ISOLATION AND PREPARATION OF A MICROBIAL CONSORTIUM TO ACCELERATE DECOMPOSITION OF PLANT MATERIALS FOR COMPOSTING

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Circular leaf spot disease (CLSD) has become a severe problem in many rubber-growing countries. Remaining fallen diseased leaves with the pathogen in the field for a longer period leads to the spread of the disease. The prolonged precence of fallen diseased leaves, containing the pathogen, in the field contributes to the further spread of the disease. Therefore, of removing the inoculum from the rubber field within a shorter period is crucial to control the disease. Mixed cultures of microorganisms are more efficient in the composting process than single cultures. Thus, the composting process can be accelerated by using a microbial consortium rather than a single culture. The main goal of this study was to develop a consortium that efficiently and sustainably decompose fallen diseased rubber leaves. The microbial consortia were prepared in clay pots containing cooked rice. Fifteen pots covered with muslin cloth, were buried in five rubber fields, as with three replicates for each field: - (Galewatta filed T1, Plant Pathology Department Field T2, Aladuwa Field T3, Dartonfield Field T4, and Plant Science Department Field T5). After five days of incubation, the clay pots were removed and colonies of microorganisms were inoculated into Potato dextrose broth (PDB). After 14 days of incubation, one homogeneous mixture was prepared for each consortium. Mass cultures were then prepared by inoculating five microbial mixtures in the PDB medium separately. The resulting consortia were filtered through Whatman No. 1 filter paper. The dry weight of the consortium and pH of the filtrate were tested. The microbial population of each consortium was evaluated using the dilution plate technique. Wood degradability and efficiency of mass loss of leaf litter were tested using standard protocols. T3 showed the highest dry weight (5.27g) and the lowest pH value (2.3682) T2 showed the lowest dry weight (3.43g). While T1 showed a pH of 2.712. T3 and T2 showed the highest (45.19%) and the lowest (35.21%) percentages of weight loss in the wood blocks, respectively. T3 was the best-performed consortium and can be developed as a potential candidate after testing the suitability for rubber plantations. It may help to control CLSD which has become a severe outbreak in the country.

Keywords: Circular Leaf Spot Disease, Microbial consortium, composting
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INTRODUCTION

Natural rubber (*Hevea brasiliensis*) which belongs to the family Euphorbiaceae is the most economically important species of the genus *Hevea*. However, diseases are one of the main factors leading to a significant losses in the rubber sector (Oktavia *et al*., 2021). *H. brasiliensis* can be affected by a wide range of diseases, mainly caused by fungal pathogens (Jayasinghe, 2001). Among them, recently reported Circular Leaf Spot Disease (CLSD) causes a huge yield loss. The disease is becoming an increasingly important pathogen in many rubber-growing countries. It was first reported in Malaysia in 2018, followed by Indonesia. Currently, all the rubber-growing countries are attempting to control the disease by developing effective disease management strategies.

The last long fallen leaves with the inoculum on the ground of the rubber plantations lead the spreading of the disease. Therefore, the leaves with the inoculum should be removed from the field to control the disease. The fallen leaves are decayed by microorganisms which play a major role in the composting process. However, the process takes longer as it naturally slow. Mixed cultures of microorganisms have been shown to enhance the degradation of lignocellulose more effectively than single cultures. Thus, developing an effective microbial consortium can be used to accelerate the composting process. The present study aimed to develop an effective and sustainable method for rapid removal of fallen diseased rubber leaves by accelerating the decomposition using a microbial consortium.

METHODOLOGY

Preparation of Microbial Consortium

Clay pots containing cooked rice were covered with a muslin cloth. They were buried in five rubber fields as five replicates in each field. Decayed rubber leaf litter from each field was placed on top of the muslin-covered pots.

Isolation of Microorganisms

The clay pots were retrieved after five days of incubation and rice-grown microorganisms were isolated into Potato dextrose broth (PDB). The cultures incubated for 14 days after which the microorganisms each of the five pots buried were mixed to prepare one homogeneous mixture. As a result, five different consortium mixtures were obtained, one from each field.

Mass Culture Preparation

Mass cultures were prepared in the PDB medium.

Comparison of Consortium Mixtures

After 14 days of incubation, a consortium in each flask and PDB itself as a control were separately ground well, and a homogeneous 05 microbial mixture was prepared. The wood degradation, leaf litter mass loss, microbial colony count and pH were assessed in each consortium. The remaining five replicates from each were filtered with Whatman No. 1 filter papers and they were taken for the comparison of the mycelial dry weight.

Comparison of the Dry Weight of the Mycelial Mat

After 14 days of incubation, the mycelia mat grown in five replicates of each consortium mixture, along with the control media were filtered through a pre-weighted filter paper (W1) and dried at 80 ˚C to a constant weight (W2). the biomass was calculated using the following equation.

$$\text{Biomass} = W2 - W1$$

Comparison of pH of Media

After filtering the mycelium mat, the pH value of each filtrate was measured using a pH meter.

Evaluation of Microbial Population of Consortium Mixtures

The dilution plate technique (Jonson and Curl, 1972) was utilized to isolate the microorganisms. The homogenized consortium mixtures were diluted as 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}. Then, 1 ml of 10^{-4} aliquot dilution was transferred into Potato dextrose Agar (PDA). The plates were incubated for four days at room temperature. The microbial count, including both fungi and bacteria was taken by colony counter.

Comparison of Wood Degradability

Rubber root pieces with pencil-liked size (7 mm thickness and 5 cm length) were taken and placed on 18 petri dishes, with as 10 pieces per dish. Each petri dish was oven-dried at 80^0C temperature until they reached constant dry weights. The wood pieces on petri dishes were put into pre-weighted and labelled polypropylene bags separately. After introducing root pieces, each bag was sealed and weighed. They were autoclaved and inoculated with 10 ml of consortium mixture of each replicate as well as the control which used PDB itself. They were incubated at room temperature in the incubator room for 12 weeks. Next, the root pieces were oven-dried at 80^0C temperature for 72 hours and weighed. The percentage of dry weight loss was calculated for each sample (James *et al.*, 1997).

Comparison of Mass Loss of Leaf Litter

Matured rubber leaves were washed with tap water followed by sterilized distilled water. The leaves were air-dried and weighed. Initial oven dry weight was calculated using sub-samples for water content determinations. The leaves were then placed into pre-weighed and labelled polypropylene bags. Each consortium mixture was inoculated into the labelled bag. The percentage weight loss of each sample and the litter decomposition rate were assessed after eight weeks of incubation. (Cornelissen and Thompson, 1997).

RESULTS AND DISCUSSION

Well-grown consortia with different types of fungi and bacteria were observed in the buried clay pots after five days of incubation. There was a large population of decaying microorganisms living on the leaf litter facilitating the composting process. In this experiment, the microorganisms living on the leaf litter easily entered to the rice medium through the muslin cloth. Bacterial growth in PDB was evident by the change in turbidity of the medium, and fungal growth was indicated by the presence of mycelial mat in the liquid medium.

According to the results of the experiment, T3 showed the highest dry weight (5.27 g) while T2 showed the lowest dry weight (3.43 g). Mycelium dry weight was used as an indicator of fungal growth. Fungi are the most effective and prevalent wood decomposers identified so far. Thus, the fungal growth rate directly correlates with the organic matter decomposition rate (Embacher *et al.*, 2023).

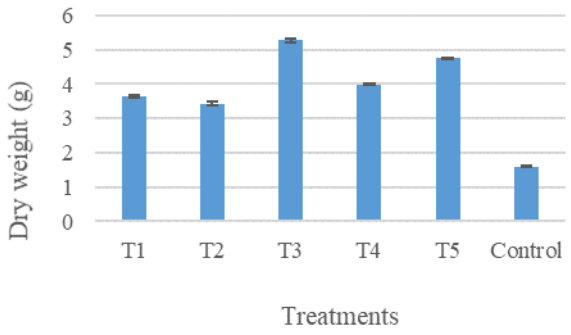

Figure 1: Graphical representation of dry weights of each treatment

According to the results, T3 showed the lowest pH value (2.3682). In contrast, statical analysis revealed that T1 exhibited a pH of 2.712, which was significantly higher than all the other

treatments. The medium become more acidic due to the growth of the consortium. This acidity increase may be caused by metabolic products and enzymes produced by the microorganisms in the consortium.

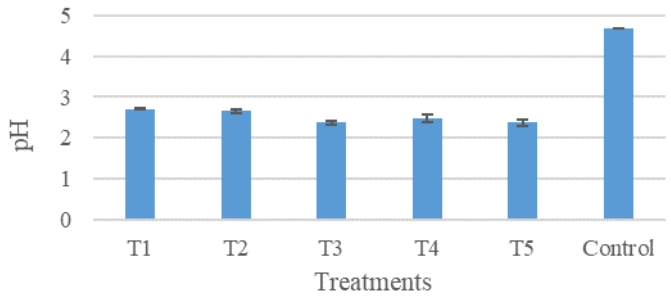

Figure 2: Graphical representation of pH of each treatment after fourteen days incubation

Bacteria, fungi and other microorganisms were found in different ratios. Bacterial colonies were more abundant in all five treatments, which indicates that they hold most of the functions in soil substance translation and are biologically the most active group as reported by other researchers. (Jonson and Curl, 1972). The second most abundant group of microorganisms was fungi.

Table 1: Microbial population of consortium treatments

Treatments	**Micro Organism**		
	Bacteria ($\times 10^4$ cfu ml^{-1})	Fungi ($\times 10^4$ cfu ml^{-1})	Other ($\times 10^4$ cfu ml^{-1})
T1	10.06±4.25	1.57±0.15	0.08±0.17
T2	11.83±2.37	1.97±0.73	0.10±0.10
T3	21.00±1.28	1.77±0.75	0.10±0.10
T4	15.60±4.33	1.37±0.38	0.30±0.30
T5	16.00±3.99	2.23±0.45	0.53±0.50

In the wood degradability test, the lowest wood degrading ability was shown by T2, as it showed the lowest weight loss of the wood block (35.21%). T3 showed the highest weight loss in the wood block. That means it exhibited the highest wood degrading ability, and it was 45.19%. Hence, T3 can convert it litter accumulated on the rubber-growing fields very quickly and convert them into nutrients. Therefore, the fallen leaves with the inoculum can be decomposed more easily, removing the disease inoculum faster compared to the natural composting process.

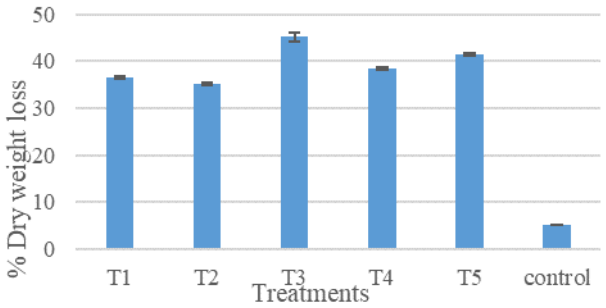

Figure 3: Graphical representation of dry weight loss of each treatment

T2 shows the lowest percentage of mass loss (35.156%) while T3 showed the highest value

(45.270%) for mass loss of leaf litter

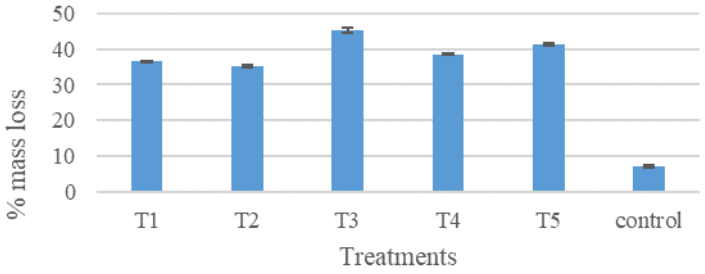

Figure 4: Graphical representation of % mass loss of each treatment

By considering the overall results of the study, the resulting consortium can be used successfully used for the leaf litter composting process. It should undergo mass culturing and be recommended for application on leaf litter with the disease inoculum. However, the product required testing for suitability. Furthermore, the selected product may assist in controlling severe outbreaks of CLSD spread throughout the rubber-growing countries. However, it should be used as part of an integrated disease management method combining chemical and biological control methods to accelerate the control process. This approach can have direct and indirect positive impacts on the country's economy.

CONCLUSIONS/RECOMMENDATIONS

According to the study, the T3 consortium which showed the highest decomposition rate can be introduced for application in rubber growing fields with infected leaf litter to control the disease to a certain extent. By using an integrated approach with chemical applications, the effectiveness of the product can be enhanced, potentially reducing chemical usage. Further studies are needed to identify microorganisms, test the suitability of the product and to expand the sample size.

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