Replacing the growing media to reduce the seedling weight of citrus (Citrus nobilis var. Microcarpa) and its effects on seedling growth

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Abstract. Seedling translocation of citrus plant is labor-intensive and expensive. It is heavy due to the large amount of soil that is needed to growth the seedlings. This research aimed to overcome the difficulties by reducing or replacing the growth media of the seedling and examined its effects on the seedling growth. The trial was conducted in Monterado Experimental Farm, Bengkayang Regency, West Kalimantan. There were two factors in the experiment, i.e. the type of growing media and biological agent. The type of growing media factor consisted of two levels, soil and cocopeat, while the biological agent factor consisted of three levels, Trichoderma, Bioboost©, and control. The sample seedlings were selected using simple random sampling method. The results showed the survival rate was 100% for all treatments. The use of soil reduced 59.25% of the initial seedling weight, while cocopeat reduced the weigh up to 77.84%. There were no significant differences in the number of leaves, leaf width, number of shoots and shoot lengths caused by different growing media and the application of the biological agents.

1. Introduction
Siam Citrus plantation is one of main commodity of West Kalimantan, producing tonnes of the exotic fruits every year. The plantation generated a valuable income for farmers as much as IDR 1,35 million per month [1]. Wiji [2] reported that the Siamese farming system in Sambas Regency is considered as highly competitive so that it’s commodity can compete in the international market and be able to finance its domestic cost. Furthermore, Sayekti, Laila [3] also confirmed that Siamese Orange farming has both competitive and comparative advantages.

There are more than five thousand hectares of citrus plantation across West Kalimantan Province. Many of them require rejuvenation every year due to poor maintenance or pest/disease-related problems [1]. The reduction of citrus production in Sambas Regency due to disease incidences has been indicated by Badan Pusat Statistik (BPS) [4]. In addition to the necessity for replacing the old plantation, there are also the needs for extensification. Suharto [1] reported around 8,000 hectares of unworked land suitable for citrus plantation are currently available in Sambas Regency. This shows that citrus seedlings are needed regularly to support the continuity of citrus production.

The translocation of citrus seedling requires many workers and high cost. It is because each citrus seedling has an average weight of two kilograms, which can give a massive burden during translocation. The cost to deliver four thousand seedlings from Sambas to Monterado Sub-District, which is only two hours driving, is IDR 1.5 million (Abu, pers. comm). In many occasions, farmers would force the load

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up to 5,000 seedlings in a truck to keep the costs down, but facing a risk of a damaged vehicle due to overload. Therefore, citrus farmers need a reliable solution to reduce the cost of seedling translocation from one area to another.

Reducing seedling weight can be done by reducing or replacing the growing media used in the polybags. Cocopeat is an excellent option in this regard since it has good physical properties, such as many pore space, high-water content, low shrinkage, low bulk density, and slow biodegradation [5]. Cocopeat has been commonly used in germination of seeds, nursery raising, cutting rooting and other vegetative plant propagation methods, hydroponic systems of plant cultivation, cultivation of glass house plants, and soil conditioning [6]. Cocopeat mixed with top soil used as growing media gave a better high increment and diameter increment of Dryobalanops aromatica seedlings compared to the use of top soil only [5].

The addition of biological agents to growing media can support the growth of the seedlings. According to Feng-de, Yong-jun [7] biological agent is a regulator beneficial for increasing survival rate and root growth of seedlings. A positive development of the root system is associated with improved plant growth and resistance to biotic and abiotic stresses [8, 9]. Trichoderma spp. have widely known as biological agent that are capable of increasing plant biomass and root growth and simultaneously protecting plants from disease through various mechanisms [10]. Meanwhile, Pseudomonas sp. and Bacillus sp. are the two commonly used plant growth promoting rhizobacteria (PGPR), a group of bacteria capable to actively colonize the plants root system and improve their growth and yield [11, 12].

This experiment aims; firstly to determine whether the citrus seedlings will survive long distance transportation after the replacement of the growing media; secondly to determine whether the replacement of the growing media will affect seedling growth; and thirdly to investigate whether the addition of biological agents will affect seedling growth and resistance.

2. Method

2.1 Experimental site
The current study, which investigated the effects of growing media replacement on seedling growth of citrus, was conducted in Simpang Monterado Experimental Farm, Bengkayang Regency, West Kalimantan from July to October 2020.

2.2 Experimental design
There were two factors in the experiment, i.e. the type of growing media and biological agent. The type of growing media factor consists of two levels, soil and cocopeat, while the biological agent factor consists of three levels, Trichoderma (formulated by BBS Seed Makasar), Bioboost© (product of K-Link Indonesia), and control. There were six treatment combinations, with each treatment replicated six times to give a total of 30 polybags of citrus seedlings, selected using simple random sampling method.

2.3 Seedling variety
The seedlings were 12-month-old Siam Citrus (Citrus nobilis var. microcarpa), produced by BPTP Kalimantan Barat. The seedlings were grown under in the shaded nursery at 27-28°C and at relative humidity of 80-90%. The average seedling height was 85 cm at the time experiment conducted.

2.4 Shoot pruning
Shoot pruning was performed by completely cutting all secondary branches of all seedlings. The shoots were cut about 1 cm below the apex. Consequently, no leaves were left in all seedlings. The pruning was done at the same time with growing media replacement.

2.5 The reduction and replacement of growing media
Depending on the treatments, the growing media (the soils) were either reduced or replaced completely. For the growing media reduction, the soils were reduced until a half left from its original volume. For
the growing media replacement, the original media were removed and replaced with about 200-300 gr of cocopeat. Then, *Trichoderma* (10 gr/l), Bioboostr® (10 ml/l) or water as control were applied, by sufficiently wetting the media with the solutions. The purpose of this treatment was to cover up the roots with the minimum amount of media, so that the seedling weight can be as light as possible. The roots covered with the media then put into the polybags and wrapped with duct tape. The seedlings were gathered in a cardboard, packed and sealed to simulate a package ready for transportation. Then, seedlings were stored for two weeks at room temperature. No treatment or additional water was given during this incubation period, which was intended to emulate the condition of long-distance transportation. After two weeks of incubation, the seedling survivals were evaluated. After that, the growing media were returned to normal by giving more soils to the polybags with the media of soil, whereas cocopeat were removed and the seedlings planted in the polybag using typical soils. Then, the seedlings were maintained normally for five weeks. After this period, the seedling growth parameters were evaluated.

2.6 Assessments
Survival rate of the seedlings was recorded at two weeks after incubation, using a simple formula as follows:

\[
\text{Survival rate} = \frac{\text{Number of seedling live}}{\text{Number of seedling observed}} \times 100\%
\]  

(1)

Seedling weight reductions were calculated from the weight loss after media replacement compared to their initial weight, using a formula as follows:

\[
\text{Seedling weight reduction} = \frac{\text{Initial weight} - \text{Weight after treatment}}{\text{Initial weight}} \times 100\%
\]  

(2)

Number of leaves on a seedling was counted from new leaves that fully developed. Leaf width was also measured from fully developed leaves. Meanwhile, number of shoots and shoot lengths were counted from newly emerged shoots, excluded the shoots that were left before the incubation periods. These measurements were done on the fifth week after seedlings maintained as normal.

2.7 Statistical analysis
Data were analyzed using an analysis of variance (ANOVA) with the type of growing media and biocontrol agent as the two factors in the treatment structure. The data (no transformation were necessary) analyses were carried out using open-source statistical software JASP 0.11.1 (Department of Psychological Methods, University of Amsterdam).

3. Results and Discussion
Table 1 shows that seedling survival was 100% for all treated seedlings. Neither different growing media nor the addition of biological agents had damaged effect on citrus seedlings within two weeks of the incubation periods.

The comparison of the average weight reduction between the use of soil and cocopeat can be seen in Fig. 1. The use of soil, by partly removing the growth media, reduced the average seedling weight by up to 59.25%. Meanwhile, replacing the growing media with cocopeat lowered the weight by as much as 77.84%.

Table 2 presents several growth parameters of the seedlings obtained after five weeks of normal maintenance. Overall, the anova test did not show any significant differences between the treatments with respect to the number of leaves, leaf width, number of shoots, and shoot lengths. There were no
positive and significant effects of *Trichoderma* and Bioboost© on the growth parameters of the seedlings, which seem to suggest that the addition of biological agents might be unnecessary.

| Table 1. Survival rate of citrus seedlings after two weeks of incubation period |
|-----------------------------------|---------------------------------|-----------------|
| Variation of treatments          | Number of seedlings | Number of life seedlings | Survival rate (%) |
| Soil without b.a. (control)      | 5                  | 5                  | 100              |
| Soil + *Trichoderma*             | 5                  | 5                  | 100              |
| Soil + Bioboost©                 | 5                  | 5                  | 100              |
| Cocopeat without b.a. (control)  | 5                  | 5                  | 100              |
| Cocopeat + *Trichoderma*         | 5                  | 5                  | 100              |
| Cocopeat + Bioboost©             | 5                  | 5                  | 100              |

1) b.a=biological agents

**Fig. 1.** The percentage of weight loss of citrus seedlings as a result of the soil reduction and replacement with cocopeat. Error bars are based on a percentage of error range of 5% from each value.

| Table 2. Effect of media replacement and the addition of biological agents on number of leaves, leaf width, number of shoots, and shoot lengths of citrus seedlings after five weeks of normal maintenance. |
|---------------------------------------------------------------|-----------------------------|-----------------------------|-----------------------------|
| Treatments                                                  | Number of leaves | Leaf width (cm) | Number of shoots | Shoot lengths (cm) |
| Soil without b.a. (control)                                | 34.00            | 1.77            | 11.20           | 3.90              |
| Soil + *Trichoderma*                                       | 24.40            | 1.43            | 8.00            | 3.84              |
| Soil + Bioboost©                                           | 32.20            | 0.87            | 12.60           | 1.60              |
| Cocopeat without b.a. (control)                            | 24.20            | 1.75            | 7.60            | 5.04              |
| Cocopeat + *Trichoderma*                                   | 26.20            | 1.74            | 10.40           | 2.78              |
| Cocopeat + Bioboost©                                       | 31.80            | 1.59            | 11.80           | 2.51              |
| Stdev                                                       | 18.53            | 0.82            | 4.69            | 2.05              |

1) b.a=biological agents
3.1 Survival rate of citrus seedlings

The result of Table 1 shows that reducing the growing media or completely replacing it did not have detrimental effect on citrus seedlings after the period of incubation. The plant resistance to the incubation period was recognized from the seedling conditions. There were no symptoms of plant stress after the seedling went through the growing media replacement and the incubation. In accordance with the present result, a study by Ma [13] has demonstrated that mechanical disturbance of roots had no effect on plant survival. However, the study also reported that the disturbance influenced the plant growth as the leaf area was reduced when measured after two weeks.

In the present study, the shoots were green and new leaves even emerged during the period of incubation. This shows that the seedling was still growing during that periods. It can thus be suggested that the method used here to reduce the seedling weight is reliable to adopt. According to Marjenah, Kiswanto [5], for seedling can survive, the root system must be viable and able to grow faster to maintain contact with the soil moisture supply. Moreover, the author found that survival and plant growth can be improved by maintaining intensive contact between the roots with soils, and the nutrients and water holding capacity of the container media. In the present experiment, soil moisture and nutrients were perfectly maintained throughout the incubation period by tightly wrapping the growing media.

The results give practical solution for practical situations. Farmers can either utilize soil or cocopeat to reduce seedling weight since both media have 100% of survival rate. Soil can simply be used when no need of massive reduction of seedling weight, while cocopeat is an excellent option to dramatically reduce the weight, as depicted in Fig 1.

3.2 Percentage of weight reductions

The most obvious finding to emerge from Fig 1 is that the amount of seedling weight reduction after the soil reduction (59.25%) and the use of cocopeat (77.84%). To put the value into perspective, the soil reduction method will lower the original weight of four tons seedlings, for example, to become only 1.63 tons. Moreover, the same amount of initial weight can be dramatically reduced to become only 886 kgs if cocopeat is going to be used. Such a reduction will cut the vehicle cost up to 45% for a carriage which relatively long-distance.

The findings reported here suggest that cocopeat is much better than soil to deliver the lighter weight possible. It reduces the seedling weight nearly 20% more than the use of soil. However, replacing the growing media with cocopeat requires more preparation time as well as additional cost for the cocopeat itself. The observations have indicated the use of cocopeat required the preparation time twice as long as the preparation of soil. In addition, the expense of cocopeat purchase is estimated about IDR 40,000 per a-100-seedlings, which may vary depending on location.

3.3 Effects of growing media replacement and shoot pruning on seedling growth

With respect to the second research question, it was found that the replacement of growing media did not affect the growth parameters of citrus seedlings, as presented in Table 2. The results indicate no harmful effect of the treatments to the root system which may cause problems to the growth of citrus seedlings. In accordance with the present results, Ma [13] reported that one factor that can cause problems to seedling growth is compact soils. Similarly, Feldman [14] found that shoot growth is reduced when seedlings are grown on compacted soils. These problems did not occur in the present experiment, which utilized loose soils and cocopeat. Furthermore, the growing media reduction and replacement were done carefully to ensure the treatments did not cause any root injuries.

Another possible explanation for this is related to the sort period of observation. Recent research has demonstrated a significant increase in the plant height and stem diameter of citrus seedlings due to mycorrhizal inoculation six months after the treatment [15]. It was revealed that the increased growth correlated with the increased phosphorus (P) uptake by the seedlings. Meanwhile, cocopeat has high P content, along with other nutrient elements [16]. Therefore, the longer period of incubation or seedling maintenance most likely will contribute to significant increases in seedling growth of citrus seedling grown in cocopeat media.
Growth reduction might have come from shoot pruning, which was aimed to prevent evaporation throughout the incubation period. The implication is that seedlings experienced a temporary growth reduction before rapid regrowth of new shoots and leaves. A research by Saifuddin, Hossain [17] has confirmed that complete pruning significantly decreased bract length, weight and bract numbers of Bougainvillea plant. However, shoot pruning on woody plants will stimulate rapid new shoot growth and resulted in the highest new shot relative growth rate [18]. The author also suggested the rapid new shoot growth will trigger a competitive inhibition of root growth, a condition of root growth reduction, which are temporary and harmless in the long run.

3.4 Effect of biological agents on seedling growth parameters
This experiment did not detect any positive and significant effects of biological agents on either the number of leaves, leaf width, number of shoots, and shoot lengths of citrus seedlings. This result may be explained by the fact that there was only short period of incubation time between the inoculation of biological agents and the examination of seedling growth. Lo et al. [19] suggested that biological agent takes time to grow and propagate and be effective. One study by Paderes, Hill [20] found a 16% increase in shoot height of Pinus radiata seedling due to Trichoderma inoculation after 9 months, although this not always the case. In a grass plant species, the significant effect of Trichoderma application on plant height was detected as early as 47 days after the introduction of the biological agent [21]. However, it is important to note that the biological agent was introduced at the time of sowing, allowing the Trichoderma to sufficiently propagate alongside the plant growth.

Trichoderma spp., Pseudomonas sp., and Bacillus sp., the last two are bacteria formulated as Bioboost©, are well known for their ability to enhance plant growth and development. These biological agents interact with the host plant through root colonization, benefiting the host plant through both direct and indirect mechanisms [9, 11]. The utilization of these biological agents for agriculture use are crucially important, which considered as the key factor in environmentally friendly strategies to minimize chemical inputs in sustainable agriculture system. Harman et al. [9] reported root colonization by Trichoderma spp. frequently enhances root growth and development, plant productivity, resistance to abiotic stresses and the uptake and use of soil nutrients. Similarly, the use of Bacillus sp. and Pseudomonas sp., either individually or in combination, enhanced nutrient uptake and plant growth under both biotic and abiotic stress conditions [22], thus playing a crucial role in plant and soil health.

4. Conclusion
Replacing the growing media of citrus seedlings is a safe and reliable method to reduce the weight, providing a solution for seedling transportation in large numbers, while lowering the cost and the risk of excessive load at the same time. The method can potentially reduce the distance limitation of the seedling transportation due to the seedling resistance to incubation periods and massive reduction of the weight. Complete shoot pruning could cause a growth reduction, hence should be avoided, while the addition of biological agents is optional, depending on the necessity. Further experiment for a longer time period is needed to affirm the reliability of the method as well as to affirm the effect of biological agents.

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