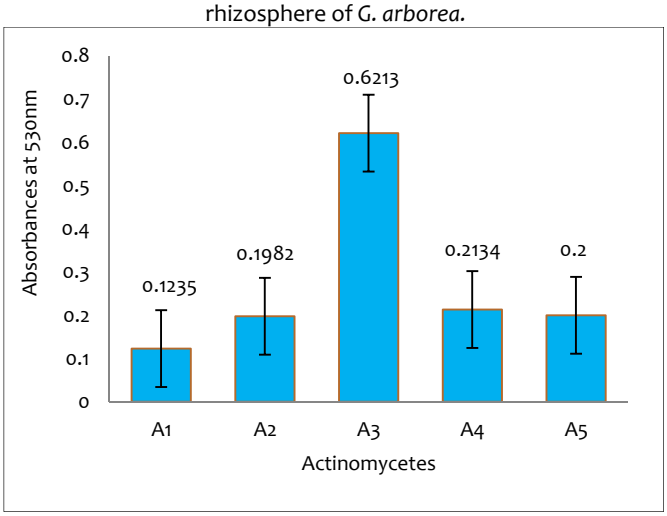

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Fig. 2. IAA production by fungal isolates obtained from rhizosphere of *G. arborea.*

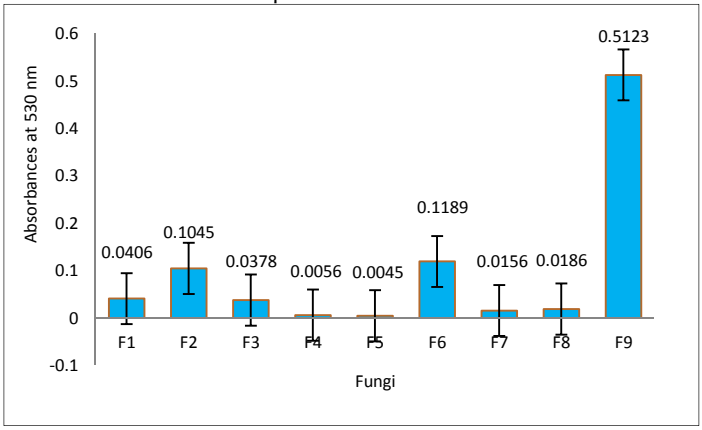


Fig. 3. IAA production by bacterial isolates isolated from rhizosphere of *G. arborea.*

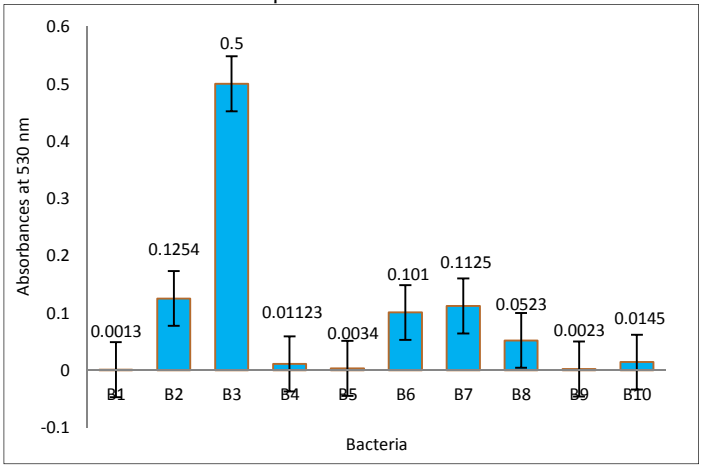
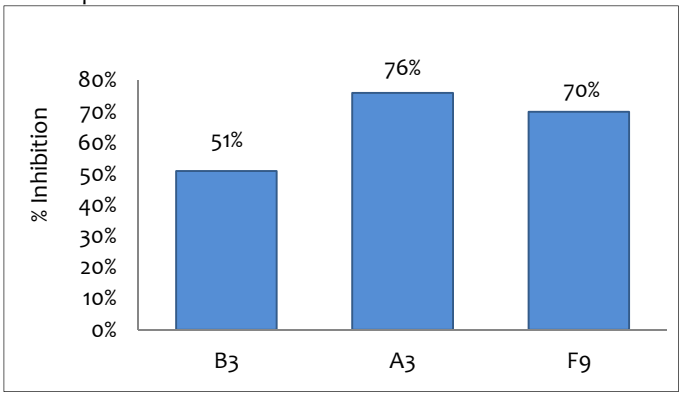


Fig. 4. Percentage inhibition of *F. oxysporum* by different rhizosphere microbes isolated from the root zone of *G. arborea.*



Antagonistic potential of selected microbes: The potential isolates selected from the study were further screened for their antagonistic potential against the plant pathogenic fungus, *F. oxysporum* and the results are presented in Fig. 4.

It was observed that the Actinomycetes isolate A3 showed maximum percent inhibition (76%), this is followed by the fungal isolate F9 (70%) and bacterial isolate B3 (51%). The findings of the study are in accordance with the observations made by other earlier researchers. Singh *et al.* (2013) reported that root diseases (rot and wilt) caused by a complex pathogenic organisms involving *Fusarium chlamydosporum* and *Ralstonia solanacearum* are serious diseases affecting the cultivation of *Coleus forskohlii*, a medicinal plant producing forskolin compound. Khamna *et al.* (2009) isolated a total of 445 Actinomycetes isolates from 16 medicinal plant rhizosphere soils. They found that among them, 23 *Streptomyces* isolates showed potential antagonistic activity against phytopathogenic fungi. Valois *et al.* (1996) reported that *Streptomyces* sp. strain has been used in China to protect cotton crops against soil-borne pathogens. Thangapandian *et al.* (2007) isolated *Streptomyces* from medicinal plant rhizosphere soils and 8 isolates had antipathogenic activity. Plant root exudates stimulate growth of rhizosphere actinomycetes that are strongly antagonistic to different fungal pathogens, while the actinomycetes utilize root exudates for growth and synthesis of antimicrobial substances (Crawford *et al.*, 1993; Yuan and Crawford, 1995).

Bio-chemical identification of microbes: The promising isolates were identified up to genes level using morphological and biochemical characterization. The Bacterial isolate (B3) was identified as *Rhizobium* sp. and Actinomycetes (A3) as *Streptomyces* sp. and Fungi (F9) as *Pencillum* sp. using morphology, staining and biochemical characterization. As similar to our work, Brown (1972), Merckx *et al.* (1987) proved the plant growth promoting property of *Streptomyces* species and Liu *et al.* (1996) proved its bio-control potential. Motaher (2014) reported that *Penicillium* species (derived from zoysia grass rhizosphere), stimulates growth and disease resistance in the cucumber plant. The use of *Pencillum* sp. with barley grain enhanced root and shoot growth and biomass of cucumber plants. Cordier *et al.* (1998) suggested that the growth substrates used in seedling production are usually devoid of sufficient beneficial microorganisms, by introducing such microorganisms to the substrates in sufficient quantity, it would be possible to lower fertilizer and pesticide inputs and grow the plants in a more sustainable way.

Conclusion

Today herbal medicines have great demand all over the world because of the awareness of side effects of modern (allopathic) medicines. There is a huge demand for the production of medicinal plants. The bioactive compounds of medicinal plants are largely affected by abiotic and biotic factors.

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The rhizomicrobiome of medicinally important plants play an effective role in improving its growth as well as medicinal values. The amendment of plant growth promoting microbes is a sustainable technology to improve the medicinal value of several important medicinal as well as other important plants. The use of chemicals fertilizers and pesticides causes serious side effects to the plants as well as the users. However, the selection and inoculation of specific and effective beneficial microbes for particular plant is important for its large scale production.

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