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Pleurotus Spent Mushroom Compost as Green Supplementary Nutrient in Tissue Culture

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Abstract. This study investigates the effect of *Pleurotus* spent mushroom compost as green supplementary nutrient in tissue culture. Different concentration of *Pleurotus* spent mushroom compost in powder (SMC) and activated carbon (SMCAC) were added into Murashige and Skoog media (MS) with 0.1 mg/L 6-benzylaminopurine (BAP). The shoot regeneration of *Clinacantus nutans* plant such as number of shoots, number of leaves and length of leaves were recorded for four weeks. Collected data was analyzed using one way analysis of variance (ANOVA) and Tukey test through the SPSS Statistics 21 software. The 0.1 g/L SMC recorded the highest leaves numbers. Meanwhile, 0.2 g/L SMC showed highest performance in length of shoot. The SMCAC showed a negative response in number of leaves and shoot as well as length of shoot. In summary, the SMC showed significant performance in number of leave and shoot length but less effective on number of shoot. In contradictory, the SMCAC reported poor performance in shoot regeneration but contribute in absorption of nutrient from environment and storage of the nutrient asfunction of biochar. Therefore, the SMC has a high potential as a green supplementary nutrient for tissue culture. The application of this material has contributes into the green technology via convert waste to product, which is in-line with zero waste concept.

1 Introduction

Tissue culture technique improves shoot regeneration of plants' growth with high proliferation rates when compared to on the soil. But, some of plants that grow in the culture media still giving low shoot regeneration rate because of low absorption of nutrients [1]. In order to increase shoots proliferation, the supplementary nutrient is added in culture media in order to reduce the use of exogenous hormones, improve performance and minimize the cost [2, 3]. Generally, the supplementary nutrient is based on synthetic chemicals [4, 5]. On the other hand, agricultural waste which has high organic compounds has been neglectedin tissue culture application [6].

Pleurotus ostreatus spent mushroom compost (SMC) is an agriculture waste that obtained from mushroom cultivation farm. For traditional on soil stem cutting study, SMC supplied nutrients to the crops and thus potential to replace inorganic fertilizer [7]. However, the activated carbon showed no detectable relationship between the activated carbon and the productivity of plant, but promised a win-win solution to energy, carbon storage and ecosystem function [8]. To authors' knowledge, there is

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lack of study in SMC and SMCAC as supplementary nutrient in tissue culture and comparison of performance.

Clinacantus nutans plant has been chosen in this study due to its medicinal properties and high demand in market [9]. The traditional on soil stem cutting technique hardly supply consistent quality and quantity ofplant. Therefore, this study focuses on the effects of SMC and SMCAC in culture medium as supplementary nutrient in tissue culture media to the *C. nutans.* Moreover, this study compares their shoot regeneration performance on the *C. nutans* through statistical analysis.

2 Methodology

2.1 Samples collection

The sample of *Pleurotus* spent mushroom compost was collected from C & C Mushroom Cultivation Farm Sdn. Bhd, Johor, Malaysia. Meanwhile, the stems of *C. nutans* as tissue culture plant were taken from plant nursery in Arau and surface sterilized by using 30% Clorox and 70% ethanol prior to subculture and plant regeneration study.

2.2 Shoot regeneration study

Nodal segments of *C. nutans* explants were cultured on full strength MS media supplemented with SMC at 0.05, 0.1, 0.2 g/L and SMCAC at 0.3, 1.25, 2.5 g/L, respectively. The media contained standardized 0.1 mg/L BAP hormone in order to induce plant regeneration. The culture jars have been incubated in growth room at adjusted temperature of 26 ± 1 ºC and under initial photoperiod of 16 hours light and 8 hours dark period. The number of shoots, number of leaves and length of shoots were observed and recorded every week for four weeks. The experiment was repeated with two nodal segments of *C. nutans* per jam jar.

2.3 Statistical analysis

Data was analyzed using SPSS Statistics 21 software for Windows 7. The values were expressed in mean ± standard error and analyzed by one-way analysis of variance (ANOVA) and tukey test.

3 Results and Discussion

Effects of *Pleurotus* spent mushroom compost (SMC) and *Pleurotus* spent mushroom compost activated carbon (SMCAC) on *C. nutans* shoot regeneration were examined through number of leaves, number of shoot and length of shoot. In addition, comparison of them were evaluated through statistical analysis.

3.1 Effect of SMC

Figure 1(a) and (b) illustrate the effects of SMC and SMCAC on *C. nutans* shoot proliferation through number of leaves and shoots as well as shoot length. In shoot proliferation, the 0.1 g/L SMC recorded the highest leaves numbers of 7 compared to 5 in other treatments. While, similar number of shoot of 2 - 3 was recorded for all treatments. Briefly, SMC enhanced number of shoot but not showed significant different performance in shoot proliferation for number of leaves and shoots amongst treatments. This revealed that the addition of SMC increased the pH values, salt contents, macro and micronutrient concentrations, thus improved shoot regeneration [10]. Wang et al. [11] supported this observation as reported that *Agaricus bisporus* SMC increased number of leaves of green cauliflower and cabbage in soil.

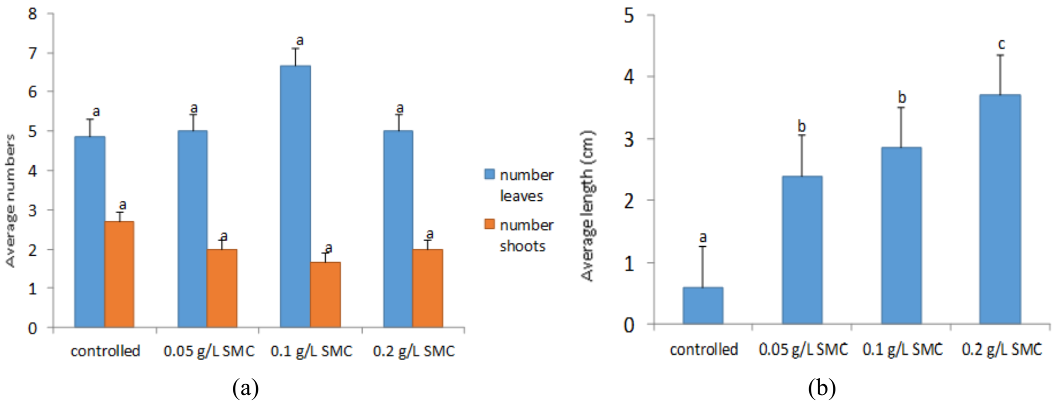


Figure 1. Effects of different concentrations of SMC on the growth performance of *C. nutans* (a) number of leaves and shoots (b) length of shoot.

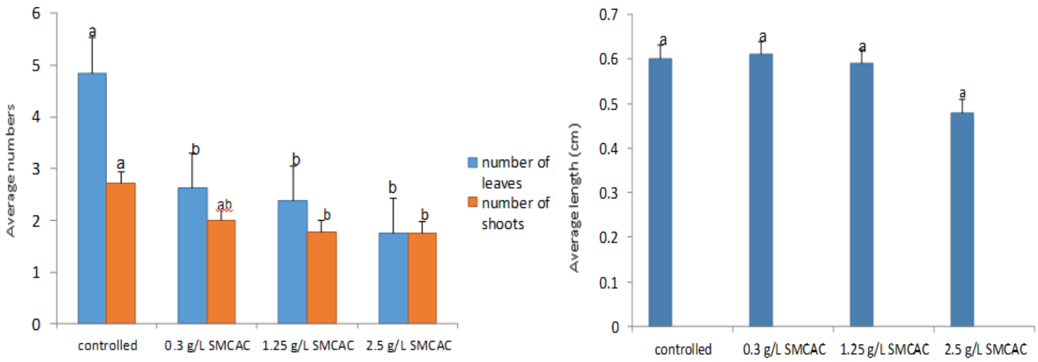
For shoot length in *C. nutans*, SMC at 0.2 g/L exhibited the highest length of shoots (Figure 1 (b)). The SMC showed significant effect on length of shoot among control, 0.05-0.1 g/L SMC and 0.2 mg/L SMC. Basically, the SMC serves as organic soil amendments to improve plant growth in agriculture [12]. Thus, the SMC improved the shoots length of *C. nutans* and directly increased the height of the plant. Increase in shoot length contributes to quantity production through higher yield of leaves obtained in order to meet market demand in a shorter time with consistent quality of culture. Similar positive observation also reported by Jonathan et al. [13] on the effects of SMC as soil conditioner for the growth of soybean plant.

3.2 Effect of SMCAC

The shoots regeneration patterns of *C. nutans* nodes segments in different concentration of SMCAC are shown in Figure 2 (a) and (b). Generally, the explants were survived in 0.3-2.5 g/L of SMCAC but showed slower in shoot regeneration than control treatment. Control and treatments of SMCAC showed significantly different in number of leaves and shoots. Meanwhile, there is no significant difference amongst treatments and control statistically for shoot length. This is because activated carbon absorbed high concentration of nutrients and growth hormone in both liquid and solid media [14]. Generally, activated carbon, a stable and recalcitrant carbon, could function as biochar which is reported as slow sequestration in carbon source [15]. Thus, such circumstances of fast absorption and slow in released of nutrients caused slower cell division which led to slower shoot regeneration. This observation was supported by Zimmerman et al. [16] who reported that the soil organic matter stimulated the co-mineralization of the labile components of biochar over the short term, while over the long term, biochar-soil interaction enhanced carbon storage through the process of organic matter sorption to biochar and physical protection.

3.3 Comparison study

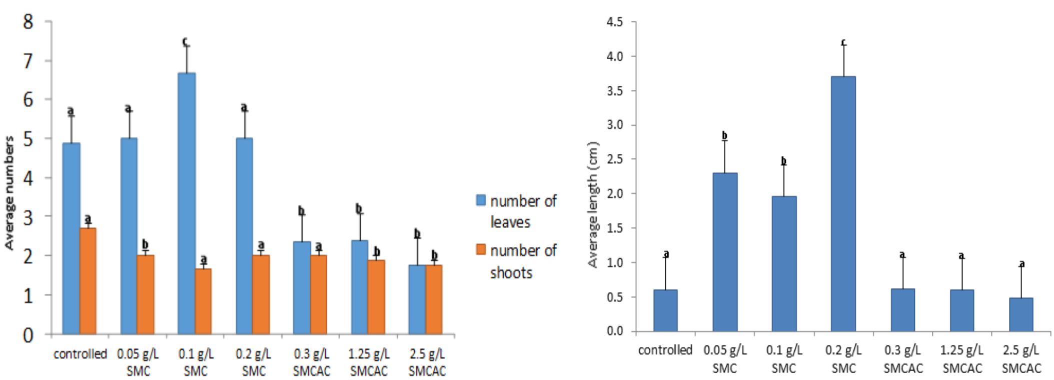
The different growth performance of *C. nutans* using SMC and SMCAC at different concentrations compared to control were demonstrated in Figure 3 (a) and (b). A 0.1 mg/L SMC showed the highest in number of leaves and significantly different statistically with control and other SMC treatments as well as SMCAC treatments. Generally, a decrease in number of shoots when adding SMC and SMCAC. The controlled treatment showed the highest shoots number among other treatments (Figure 3 (a)).



(a) (b)

Figure 2. Effects of different concentrations of SMCAC on the growth performance of *C. nutans* (a) number of leaves and shoots (b) length of shoot.

Overall, the control showed significantly different with SMC and SMCAC in number of shoot. The explants in 0.2 g/L of SMC showed the highest length of shoots with increment of two times when compared to the control and SMCAC treatments (Figure 3 (b)). It can be summarized that SMC is an excellent candidate as supplementary nutrient in tissue culture technique for *C. nutans* as it exhibited highest and significant performance in number of leaves and length of shoots compared to other treatments. The application of SMC in tissue culture technique not only significantly improves plant shoot regeneration and plant growth but also convert agricultural waste to product which is in-line with green technology development and zero waste concept.



(a) (b)

Figure 3. Effects of different concentrations of SMC and SMCAC on the growth performance of *C. nutans* (a) number of leaves and shoots (b) length of shoot.

4 Conclusion

The potential of SMC and SMCAC as green supplementary nutrient in tissue culture were evaluated. This study exhibit that SMC was found to be increased number of leaves and length of shoot of *C. nutans*. The highest number of leaves and length of shoots were recorded at 0.1 /L SMC and 0.2 g/L SMC, respectively. But, SMC showed a reverse relationship for number of shoots with increase of SMC concentration. On the other hand, SMCAC revealed a negative performance in shoot regeneration in terms of number of leaves, number of shoots and length of shoots. In conclusion, SMC is a potential green supplementary nutrient in tissue culture with significant performance in number of leaves and length of shoots. Meanwhile, SMCAC was found to be a good absorbent as potential activated carbon functions as biochar for nutrient adsorption from environment. The application of SMC and SMCAC not only develop green biotechnology but also apply zero waste concept by convert waste to product.

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