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Suppression of seedling diseases of silk cotton (*Bombax Ceiba*) and eucalyptus (*Eucalyptus tereticornis)* by *Pleurotus Sajorcaju* decomposed coir pith and Plant Growth Promoting Rhizobacteria (PGPR) based potting media

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Abstract

The studies on the decomposition of coir waste by yeast slurry and *Pleurotus Sojar caju* showed that it was found to decompose the coir waste effectively. The nutrient analysis of the decomposed coir pith showed that the *Pleurotus* decomposed coir pith (PDCP) recorded a higher nutrient status than the yeast slurry decomposed coir pith (YDCP). The effect of decomposed coir pith, biocontrol agents and Plant growth promoting Rhizobacteria (PGPR) based potting media on forest tree seedling disease and their growth was studied. Furthermore, the various combinations of normal nursery mix and *P. Sojar caju* decomposed coir pith (PDCP) used were 0:100, 25:75, 50:50 and 100:0. The media with equal proportions of normal media mix (NMM) and (PDCP) at 50:50 recorded maximum germination in forest tree seeds, *viz.,* Eucalyptus (*Euclayptus tereticornis),* Silk cotton (*Bombax ceiba*). The treated seeds with *Trichoderma viride* were sown in a PDCP 50:50 ratio along with the amendment of *Pseudomonas fluorescens+ Azospirillium + Phosphobacteria* @ 10 % (v/v) were recorded the maximum shoot and root growth in both the seedlings of silk cotton and eucalyptus and also resulted in a minimum disease incidence of 2.36 and 1.36 percent in Eucalyptus wilt (*Fusarium oxysporum*) and silk cotton root rot (*Verticillium albo-atrum),* respectively. These findings highlight an eco-friendly approach to disease suppression in forest nurseries, offering a sustainable alternative to chemical fungicides.

Keywords

forest tree seedling; PGPR; potting medium; suppression of disease; yeast slurry

Introduction

Soil-borne pathogens are responsible for root diseases in forest nurseries around the world. The tree seedlings are subjected to serious soil-borne pathogens that are causing serious damage every year. Treatment of soil, seeds and seedlings with fungicides is traditionally used to control the causative agents. However, fungicides do not possess selective action and kill useful epiphytic and saprotrophic microbiota and mycorrhizal fungi (1). One possible way to control phytopathogens and not affect beneficial soil microorganisms is to use biological methods of control (2), such as the introduction of anti-phytopathogenic microorganisms into the soil with an appropriate potting medium.

Potting media can influence the equality of seedlings to a great extent. Seedlings raised in good medium can ensure better establishment and growth when planted out in the field. Plant growth-promoting rhizobacteria (PGPR) are naturally occurring soil The beneficial bacteria that can aggressively colonize plant roots and stimulate plant growth when applied to root and seed zones (3,4). PGPR can control soil-borne fungi, enhance plant survival and induce systemic resistance to pathogens (5). The utilization of PGPR has the potential to facilitate the development of desirable root characteristics that enhance the efficient utilization of soil resources, hence enabling the achievement of sustainable agricultural output (6,7,8).

The information on the effect of media mixes on the growth of seedlings is essential for large-scale production of healthy seedlings in the nursery. A cheap and successful medium will enable the farmers and forests to produce high-quality seedlings of forest tree species for extensive planting under social/ agroforestry planting programs. Likewise, (9,10) reported that organic amendments such as coir pith and biochar enhance soil microbial diversity and activity, contributing to improved plant health and disease suppression.

The current study explores the efficacy of coir waste decomposed by *Pleurotus sajor-caju* in suppressing soil-borne diseases in forest tree seedlings. Specifically, it examines the synergistic effects of PGPR, biocontrol agents and nutrient-rich potting media on the growth and health of Eucalyptus (*Eucalyptus globulus*) and Silk cotton (*Ceiba pentandra*) seedlings. By integrating organic amendments and biological control measures, this research aims to provide a cost-effective, eco-friendly solution for forest nurseries to combat soil-borne diseases and enhance seedling quality for large-scale planting programs.

Materials and Methods

Isolation of pathogens

The diseased seedlings of *Ceiba petandra* and *Eucalyptus globules* were collected from different locations such as killikulam (Thirunelveli district), Forest College of Research Institute (FCRI), Mettupalayam and Botanical Garden Nursery (Coimbatore) and causal agents were isolated from infected plant parts by half plate technique using Potato Dextrose Agar (PDA) (11). The cultures were maintained in PDA slants at 4 °C for further studies.

Isolation of antagonists

Fungal and bacterial antagonists were isolated from the soil samples collected from the rhizosphere of forest nursery namely Culture Collection Centres of the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore for biocontrol agents and the bio-inoculants were obtained from the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore seedlings. The antagonists were isolated by serial dilution method. The dilution range adopted was 10^{-3} to 10^{-4} and 10^{-5} to 10^{-6} for fungi and bacteria, respectively. The soil antagonistic microbes *viz*., *Pseudomonas fluorescens*, *Trichoderma viride* were isolated from the soil by using King's B (KB) medium (12) and Trichoderma Special medium (TSM) (13), respectively. The morphological identification was made through a microscope (Lawrence & Mayo Binocular Microscope LM 52 1704, Halogen, 40X -1000X) and pure cultures were maintained on respective agar slants at 4° C for fungal biocontrol agents. The *Pseudomonas fluorescens* strains were confirmed by the biochemical method explained by (14).

In vitro screening of biocontrol agents against isolated pathogen

Both fungal and bacterial biocontrol agents were tested against *Fusarium oxysporum* and *Verticillium albo-atrum* by dual culture method on PDA medium (15). A nine mm disc of fungal antagonist was placed at one end of the Petri dish over the PDA medium and just opposite to that a nine mm disc of pathogen was placed concomitantly. In the case of the bacterial antagonist, the bacterial culture was streaked at one side of Petri dish (1 cm from the edge of the plate) containing PDA medium and a mycelial disc (9 mm) of seven-day old culture of the pathogen was placed on the opposite side of Petri dish. The plates were incubated at room temperature (28±2° C) and the pathogen inhibition was measured when control (mycelial growth in plate inoculated with pathogen alone) reached maximum. Per cent inhibition of pathogen by biocontrol agents was calculated as follows.

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

Were, C- mycelial growth of pathogen in control

T- Mycelial growth of pathogen in dual plates.

Decomposition of coir waste

The decomposition of coir pith was procured Coconut Research Station, Aliyar, Pollachi was done as described by (16). One hundred kg of coir pith was spread in an area of 2 x 1 m^2 under shaded conditions 26C with relative humidity of 67-73% after removing weeds. The decomposing agents viz., Yeast slurry (200 g) and one packet of basidiomycetous fungus, *Pleurotus Sojar caju* (300 g grain spawn) were sprinkled over the coir pith into two different heaps and then hundred kg of coir pith was again distributed over *decomposing agents*. Then, urea (1 kg) was sprinkled over the bed and covered by a hundred kg of coir pith. Likewise, one ton of coir pith was sandwiched and watered at five-day intervals. The heap was after 60 days samples were taken from the heap for detailed nutrient analysis as the procedure of (17). The plant nutrient content of the decomposed coir waste is presented in Table 1.

Selection of appropriate medium in vitro

The coir pith was decomposed by using yeast (Yeast slurry decomposed coir pith-YDCP) and *Pleurotus* (*Pleurotus* decomposed coir pith -PDCP) respectively and mixed with the Standard Media Mixture (SMM) [(1 (red soil):1(Farmyard manure):1 (sand)] separately in different ratios *viz.,* 100:0, 25:75, 50:50, 75:25 and 0:100. The mixtures were filled in plastic seedling trays and the seeds of silk cotton *and Eucalyptus* were dibbled. The germination of the seeds was observed regularly at two-day intervals.

Combination of Decomposed coir waste, biocontrol agents and PGPR on forest tree seedling growth

The influence of formulations biofertilizers such as *Azospirillum brasilense* and Phosphobacteria (*Bacillus megaterium*) obtained from the Department of Agricultural Microbiology

Table 1. Chemical and nutrients Profiles of decomposed coir pith by Pleurotus sajor-caju and Yeast Slurry Methods on a dry weight basis

S. no	Chemical properties	Yeast Slurry based decomposed coir pith (YDCP)	Pleurotus Sojar caju based coir pith (PDCP)
1	pH	6.4	6.8
2	EC (dSmÿ1)	0.11	0.13
3	Organic carbon (%)	27	29.3
4	Nitrogen (%)	1.04	1.10
5	Phosphorus (%)	0.16	0.18
6	Potassium (%)	0.80	0.86
7	Calcium (%)	1.90	1.94
8	Magnesium (%)	0.91	0.96
9	C/N ratio	25.96	26.63
10	Iron ppm	3956	4236
11	Zinc ppm	36	41
12	Copper ppm	609	694

either single or in combination with the biocontrol agents were added (10 % v/v) with the best-decomposed coir waste and used as potting media in glasshouse conditions. The disease incidence shoots and root length, dry matter production, germination percentage and vigour index of Eucalyptus and Silk cotton, were recorded.

Statistical analysis

Statistical analysis was performed using analysis of variance was applied and the significance between the means was compared using one-way ANOVA and the student t-test at the significance level of 0.05 in GRAPES Software.

Results

Isolation of pathogens and antagonists

In the present studies, three different major soil-borne pathogens were isolated from diseased tree seedlings. The soil-borne pathogens like *Fusarium oxysporum* from silk cotton and *Verticillium albo-atrum* from eucalyptus were found more frequently associated with the diseased seedlings. The damping of fungi, *Pythium aphanidermatum* was also noticed in the diseased part of the seedlings.

The predominant antagonists found in the rhizosphere of the forest seedlings were *Pseudomonas fluorescens* and *Trichoderma viride*. They were found more frequently compared to other antagonists.

In vitro assay of antagonists against forest nursery pathogens

The efficacy of biocontrol agents was tested against *F. oxysporum and V. albo-atrum*, the highest per cent inhibition was recorded by *T. viride* isolate TV1 (83.3 %) followed by *P. fluorescens* Pf1 (72.3 %). To compare the efficacy of *Pythium aphanidaermatum* was also effectively inhibited by *T. viride* TV1 (71.2 %) followed by *P. fluorescens* Pf1 (69.5 %) and these two isolates were selected for greenhouse studies two times.

Decomposition of Coir pith

The results showed that the coir waste was effectively decomposed by *Pleurotus Sojar caju*. The nutrient analysis showed that *Pleurotus Sojar caju-based* coir waste recorded a maximum content of Organic carbon (29.3 %), Nitrogen (1.10 %), Phosphorus (0.18%) and Potassium (0.86 %) when compared to Yeast slurry decomposed coir pith (Table 1). The trace elements such as calcium and magnesium were increased in PDCP. In this study, PDCP was selected to assess the combination of biocontrol agents *Trichoderma viride* TV1 and Plant Growth Promoting Rhizobacteria *viz., P. fluorescens, Azospirillium, Phospho bacteria.*

Effect of combining biocontrol agents and PGPR on forest tree seedlings

The treatment consisting of a media mix of 25:75 with Phospobacteria and seed treatment with *Pseudomonas fluorescens* showed maximum root and shoot length in *Eucalyptus* and silk cotton which was on par with 50:50 (Table 2). Similarly, the above-mentioned treatments exhibited good performance in the case of germination percentage and vigour index than other treatments.

Discussion

The potential for using decomposed coir waste along with PGPR strains in silk cotton and Eucalyptus was demonstrated, as all rhizobacteria increased the speed of germination and vigor of the seedling. In addition, more rapid and uniform germination of seeds has been shown to result in a significant decrease in mortality caused by soil-borne fungus. Studies have shown that the positive aspects of these rhizobacteria are growth promotion under field conditions (18,19).

Compared to the results of (20) on the effect of supplementation of decomposed coir waste @ 25 g/3 kg of soil along with inorganic fertilizer (NPK) on the growth and quality of leaves of the mulberry plant *Morus alba* L., in the present investigation enhanced the quality of seedlings and provides all the essential nutrients required for growth and yield of the mulberry plant, with an added advantage of protecting the physio-chemical and biological characteristics of the soils

In Canada, the use of *Pseudomonas fluorescens* resulted in a significant increase in the biomass of lodgepole pine (*Pinus contorta)* and white spruce (*Picea gluca*) seedlings 8 weeks after sowing (21). Likewise, pyoverdin mutant MPFM1 of *P. aeruginosa* strain 7NSK2 produces the siderophore pyochelin (22) and inhibits the growth of *R. solanacearum*, whereas mutant KMPCH that lacks both pyoverdin and pyochelin did not inhibit growth. Strain *P. fluorescens* CHA0 can produce several antibiotics, including 2,4-diacetyl phloroglucinol, pyoluteorin, pyrrolnitrin and HCN (23,24). Because growth inhibition by *P. fluorescens* CHA0 was not iron-dependent, one or more of these antibiotics were likely responsible for its antagonistic activity towards *R. solanacearum*. The strong antagonistic activity of *P. putida* WCS358 suggests that the growth of *R. solanacearum* is very sensitive to 2,4- diacetylphloroglucinol and that this metabolite may explain the growth inhibition by CHA0.

Another report on seedlings treated with this *P. fluorescens* showed an increase in the rate and percent germination when treated before sowing. This effect is similar to the data reported for PGPR combination with potting media in this study. Yet another PGPR isolate, *Burkholderia cepacia*, used in containerized nurseries in British, resulted in a significant decrease in the amount of *Fusarium oxysporum* on *Pseudotsuga menziesii* roots, increased seedling stands and biomass (25). Finally, the integration of organic amendments with microbial

Table 2. Efficacy of different suppressive potting medium combinations against forest seedling diseases and its growth parameters

Sl. No	Treatment*	Germination percentage		Shoot length *(cm)*		Root length *(cm)*		Disease incidence		Vigor index	
		Eucalyptus	Silk cotton	Eucalyptus	Silk cotton	Eucalyptus	Silk cotton	Eucalyptus	Silk cotton	Eucalyptus	Silk cotton
T1	Standard Media Mixture (Red soil +sand + FYM_ @ 1:1:1	30.0 (29.2)^{d}	15.0b^{c} (16.7)	5.0	6.9	2.2	3.2	58.4 (4.8)	61.2 (52.4)	266^{e}	285de
T2	Standard Media Mixture –with Seed treatment *T.viride*	35.0 (35.7)^{cd}	25.0b^{c} (22.7)	4.3	6.4	3.1	3.6	35.6 (38.3)	37.6 (37.7)	315de	287de
T3	PDCP (25:75)- with seed treatment	30.0d (29.2)	29.8^{bc} (24.9)	4.6	8.9	3.6	4.7	27.4 (32.7)	24.4 (29.6)	401de	636^{be}
T4	PDCP (50:50)- with seed treatment *T.viride*	64.0$^{a\text{-}d}$ (51.0)	40.0^{abc} (32.0)	7.4	11.5	3.9	5.5	21.3 (27.1)	22.6 (28.2)	544^{d}	512^{cde}
T5	PDCP (75:25)- with seed treatment *T.viride*	72.0$^{a\text{-}d}$ (60.2)	35.0^{abc} (28.0)	7.7	12.1	3.9	5.6	16.8 (24.3)	20.3 (27.1)	721^{d}	811$^{a\text{-}e}$
T6	PDCP alone	95.0^{a} (80.1)	35.0^{abc} (28.8)	7.3	10.6	4.4		9.1 (18.8)	19.2 (26.3)	1520^{b}	926^{ac}
T7	T4+*P. fluorescens* @ 10 % (v/v)	85.0^{abc} (73.5)	50.0^{ab} (45.2)	8.3	12.1	5.9	6.8	7.5 (15.2)	14.2 (22.2)	1332^{c}	1319^{ad}
T8	T4+*Azospirillium* @ 10 % (v/v)	60.0^{bc} (51.1)	65.0^{ab} (54.3)	9.9	11.8	5.4	5.1	11.4 (20.1)	17.4 (24.4)	1159^{bc}	1475^{abc}
T9	T4+Phosphobacteria @ 10 % (v/v)	75.0^{abc} (67.6)	55.0^{bc} (44.0)	10.5	11.6	5.1	5.3	14.2 (22.3)	15.3 (23.2)	1170^{bc}	1775^{ab}
T10	T4+ *Pseudomonas*+ *Azospirillium*+ *Phospho bacteria* @ 10 % (v/v)	95.0^{a} (83.5)	80.0^{a} (66.9)	10.9	17.5	6.2	7.5	8.6 (16.6)	6.3 (14.2)	1550^{a}	1972^{a}

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

Values in parentheses are arcsine-transformed value

*-Mean of three replications

biocontrol aligns with recent advances in soil microbiome research, highlighting the role of microbial diversity in plant fitness and disease suppression (9). The reduced disease incidence observed here may be attributed to microbial competition for resources and the induction of systemic resistance, mechanisms widely supported in contemporary studies on PGPR and organic amendments. (26) observed maximum height growth in eight-week-old *Acacia mangium* seedlings grown in soil mixed with sawdust or coir dust and in pure peat. Maximum diameter in *Atrocarpus hirsutus* was shown by a media of soil + sand + cow dung (27). This study and these examples of PGPR strains strongly suggest that the beneficial effects of using PGPR are much better in terms of the growth and vigour of seedlings.

The biocontrol agents *viz., Pseudomonas fluorescens* and *Trichoderma viride* are potential inoculants which suppress the soil-borne plant pathogens by producing antibiotics, mycoparasitic nature with competitive colonisation around the root region and production of iron chelating siderophore *viz.,* pyrrolnitrin which sequesters available iron from the soil. This aligns with studies that show siderophore-producing PGPR strains are particularly effective against pathogen inhibition by depriving them of essential nutrients (28). The observed reduction in disease incidence in Eucalyptus (2.36%) and Silk cotton (1.36%) seedlings highlights the practical relevance of this mechanism in creating a disease-suppressive PGPR.

The scalability and cost-effectiveness of PGPR strategies are crucial for their widespread adoption. These technologies must be versatile enough to suit various crops, environments and farming practices. Achieving this requires continuous research and development to customize PGPR solutions for specific agricultural needs. Moreover, reducing production costs and enhancing accessibility, especially for farmers in developing countries, is essential. Collaboration between researchers, farmers, governments and industry stakeholders is key to creating affordable and sustainable PGPR products and practices.

Conclusion

The plant pathogenic suppressive medium developed from our study enriched with *P. fluorescens*, *T. viride*, *Azospirillum*, *Phosphobacteria* is a potential potting medium for the production of healthy forest seedlings. The proposed formula could be used to develop the new potting medium product as a ready reckoner for nursery growers. In the future, the enriched coir pith-based medium will be a novel value addition to the coir industry which will have global demand for the production of healthy and disease-free seedlings production.

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Authors' contributions

VS conceived and planned the design of study, carried out the experiment and SKM reviewed the manuscript. RR performed statistical analysis and prepared the manuscript with inputs. All the authors approved and read the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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