APPLICATION OF MICROBIAL CONSORTIA IN COCOA SEEDLING GROWTH

Nurfadzilah M.[1*] & Ishak Z[2].
[1] Division of Cocoa Upstream Technology, Cocoa Research, and Development Centre, Malaysian Cocoa Board, Jalan Jengka 23, P.O. Box 34, 28000 Temerloh, Pahang, Malaysia
[2] Division of Biotechnology, Cocoa Innovation & Technology Centre, Malaysian Cocoa Board, Lot Pt 12621, Nilai Industrial Park, 71800 Nilai, Negeri Sembilan, Malaysia
Corresponding author: nurfadzilah@koko.gov.my

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ABSTRACT – *Biofertilizers are defined as formulations containing living microorganisms or latent cells having the potential of colonizing the roots of crops and plants and promoting growth by improving nutrient availability and acquisition. Most commercially available biofertilizer products are based on single species and have shown success in achieving specific goals following validation, as evidenced by several recent meta-analyses. The main purpose of this present study is to enhance the idea of mixing multiple species with complementary traits which is known as consortium. Furthermore, the use of agricultural waste as a carrier material will help in increasing the efficiency of the biofertilizer and allowing easy handling and prolong the shelf life of the biofertilizer. The research was conducted in the Cocoa Research and Development Centre greenhouse, Jengka, Pahang. Five different formulations of the microbial consortium were prepared, using rice husk char and packed using polyethylene bags, and sterilized at 121°C or 20 minutes before microbial consortia formulations were added according to the treatments. The formulation was added to the pot after two months of plantings. The results of the pot study showed the application of microbial consortia of UL and B7 with half amount of normal inorganic fertilizer applications has an equivalent result with the application of normal applications of inorganic fertilizer but with a lesser amount of inorganic fertilizer (12:12:17:2 +TE).*

Keywords: Microbial consortia, Plant growth promoting rhizobacteria, cocoa seedling, and plant growth

INTRODUCTION

The use of inorganic fertilizers in conventional agriculture have increased due to significant human population growth. Therefore, it is important to increase the food supply by upgrading our plant nutrition. However, prolonged use of inorganic fertilizer leads to water eutrophication, soil acidification, groundwater contamination, and atmospheric contamination. In addition, the usage of inorganic fertilizers affects microbial biodiversity by reducing the viability of beneficial microbes in the soil (Vassilev *et al.*, 2015). Therefore, there was an urgent need to find alternative strategies to ensure competitive crops yield, reduce inorganic fertilizer usage and maintain a long-term ecological balance in the agroecosystem.

Plant Growth Promoting Rhizobacteria (PGPR) are bacteria that can colonize a plant's root system and promote plant growth. Plants are positively impacted by PGPR in a variety of ways, from direct processes that regulate plant nutrition and growth to indirect effects related to biocontrol activity. As a result, PGPR seems to encourage plant growth by serving as both biofertilizer and biopesticides. The use of PGPR in agriculture is tremendously increasing, and various studies have shown that rhizobacteria can stimulate alternate nutrient uptake pathways, which has the effect of stimulating their growth.

According to numerous recent meta-analyses, the majority of commercially accessible products are based on a single species and have demonstrated efficacy in accomplishing particular objectives after validation (Sun *et al*., 2022. However, individual species do have restrictions on their roles and ecological niches. In the soil environment, this may result in fierce rivalry between inoculant and native communities, which could impair survival and usefulness. As a result, the concept of combining various species with complementary traits to form a consortium has grown during the past ten years.

In a consortium, two or more microbial groups live symbiotically and this consortium is efficient, robust, modular, and reliable in nature (Ram *et al.*, 2022). Consortia are anticipated to have several benefits over single species. First, diverse members of a consortium can make up for qualities that some members lack, resulting in improved overall results. Consortia made up of isolates that generate indole-3-acetic acid and solubilize inorganic phosphates, for instance, were more effective at promoting growth than the strain used alone (Kumar *et al.*, 2016). Furthermore, soil enzyme activities were higher with the Plant Growth Promoting Bacteria and Arbuscular Mycorrhizal Fungi (AMF) combination leading to a positive correlation between plant growth, grain yield, and soil physicochemical parameters.

Second, through synergistic collaboration, consortium members can aid in the development and operation of the target strain. Sun *et al.* 2022 claimed that by secreting compounds that improve the diversity of native plants, the microbial community will be better able to encourage plant growth and reduce salt stress. Third, quorum sensing and antibiotic release within bacterial consortia are more likely to mediate interactions between inoculants and native species (Santoyo *et al.,* 2021). According to Khan (2022), these aspects rely on support by using microbial consortia to achieve stability and effective outcomes.

Therefore, this study is designed to enhance the idea of mixing multiple species with complementary traits and determine the effect of microbial consortium in cocoa seedling's growth. The use of multiple bacteria is expected to obtain several advantages over single species. Furthermore, the use of agricultural waste as a carrier material is also a good idea because it helps increase the efficiency of the biofertilizer allows easy handling and prolonged the shelf life of the biofertilizer.

MATERIALS AND METHODS

Pot experiments were carried out in the Cocoa Research and Development Centre greenhouse, Jengka, Pahang. Pots were filled with 3 kg of sterile soil. The soil was sieved, placed into clean plastic pots, and arranged in an experimental design layout. Cocoa seeds of BR25 clone were surface sterilized for 15 min using 70% ethanol. The seeds were put into a double layer of wet gunny sacks for germination. After three days, two germinated seeds were transferred into pots and watered at field capacity. After a month of sowing, the seedlings were thinned out and only one healthy and uniform seedling was retained in the pots.

The selected microbial consortia effect was studied on 2-month-old cocoa seedlings. A total of 189 pots were sown with nine seedlings for each treatment/replication were arranged in a randomized complete block design (RCBD). The treatment details are shown in Table 1.

Bacterial cultures and fertilizer were applied after 2 months of planting (after the cotyledon had fallen) and microbial consortia were applied to the seedlings near the root zone of the cocoa seedlings. The plants were harvested 6 months after planting. This study was conducted by using a Randomized Complete Block Design (RCBD) with three replications. The parameters studied were plant height, plant girth, and total fresh and dry weight of cocoa seedlings and data were recorded biweekly for six months. For the total fresh and dry weight, the data was recorded after cocoa seedling harvesting and the plant was oven-dried at 70°C for 96 h.

Table 1: List of treatments for efficacy of bacterial consortia

No.	Treatment	Details
1	T1	30 gm of Consortium 1 (2 endophytes BL & UL and 2 Rhizobacteria B7 & B1) + 2.5gm of inorganic fertilizer (12:12:17:2+TE)
2	T2	30 gm of Consortium 2 (single endophyte [BL] and single rhizobacteria [B7]) + 2.5gm of inorganic fertilizer (12:12:17:2+TE)
3	T3	30 gm of Consortium 3 (single endophyte [UL] and single rhizobacteria [BL]) + 2.5gm of inorganic fertilizer (12:12:17:2+TE)
4	T4	30 gm of Consortium 4 (single endophyte [BL] and single rhizobacteria [B1]) + 2.5gm of inorganic fertilizer (12:12:17:2+TE)
5	T5	30 gm of consortium 5 (single endophyte [UL] and single rhizobacteria [B7]) + 2.5gm of inorganic fertilizer (12:12:17:2+TE)
6	T6	5 gm of inorganic fertilizer (12:12:17:2 + TE)
7	T7	5 gm of inorganic fertilizer (15:15:15)

RESULTS AND DISCUSSIONS

Biweekly plant girth and plant height were recorded accordingly. Figure 1 shows the total plant height increment for cocoa seedlings from 1st data taken until the 11th recorded data. First data were taken after 1 week of treatment applications. Based on the figure below, the T6 application of inorganic fertilizer (12:12:17:2+ TE) showed the highest plant girth increment, followed by T5, T1, T3, T4, T2, and lastly T7. Figure 2 shows the total cocoa seedling girth increment from the 1st until the 11th data recorded. The results showed that there was no significant effect on the total girth increment of cocoa seedlings girth. Based on the figure below, T5 and T6 showed a higher girth increment, followed by T1, T3, T2, and lastly T4 and T7. Meanwhile, for fresh weight of cocoa seedlings, there is a significant different of treatment where T6 showed heavier fresh weight, followed by T5, T1, T4, T3, T7 and lastly T2 (Table 2). There is no significant different in treatment of cocoa seedlings total dry weight. However, T5 showed heaviest dry weight followed by T6, T1, T4, T3, T7 and lastly T2. In spite of that, there were some treatments slightly affected the plant girth and height by showing similar result such as in T5. Such treatment should give a positive effect to the quality of microbial consortium apply and it is relatively important particularly in reducing the amount of inorganic fertilizer application in cocoa seedlings. This might be the synergistic effect of both bacteria increases the content of chlorophyll and chloroplasts, and the process of photosynthesis to give a better plant growth. This is similar to the finding of Fitriatin *et al.*, (2019), where a combination of synergistic microbial consortiums was able to increase rice growth and weight of grain weight in the minimal application of inorganic fertilizer.

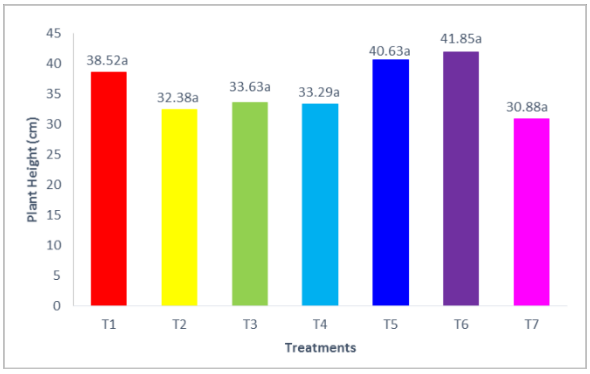

Figure 1. Total plant height increment of cocoa seedlings for recorded data

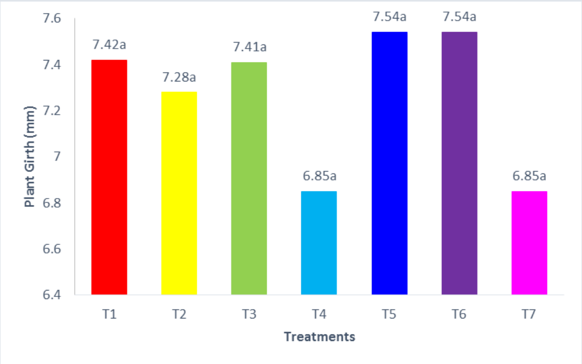

Figure 2. Total plant girth increment of cocoa seedlings for recorded data

Table 2. Total fresh and dry weight weight of cocoa seedlings**.**

Treatment	Total Fresh Weight	Total Dry Weight
T1	96.00 ± 28.69ab	29.20 ± 13.78a
T2	67.68 ± 5.55b	21.50 ± 3.81a
T3	86.22 ± 20.19ab	25.60 ± 3.82a
T4	89.55 ± 13.36ab	29.05 ± 4.17a
T5	102.44 ± 9.37a	32.16 ± 4.00a
T6	107.11 ± 14.75a	31.35 ± 3.20a
T7	83.46 ± 13.87ab	25.46 ± 4.85a
Corrected Value	19.35	24.60
F-Value	1.36*	0.72*

CONCLUSIONS

In the present study, the application of 5gm of NPK Blue fertilizer (12:12:17:2+TE) showed better plant height and plant girth. However, application of T5 (combination between bacteria UL and B7) showed similar plant height, girth data, total fresh and dry weight and this formulation combination only requires 50% of inorganic fertilizer (12:12:17:2+TE). Therefore, it can be concluded that the application of T5 able to give similar results with control treatment but with a lesser amount of inorganic fertilizer (12:12:17:2 + TE).

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