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Stems Extract of Kemuning cina (*Catharanthus roseus*) as Biofungicides against White Root Fungal (*Rigidoporus microporus*) of Rubber Trees (*Hevea brasiliensis*)

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Abstract

White root disease which cause by *Rigidoporus Microporus* fungus are the most destructive and serious disease among the three major root disease in rubber plantation. Biological control using stems extract of kemunting cina (*Catharanthus roseus*) have been used as a healing agent of infected rubber trees. The objectives of the study are to determine the ability of stems extract from kemunting cina to control white root disease of rubber trees and to observe the plants performance towards the fungus. Stems of the plant were soaked in dichloromethane (DCM) solution because the solvent was found as the best extraction for the treatments. Three batches of rubber trees were prepared in the research, which consists of five trees. Group A was a control, while the trees in Group B were planted with fungus and the trees in Group C was applied with the stems extract together with the fungus. Five hundred ml of extracts was used as biofungicides against white root disease. The chlorophyll, diameter, height and number of leaves were recorded. Among the three groups of rubber trees, Group C shows the ability of extract to inhabit against *Rigidoporus Microporus* growth refers to the improvement of growth performance of the plants. The stem extracts of kemunting cina were antagonistic inhibited against *Rigidoporus microporus* fungus.

Keywords: White root disease; *Rigidoporus Microporus*; Kemunting cina (Catharanthus roseus); Dichloromethane; Biological control

Introduction

The agriculture sector was indeed the most important sector for developing countries like Malaysia. In fact, it was one of the most important features that differentiate status between developing countries and developed countries. These sectors contributed to the foundation of Malaysian economy in the post-independence era where the majority of the population-based activities focused on agriculture and mining. The Prime Minister of Malaysia, Datuk Seri Abdullah bin Hj. Badawi announced that the agricultural sector will developed in the Ninth Malaysia Plan in 2005 (9MP) [1]. Since the announcement, many government agency and non-government organization get involved in this sector. However, the rapid development and high progressive in the sector, excessive agricultural activities will cause disturbance to the ecosystem stability. Therefore, some arguments regarding to the issues could threaten the health of ecosystem in Malaysia.

Agriculture sector are closely related to the environment and the most important sectors to generate food security for human and animals. Plant infected with fungus can reduce the productivity of agriculture. Fungicides are able to treat the plant disease and improve crop quality and supplies. Fungicide is an agent that been used to kill fungi, bacteria and viruses that generally harm human and environment as well. Most of the fungicide that commonly used was chemical fungicides. However, prolonged usage of the chemical fungicide not only kills the fungus but also other wildlife. Apart from that, it can trigger human health. Chemical fungicide also can cause cancer and lead to birth defects on baby. Therefore, the researches on substances with antimicrobial activity are frequently studied, and medicinal plants have been considered used to remedies for many infectious diseases [2].

Hevea brasiliensis or well known as rubber trees was the most common plant that have been planted since 1877 in Kuala Kangsar, Perak. Rubber trees are well known due to its important in producing latex that is useful for industrial use in Malaysia. Although, the production of rubber was highly at one time, but due to the attack of pest and plant diseases, the number of rubber trees present nowadays is decreasing. Large number of pest and disease problems occurs mainly in man-made forest [2]. Monoculture plantations of rubber trees also show clear evidence to promote higher outbreaks of pests and disease compared to mixed culture plantations [3]. According to Wingfield et al. [4], most of the failures occurs in the early phase of forest plantation development has been linked to the serious problems with root disease. White root disease which caused by *Rigidoporus microporus* fungi are known to be the most serious and dangerous to plants as it can spread the disease very quickly and are often fatal.

Kemunting cina (*Catharanthus roseus*) is a tropical plant was important as medicinal plant from *Apocynaceae* family. It was cultivated mainly for its alkaloids due to the presence of indispensable anti-cancer, drugs, vincristine and vinblastine. The content of alkaloid in root of the plant is the main source of anti-hypertension [5]. It also known as ornamental plants among the community as there are commonly have two varieties of plant based on the flower color which is pink and white in color [6]. The plants have a lot of advantages especially in the medical field due to the ability to treat several types of disease. Some parts of the plants like the leaves and stems are the sources of dimeric alkaloids, vinacristine and vinblastine that are indispensable cancer drugs. And

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its roots have antihypertensive, ajmalicine and serpentine [7]. It also was used as a folk remedy for diabetes in Europe for centuries. In India, juice from the leaves was used to treat wasp stings. In Hawaii, the plant was boiled to make a poultice to stop bleeding. In China, it was used as an astringent, diuretic and coughs remedies [8]. Furthermore, its leaves can be used to produce the Jamaican tea that can treat diabetes.

Catharanthus roseus also has been categorized as poisonous plants due to the present of catharanthine, leurosine, norharman, lochnerine, tetrahydroalstonine, vindoline, vindolinine, akuammine, vincamine, vinleurosin and danvinrosidin. It can only be taken in dosages that have been defined and allowed only. However, there are a few studies that revealed the ability of the plant to treat fungus diseases for infected plant. Junaid et al. [9] have studied about *Catharanthus roseus*, an important drug: its application and production. Antibiogram of *Catharanthus roseus* extracts also have been analyzed by Sathiya et al. [10]. According to all of the researches above, *Catharanthus roseus* are able to apply as effective biological tool in treating the plant disease [11].

The objectives of the research are to identify the ability of *Catharanthus roseus* stems extract to control white root rot disease of rubber trees and to observe the performance of rubber plant towards the *Rigidoporus microporus*. The Catharanthus roseus stems extract was selected as a healing and control agent for *Rigidoporus microporus*fungus, which causes white root diseases to all rubber trees. The research was conducted in a small scale study using the same height of rubber trees. The chlorophyll, diameter, height and number of leaves were recorded to reveal the effectiveness of the extracts.

Material and Methods

Samples collection

The fungus of *Rigidoporus microporus* and rubber trees species (Hevea brasiliensis) has been provided from Forest Research Institute of Malaysia (FRIM) for the research purposes. The healthy and fresh stems of *Catharanthus roseus* were collected from Terengganu coastline and from the residential area located near Universiti Putra Malaysia (UPM).

Preparation of *Catharanthus roseus* extracts

The stem samples of *Catharanthus roseus* were air dried for almost 4 week until the stems were fully dry. The stems of *Catharanthus roseus* were used in this study to determine the potential of stems part as biopesticide. The samples were grinded to powder using mortar. Then, the powder samples were soaked with dichloromethane (DCM) almost for four days to make sure all of bioactive compounds dissolved in the solvent. The solutions were filtered with whatman No. 1 filter paper to separate between the powdered and liquid. Then, rotary evaporator was used to remove DCM solvent from the extracts. The crude extracts were dissolved using sterile distilled water to a final concentration of 20 g/L. The 20 g/L of crude plant extract of *Catharanthus roseus* species was used in the study because the concentration are capable of inhibiting the growth of *Rigidoporus microporus.*

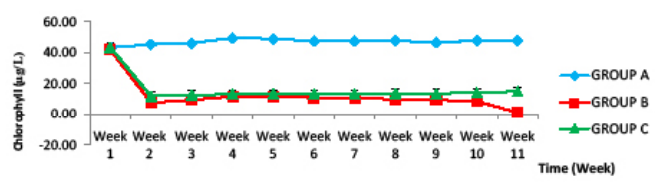

Figure 1: The changes of chlorophyll in 11 weeks.

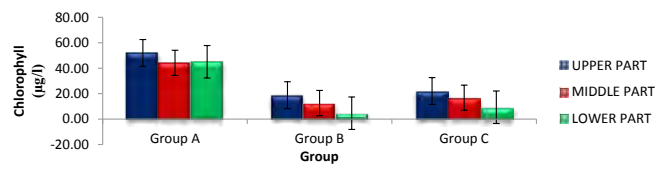

Figure 2: The distribution of chlorophyll based on the different part of the leaves.

Preparation of inoculum blocks

According to Lee and Sikin [12], young rubber branches were cut into wood blocks measuring approximately 2.0-3.0 cm diameter by 8 cm length. Then, it were placed in vertical condition in autoclavable plastics bags for the colonization process to take place. One to four fungal discs were taken from the fungus specimen and inserted into the autoclavable plastics bags using sterilized needle. The plastics bags were then stoppered back with cotton wool and sealed with parafilm to avoid from outside contamination. The blocks were incubated in the dark at room temperature for one month to allow for the colonization process with the fungi to occur.

Planting of rubber trees with colonize wood blocks

Five trees per group were prepared for the study. The rubber trees in group B and C were replanted together with the colonized blocks. Eight inoculum blocks were tied together and placed in contact with the taproot.

Application of *Catharanthus roseus* stems extract to rubber trees

Group A of rubber tree as a control without any application of fungus and stems extract, while Group B, the trees were planted with fungus as mention above without any extracts application. The trees in Group C were applied with the stem extracts together with the fungus. About 500 ml of the extract were applied to each rubber tree in Group C three times before replanting procedure. The extracts were applied before the fungus was fully reproduced on the root. The *in-situ* parameter of measurement for plants such as chlorophyll, height, diameter and number of leaves were measured using chlorophyll meter SPAD-502, measuring tape and vernier caliper through observation. The measurements of each parameter were carried out for 11 weeks and the data were collected once a week at the same time. All of 15 rubber trees were placed in the nursery of Forestry Faculty, Universiti Putra Malaysia.

Statistical analysis

Bar chart was used to provide clearer view and to show general trends of the data collected. Based on the statistical analysis using ANOVA single factor, it generates p-values, one for each parameter independently.

Chlorophyll

The chlorophyll reading in Group C was increased at the end of the experiment as shown in Figure 1. The stems extract was ability to inhibit the growth of fungus rather than Group B, which shows negative results due to the fungus attack. While plants in Group A, shows the constant reading and not much different throughout the 11 weeks of observation time because there is no disturbance to the plants. The chlorophyll reading was based on the concentration of the green pigment, which

determines the color of leaves. There are significant differences ($p \leq 0.05$) in chlorophyll reading between all of the plant.

Figure 2 shows the upper part of leaves shows the highest reading followed by the lower one and the middle leaves. The upper part of plant consist of the youngest leaves, the middle and lower part consist of the older leaves. Based on the previous study [13], the concentration of the green pigment were different based on the different in color of the leaves.

Height

Figure 3 shows the changes of height were increased in Group A throughout the observation weeks. The result shows that the plants have enough nutrient and resources for their growth without any disturb from fungus infection. Meanwhile, height of the plant in Group C showed the increased at the end of the experiment. The presence of stems extract was capable to inhabit the growth of fungus and improved the growth of the rubber trees. The changes of height were constant in Group B where the fungus is become dominant and affect the growth of rubber trees. Thus, the growth of plant was retarded and finally, the rubber trees become death. The presences of the fungus will harm the rubber trees because fungus relied on the host plant for their supply of food [14]. The result shows that there was significant difference ($p \leq 0.05$) in height of plant between the entire plant groups.

Diameter

According to Figure 4, the diameter changes in Group A and C were increased throughout the 11 week of study. However, the diameter is almost the same every week. The stems extract was much influenced in diameter changes of the plants. Meanwhile, the result in Group B shows the diameter changes are remains constant starting from week 6 onwards. The plants begin to dieand the growth was stunted due to the fungus attacked. There was a significant difference ($p \leq 0.05$) in diameter between all the tree groups.

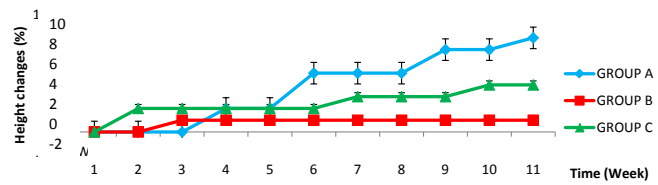

Figure 3: The changes of height until week 11.

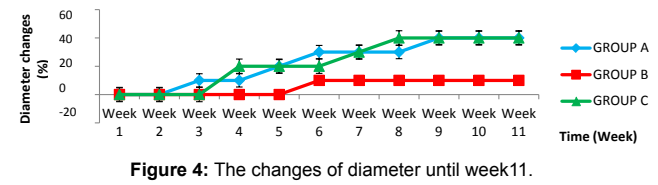

Figure 4: The changes of diameter until week11.

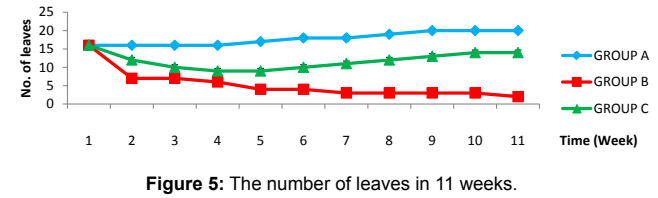

Figure 5: The number of leaves in 11 weeks.

Number of leaves

According to Figure 5, the result shows that the number of leaves in Group A and C was increased until the end of the experiments. Application of the stems extract seems possible to enhance the shoot growth of rubber trees after the infection even though at the beginning showed there was reduction in the number of leaves. In Group B, there was reduction in number of leaves throughout the week. The reduction occurs due to fungus infection and also water stress. There was significant difference in number of leaves between all the groups. The symptoms appeared were wilting and yellowing of the leaves, defoliation and white mycelium on the root system after infection.

Discussion

According to Lee and Sikin [12], usually the fungus will start to attack after 2 week the inoculum been planted, but it depends on the plants as every plant have its own defense to the diseases. Moreover, since the *Rigidoporus microporus* do not contain chlorophyll, so it cannot produce their own food and they need to rely on their host (rubber plants) to provide them with food. The color of leaves also influence the chlorophyll reading for the whole plant. When the leaves was old and there was damaged to the leaves, it may turn yellow or orange in color. The chlorophyll was translocated out of the leaves and appeared yellow before death. Meanwhile for Group C, the chlorophyll reading shows slightly increased until the end of the observation. This proved that the extracts were able to act as an agent to reduce the effect of white root diseases. The early application of the extract makes the plants to build its own resistance towards the diseases.

The plant height in Group B shows that no changes at the first3weeks until the end of observation. *Rigidoporus microporus* attacks at its root system influence the performance and growth of the tree. The root system got damaged as the fungus become dominant. In order for the fungus to grow and live, they need to rely on the other plants to get the nutrients thus inhibit the plant growth. But, the rubber trees were capable to keep growing due to the presence of the *Catharanthus roseus* extracts (Group C). Thus, the result proved that the *Catharanthus roseus* stems extract was capable to improve the life span of the rubber trees.

The diameter of tree stems changes with time are the first been used to detect plant growth [15]. The changes are influence by a lot of factors and one of the major factors is water content and water tension inside the rubber plants. These changes have been detected since the late 19th century and their connection with environmental factors have been reported. The funguses that have been attacked to the rubber tree in Group B were influenced to the diameter changes. Plants in Group B were trying to adapt to the new environment due to the presence of *Rigidoporus microporus* fungi which absolutely give changes to the life span of the tree as well as the tree diameter. The fungus gets their resources to survive and the rubber plants were become as their host for food and water. Moreover, fungus usually loves to lives on moist or damp environment. Finally, the fungus was become dominant and able to conquer that particular area. The plants in Group C were trying to fight against the *Rigidoporus microporus* fungus in the presence of stems extract. After week 3, the diameter of plants was increased where the plants tried to manage the stems extract as their weapon to avoid and slow down the fungus activity.

The numbers of leaves were increased form the plants in Group C have been recorded throughout the 11 weeks of observation time. Several new shoots have been observed after week 4 shows the rubber plant tried to recover. The presence of stems extract inhabits the growth

of fungus at the plant root. However, the plants in Group B shows almost lost their leaves week by week. Starting from week 2 the number of leaves decreased dramatically. The fungal attacked to the plant root causes loss their function to carry water and nutrients from soil. The fungus was harmed the life span of the plants.

Chlorophyll contents in the leaves also influence the leaves performance. If the level of chlorophyll decreases, the leaves will become yellow. The possibility of the leaves to fall is higher. Weather and climate also play a huge role in causing the leaves to fall. Sudden changes in temperature can lead the leaves to turn yellow or brown and thus cause the leaves to drop.

Conclusion

White root disease caused by *Rigidoporus microporus* fungus can be recognized by the presence of white rhizomorphs on the tree root surface. The stems extract of *Catharanthus roseus* medicinal plant are able to reduce the effects of white root diseases. The results confirmed that stems extract were antagonistic inhabited against the diseases. The presence of the stem extracts showed that the plants have the ability to improve the rubber trees performance after infection refer to positive result of chlorophyll density, height, diameter and number of leaves as shown in this study. The plants in Group C tried to survive after exposed with fungus and *Catharanthus roseus* stems extract compared to Group B without extract. Thus, the stems extract of *Catharanthus roseus* was suitable to become as one of the biological control of plants diseases especially for root rot diseases.

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