Research article

Plant Growth Promoting Microbes in the Future Management of Indonesian Estate Forests

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Abstract.
Plant growth promoting microbes (PGPM) are soil-borne microbes that colonize plant internal tissues as endophytes or live on root surfaces. They are classified into three main groups based on their growth promoting mechanisms, namely biofertilizers, biostimulants, and biocontrol agents, each of which contribute directly or indirectly to improved plant growth and productivity in a variety of ecosystems. PGPM development as biocontrol agents in the context of integrated pest and disease management has been a research program initiative in several forestry companies for quite some time. Adoption of PGPM as biofertilizers and/or biostimulants, particularly in nursery operations to produce vigorous and healthy seedlings, has recently gained traction. The current state and potential future roles of PGPM in the sustainable production of Indonesian estate forests are discussed in this paper.

Keywords: *Acacia*; biocontrol agent; biofertilizer; biostimulant; *Eucalyptus*

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1. Introduction

Plant growth promoting microbes (PGPM) live on the surface of the roots or in the internal plant tissues as endophytes. They serve varied ecological roles in different ecosystems. PGPM can be fungi (PGPF) or bacteria (PGPB/PGPR) and classified into three groups based on the mechanisms involved in the growth promoting processes, i.e. (i) biofertilizers (improving nutrient availability and utilization), (ii) biostimulants (providing beneficial substrates), and (iii) biocontrol agents (inhibiting pathogen growth through the generation of antimicrobial substances or space and food competition) [1]. Some of PGPM display two or even three different activities. PGPM may boost plant growth and health by direct and/or indirect mechanisms. The direct processes improve plant growth by producing minerals or by yielding growth regulators, while indirect modes enhance plant resilience in the existence of both biotic and abiotic stresses [2-3].

In 2018 the coverage of Indonesian estate forests exceeded 8,5 million ha [4], most of which were situated on the Sumatera and Kalimantan islands. In that same year the Forestry Sector shared ca. USD 12,2 billion to the country's income [5]. One limiting factor in the sustainable management of estate forests in Indonesia has been pest and disease attack. Introduction of new plant species has frequently led to the emergence of new and threatening pests and diseases. Several pathogens have been reported since the introduction of fast-growing acacias and eucalypts. Abiotic stresses have also been identified to cause reduced tree growth rate or even mortality in certain areas. Utilization of plant resistance [6-7] and development of PGPM as biocontrol agents [8-16] in the scenario of integrated pest and disease management have for some time been one of research program initiatives in a number of forestry companies. Recently, however, adoption of PGPM as biofertilizers and/or biostimulants, especially in the nursery operations to produce vigorous and healthy seedlings, has also regained momentum [17-19]. This paper is aimed at briefly discussing current status and potential future roles of PGPM in the sustainable production of Indonesian estate forests.

1.1. Endophytic Trichoderma

In this paper the discussion is focused on endophytic *Trichoderma* isolates as they are considered more stable and have a wider plasticity than the free-living isolates. They are also better protected from abiotic stresses compared to the rhizospheric mates. They have capacities to improve both plant health and plant growth as well as to exist in the plant root through the rotation [20]. More than 200 putative isolates were collected and isolated from different ecosystems and localities in Riau, Indonesia (Figure 1), and subsequently screened (Figure 2). Out of those isolates, some were able to increase the growth rate of *Acacia mangium* seedlings (height up to 26% and diameter up to 10%) and reduce significantly the red root rot disease incidence in the nursery (Figure 3) [11-17].

1.2. Organic Biofertilizer LOB-BS

A commercially available organic biofertilizer (LOB-BS) developed by LIPI (Indonesian Institute of Sciences) [21-22] was recently evaluated for their ability to enhance the growth and health of *A. crassicarpa* seedlings in nursery [17-18]. Figure 4 (top) shows that the LOB-BS was able to increase germination rate of up to 3.5 % [18]. The LOB-BS also had a positive impact on the growth of *A. crassicarpa* seedlings. As presented

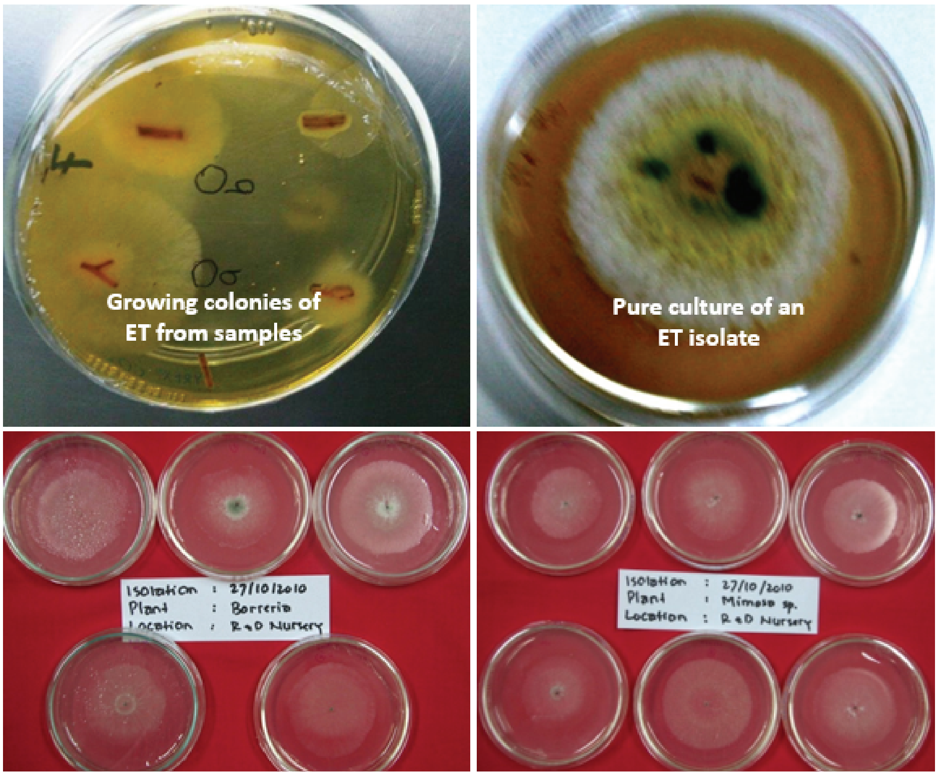

Figure 1: Isolation and purification of putative endophytic *Trichoderma* isolates [11-17].

Figure 2: Nursery screening of endophytic *Trichoderma* isolates [11-17].

in Figure 4 (bottom) below, the height of *A. crassicarpa* seedlings at 12 weeks after application was increased at the rate of 20 % [18]. The microbes in the LOB-BS play a critical role in reducing immobilization of available nutrients in the media, increasing nutrient absorption leading to increased height of *A. crassicarpa* seedlings.

Observations were also made on the incidence of major diseases of *A. crassicarpa* seedlings, i.e. Xanthomonas leaf blight, Fusarium wilt and leaf spots caused by pathogens *Pestalotiopsis* sp. and *Phaeotrichoconis* sp. Figure 5 (top) shows that the LOB-BS reduced the incidence of major diseases on *A. crassicarpa* seedlings of up to 40 % [18]. The ability of the LOB-BS to improve seedling vigor was also obvious

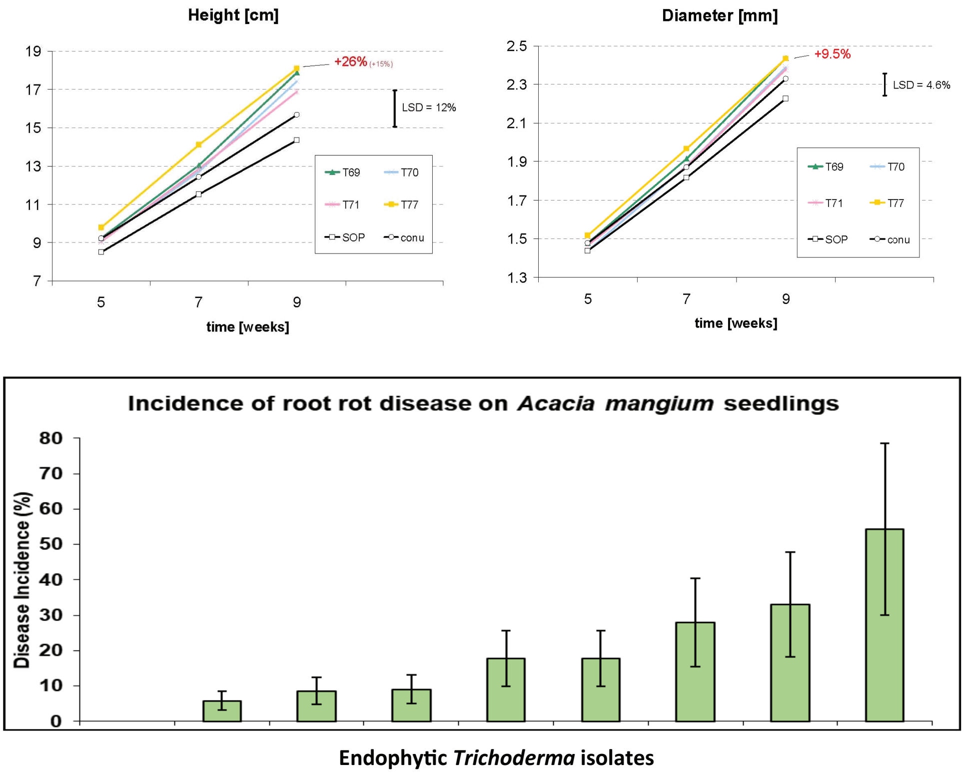

Figure 3: Some endophytic *Trichoderma* isolates were able to to improve the growth rate (top) of and reduce significantly or even eliminate red root rot disease incidence (bottom) on *Acacia mangium* seedlings in the nursery [11-17].

[17]. Figure 5 (bottom) shows the effect of the product on increasing the vigor of *A. crassicarpa* seedlings in nursery, providing in turn more resistant stands against abiotic and biotic factors when later planted commercially in estate forests.

1.3. Arbuscular Mycorrhiza

Artificial chemicals have been routinely used in nursery operations to maintain nursery productivity. The utilization of chemical fertilizers and pesticides, however, has posed environmental concerns. Therefore, nursery should be managed in an environmentally justified manner. Utilization of PGPM as one option in this scenario has recently been reviewed in different ecosystems [23-26]. Application of mycorrhizae to form symbiotic association with plant roots is an example. It has been demonstrated that mycorrhizal association increases plant resistance or tolerance against biotic or abiotic stresses [27-29]. A number of different species of arbuscular mycorrhizae (AM) were recently tested in nursery for their ability to enhance growth of *Eucalyptus pellita* seedlings under lower consumption of chemical fertilizers and zero pesticides [17,19]. As it can be seen in Table

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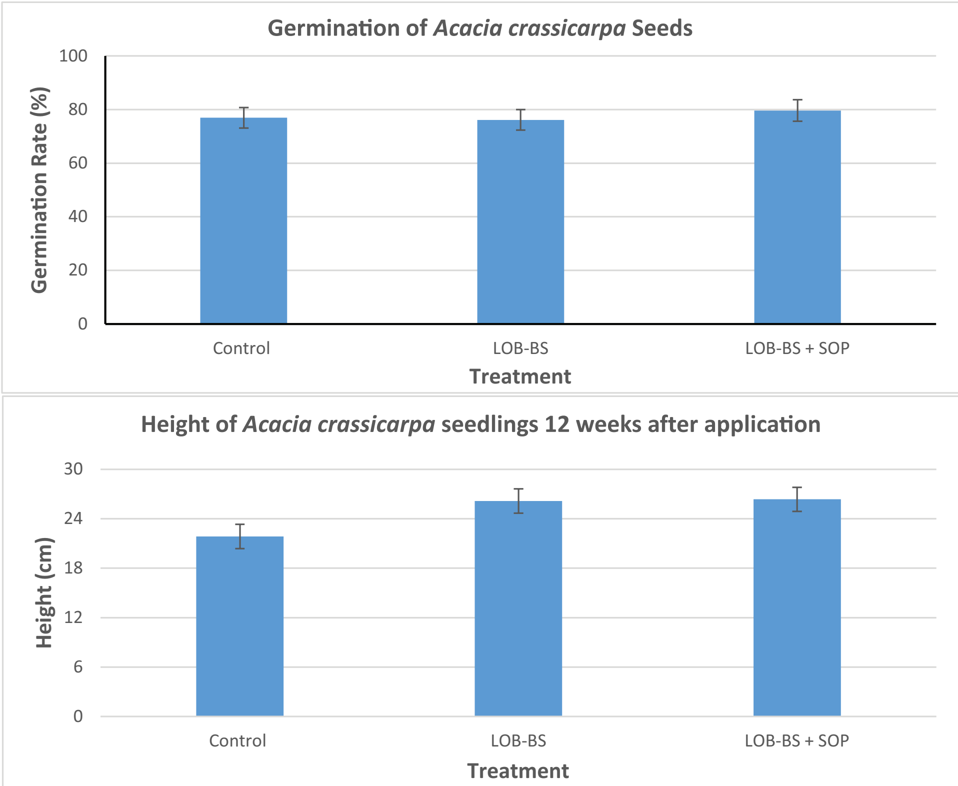

Figure 4: Effect of LOB-BS on the germination rate (top) and on the growth 12 weeks after application (bottom) of *Acacia crassicarpa* seedlings [18].

1, the two AM products tested, AM1 and AM2, were not able to compensate the reduced doses (both to 50 % and 25 %) of the current fertilizer regime (SOP) in term of seedling height and root collar diameter of *E. pellita* seedlings [19]. More research is required to explore possible reduction of fertilizer regimes through application of the AM products.

Table 1: Height and root collar diameter of *Eucalyptus pellita* seedlings at 90 days old in different treatments of arbuscular mycorrhizae, adapted from [19].

Treatment	Height (cm)*	Diameter (mm)*
AM1 + SOP	48.44a	4.97a
AM1 + 50 % SOP fertilizer regime	40.67b	4.30bc
AM1 + 25 % SOP fertilizer regime	33.46cd	3.58d
AM2 + SOP	49.64a	4.98a
AM2 + 50 % SOP fertilizer regime	36.37c	3.90cd
AM2 + 25 % SOP fertilizer regime	32.05d	3.46d
SOP (current fertilizer regime)	48.15a	4.76ab

*Numbers in the same column followed by the same letter are not statistically different (p-value < 0.05).

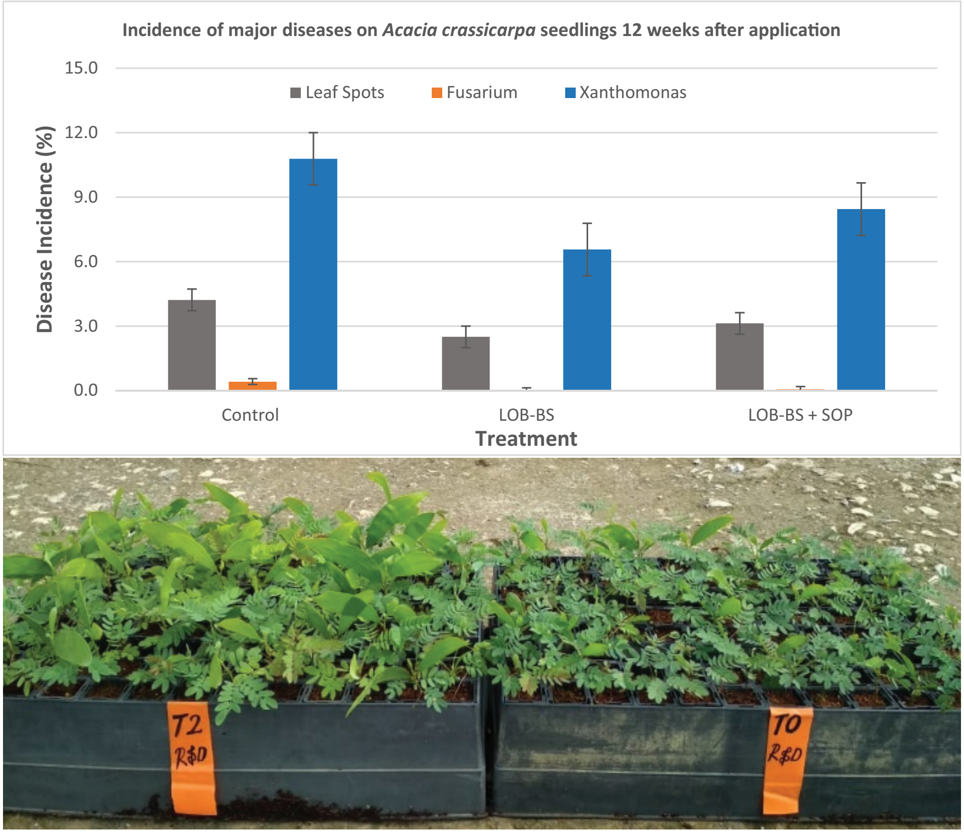

Figure 5: Incidence of major diseases on *Acacia crassicarpa* seedlings 12 weeks after application (top) and vigor of *A. crassicarpa* seedlings with (bottom left) and without (bottom right) application of LOB-BS [17].

1.4. Future Potentials

The role of PGPM in different ecosystems has been adequately reviewed. Little is known, however, about their application in plant micropropagation or tissue culture processes. A special discussion on this topic was recently published [1]. The authors reveal that the strictly sterile conditions of the *in vitro* conventional tissue culture methods deserve to be revisited to provide the *vitro*-plants with the positive impacts of PGPM. They further mention that PGPM should increase the efficiency of plant tissue culture techniques, leading to reduced production cost of *vitro*-plants. The utilization of adequate PGPM in micropropagation is called “biotization” [30], which should reduce chemical use in plant production [31].

Biotization is gaining attention from researchers of different fields including forestry. In nature, all species of forest trees depend on symbiotic associations with endomycorrhizal fungi [32]. *In vitro* mycorrhization of micropropagated plants prior to acclimatization improves survival and resilience to both biotic and abiotic stresses and enhances the functionality of the root system [276-29, 33]. In the forestry sector, *in vitro* mycorrhization techniques have indeed been developed for several trees including *Eucalyptus* [34-35]. Application of mycorrhiza enables *E. tereticornis* micropropagated plantlets to survive and grow better [36]. Identification and selection of effective PGPM isolates with ecological plasticity for plant tissue culture use are the main challenges in the forestry sector, putting the benefits of consortiums rather than single strain inoculums into consideration. In addition, efforts should be made toward nanotechnology-based formulations of the PGPM. Genetically modified inoculants should also provide opportunities to improve the quality of the PGPM isolates [1].

In recent years PGPM, especially bacteria, have been utilized as the priming agents both as *in vitro* co-cultures and on transplanting. The process, often referred to as biological hardening or biopriming, is aimed at increasing plant growth and health at a reduced chemical input. Plant growth promoting rhizobacteria (PGPR) and mycorrhiza are among the PGPM used to accelerate the seedling production processes in nurseries by reducing the time required for lignification of the micropropagated plantlets. Research indicates that the beneficial microbes are sustained even after exposed to the field conditions [37]. Despite this fact, however, the inoculation of tissue culture propagules to enhance plant fitness, productivity and resistance against biotic stresses is still relatively new [38].

Pseudomonas and *Bacillus* have been the most widely studied organisms as potential priming agents. In tea, for example, *Bacillus subtilis*, *Bacillus* sp. and *Pseudomonas corrugata* were reported to enhance survival (up to 100 %) compared to control plants [39]. Bacterial inoculations of tissue culture propagules also increased tea growth [38]. Similarly, mixtures of endophytic *B. pumilus*, *B. subtilis* and *P. fluorescens* were effective in increasing the growth and leaf number of micropropagated banana plants under greenhouse study. The application of the bacterial consortia also resulted in 61.62 % disease reduction over control [40].

In certain estate forest areas, acidic sulphate soil is the dominant site. This type of soil, particularly that with high pyrite content, is not conducive for optimum tree growth. The application of sulphate-reducing bacteria (SRB) has been suggested to mitigate the negative impacts of pyrite on tree growth. SRB inhibits the growth of the Gram-negative, acidophilic organism *Acidithiobacillus* spp., which is capable of expediting pyrite oxidation. Thus, SRB will lead to the limited production of acid resulted from the process. In this scenario, *Desulfovibrio vulgaris* and *D. caledoniensis* are SRB species widely studied. The two species were able to increase their environmental pH from 4 to 7.4 and from 6 to around 7.5, respectively [41].

2. Conclusion

The utilization of PGPM in the management of Indonesian estate forests has been initiated. Biocontrol agents provide a significant contribution to the integrated disease management. Although research on organic biological fertilizer and mycorrhiza is still in its infancy and our knowledge is still limited, some promising results have been obtained.

Future work should also be directed to explore other roles of PGPM to ensure the sustainable management of Indonesian estate forests. This may include biotization and biopriming agents to enhance plant growth and health, as well as sulphate reducers to mitigate negative impacts of acidic sulphate soil with high pyrite contents.

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References

[1] Soumare A, Diédhiou AG, Arora NK et al. Potential role and utilization of plant growth promoting microbes in plant tissue culture. Frontiers in Microbiology. 2021;12:1-13. https://doi.org/10.3389/fmicb.2021.649878

[2] Arora NK, Fatima T, Mishra J et al. Halo-tolerant plant growth promoting rhizobacteria for improving productivity and remediation of saline soils. Journal of Advanced Research. 2020;26:69–82. https://doi.org/10.1016/j.jare.2020.07.003

[3] Goswami D, Thakker JN, Dhandhukia PC. Portraying mechanics of plant growth promoting rhizobacteria (PGPR): A review. Cogent Food & Agriculture. 2016;2:1–19. https://doi.org/10.1080/23311932.2015.1127500

[4] Badan Pusat Statistik. Statistics of timber culture establishment – 2018. Jakarta: BPS RI; 2019.

[5] Pejabat Pengelola Informasi dan Dokumentasi, Kementerian Lingkungan Hidup dan Kehutanan. Available from: http://ppid.menlhk.go.id/siaran_pers/browse/1724

[6] Gafur A, Syaffiary A, Nugroho A, Wong CY, Sharma M. Plant tolerance as a component of *Ganoderma philippii* management in *Acacia mangium* plantations. Paper presented at: Proceedings of the Genetics of Tree-Parasite Interactions Meeting; 2015 Aug 23–28; Orleans, France.

[7] Gafur A, Nasution A, Yuliarto M, Wong CY, Sharma M. A new screening method for *Ganoderma philippii* tolerance in tropical Acacia species. Southern Forests: A Journal of Forest Science. 2015;77:75–81.

[8] Yuvika, Nasution A, Gafur A. Actinomycetes isolation and in vitro screening for *Xanthomonas* biocontrol. Jurnal Fitopatologi Indonesia. 2013;9:124–129. https://doi.org/10.14692/jfi.9.5.160

[9] Sastrini T, Nasution A, Gafur A. Isolation and screening of microbial antagonists for control of bacterial wilt of eucalyptus caused by *Ralstonia* spp. Paper presented at: Proceedings of the 5th Asian PGPR International Conference for Sustainable Agriculture; 2017 Jul16–19; Bogor, Indonesia.

[10] Nasution A, Glen M, Gafur A, Evans K, Mohammed CL. Endophytic bacteria as potential biological control agents. Paper presented at: Proceedings of the Ceratocystis International Workshop; 2016 Feb 15–16; Yogyakarta, Indonesia.

[11] Gafur A, Nasution A, Wong CY, Sharma M. Development of biological control agents to manage *Ganoderma philippii* in tropical *Acacia mangium* plantations. Paper presented at: Proceedings of the Genetics of Tree-Parasite Interactions Meeting; 2015 Aug 23–28; Orleans, France.

[12] Gafur A, Nasution A, Wong CY, Sharma M. Diversity of endophytic *Trichoderma* isolated from various ecosystems in Riau, Indonesia. Paper presented at: Proceedings of the 2nd International Conference on Life Sciences and Biotechnology, ICOLIB–2017: Integrated Biological Sciences for Human Welfare; 2017 Aug 7–8; Jember, Indonesia.

[13] Gafur A. Endophytic *Trichoderma* as biocontrol agents of red root rot disease in tropical plantation forests. Paper presented at: Proceedings of the 6th PGPR International Conference; 2019 Aug 18–22; Tashkent, Uzbekistan.

[14] Gafur A. Development of biocontrol agents to manage major diseases of tropical plantation forests in Indonesia: A Review. Environmental Sciences Proceedings. 2020;3:1-7. https://doi.org/10.3390/IECF2020-07907

[15] Gafur A. Plant growth promoting microbes (PGPM) for the sustainability of tropical plantation forests in Indonesia. Academia Letters. 2021;1308:1-3. https://doi.org/10.20935/AL1308

[16] Siregar BA, Liantiqomah D, Halimah, Gafur A, Tjahjono B. Screening of endophytic *Trichoderma* isolates to improve the growth and health of eucalyptus pellita seedlings. IOP Conference Series: Earth and Environmental Science. 2021;74(012084):1-8. https://doi:10.1088/1755-1315/974/1/012084

[17] Gafur A, Naz R, Nosheen A, Sayyed RZ. Role of plant growth promoting microbes (PGPM) in managing soil-borne pathogens in Indonesian estate forests. In Mawar R, Sayyed RZ, Sharma SK, Sattiraju KS, editors. PGPM in Arid Ecology: Status and Prospects. 2022; Springer Nature, Singapore, in press.

[18] Syaffiary S, Antonius S, Said D, Nugraha AK, Gafur A. Effect of organic fertilizer products on the growth and health of *Acacia crassicarpa* seedlings. 2022; KnE Publisher, in press.

[19] Arifin Z, Siregar BA, Rosanti N et al. Effect of arbuscular mycorrhizae on the growth of *Eucalyptus pellita* seedlings. 2022; KnE Publisher, in press.

[20] Hill R. *Trichoderma* root endophytes enhance plant health and vigour. Paper presented at: Proceedings of the 12^{th} International Trichoderma and Gliocladium Workshop; 2012 Aug 27–30; Christchurch, New Zealand.

[21] Antonius S, Agustiyani D, Dewi TK, Laili N, Osaki M. Tropical peatland eco-management. Osaki M, Tsuji N, Foead N, Rieley J, editors. 2021;Springer, Singapore. https://doi.org/10.1007/978-981-33-4654-3_9

[22] Pembuatan Pupuk Organik Hayati (POH) Beyonic-Startmik LIPI di BPP-PU Tanding. [cited 2021 May 11]. Available from: https://mablu.wordpress.com/2016/06/04/pembuatan-poh-byonic-startmik-lipi-di-bpp-pu-tanding

[23] Hamid B, Zaman M, Farooq S et al. Bacterial plant biostimulants: A sustainable way towards improving growth, productivity, and health of crops. Sustainability. 2021;13(2856):1-24. ttps://doi.org/10.3390/su13052856

[24] Arora H, Sharma A, Sharma S et al. Pythium damping-off and root rot of *Capsicum annuum* L.: Impacts, diagnosis, and management. Microorganisms. 2021;9(823):1-17. https://doi.org/10.3390/microorganisms9040823

[25] Saboor A, Ali MA, Hussain S et al. Zinc nutrition and arbuscular mycorrhizal symbiosis effects on maize (*Zea mays* L.) growth and productivity. Saudi Journal of Biological Sciences. 2021;28(11): 6339-6351. https://doi.org/10.1016/j.sjbs.2021.06.096

[26] Sarkar D, Sankar A, Devika OS et al. Optimizing nutrient use efficiency, productivity, energy use efficiency, and economics of red cabbage following mineral fertilization and biopriming with compatible rhizosphere microbes. Scientific Reports. 2021;11(15680):1-14. https://doi.org/10.1038/s41598-021-95092-6

[27] Gafur A, Schützendübel A, Langenfeld-Heyser R, Fritz E, Polle A. Compatible and incompetent *Paxillus involutus* isolates for ectomycorrhiza formation in vitro with poplar (*Populus* x *canescens*) differ in H_2O_2 production. Plant Biology 2004;6:91–99.

[28] Gafur A, Schützendübel A, Polle A. Peroxidase activity in poplar inoculated with compatible and incompetent isolates of *Paxillus involutus*. Hayati Journal of Biosciences. 2007;14:49–53.

[29] Langenfeld-Heyser R, Gao J, Ducic T, Tachd P, Lu CF, Fritz E, Gafur A, Polle A. *Paxillus involutus* mycorrhiza attenuate NaCl-stress responses in the salt-sensitive hybrid poplar *Populus* x *canescens*. Mycorrhiza. 2007;17:121–131.

[30] Nowak J. Benefits of in vitro “biotization” of plant tissue cultures with microbial inoculants. In Vitro Cellular & Developmental Biology – Plant. 1998;34:122–130. https://doi.org/10.1007/BF02822776

[31] Kanani P, Modi A, Kumar A. Biotization of endophytes in micropropagation: a helpful enemy. In Kumar A and Singh VK, editors. Microbial Endophytes: Prospects for Sustainable Agriculture, Food Science, Technology and Nutrition. 2020; Woodhead Publishing – Elsevier, Cambridge:357–379.

[32] Brundrett MC. Mycorrhizal associations and other means of nutrition of vascular plants: Understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant Soil. 2009;320:37-77. https://doi.org/10.1007/s11104-008-9877-92009

[33] Siddiqui ZA and Pichtel J. Mycorrhizae: an overview. In Siddiqui ZA, Akhtar MS, Futai K, editors. Mycorrhizae: Sustainable Agriculture and Forestry. 2008; Springer, Netherlands:1–35.

[34] Freire CG, Giachini AJ, Gardin JPP et al. First record of in vitro formation of ectomycorrhizae in *Psidium cattleianum sabine*, a native myrtaceae of the Brazilian Atlantic forest. PLoS One. 2018;13(5):1-15. https://doi.org/10.1371/journal.pone.0196984

[35] Di-Gaudio AV, Tubert E, Laino LE et al. New and rapid micropropagation protocol for *Eucalyptus grandis* Hill ex Maiden. Forest Systems. 2020;29(1):1-6. https://doi.org/10.5424/fs/2020291-15965

[36] Reddy MS, Satyanarayana T. Inoculation of micropropagated plantlets of *Eucalyptus tereticornis* with ectomycorrhizal fungi. New Forests. 1998;16:273–279. https://doi.org/10.1023/A:1006589310990

[37] Kavino M, Harish S, Saravanakumar D, Jeyakumar P, Kumar N, Samiyappan R. Biological hardening – A new approach to enhance resistance against biotic and abiotic stresses in micropropagated plants. Tree and Forestry Science and Biotechnology. 2010;4(Special Issue 1):11-21.

[38] Nowak J, Shulaev V. Priming for transplant stress resistance in in vitro propagation. In Vitro Cellular and Developmental Biology – Plant. 2003;39(2):107-124.

[39] Pandey A, Palni LMS, Bag N. Biological hardening of tissue culture raised tea plants through rhizosphere bacteria. Biotechnology Letters. 2000;22:1087–1091.

[40] Jebakumar RM, Selvarajan R. Biopriming of micropropagated banana plants at pre- or post-BBTV inoculation stage with rhizosphere and endophytic bacteria determines their ability to induce systemic resistance against BBTV in cultivar Grand Naine. Biocontrol Science and Technology. 2018;28(11):1074-1090. https://doi.org/10.1080/09583157.2018.1514583

[41] Tran TTT, Kannoorpatti K, Padovan A, Thennadil S. Sulphate-reducing bacteria's response to extreme pH environments and the effect of their activities on microbial corrosion. Applied Sciences. 2021;11:1-19. https://doi.org/10.3390/app11052201