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The correlation between phase formation and the structure of the pellets with the fungal immobilization study as a commercial substrate culture/planting material

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Abstract

Calcined clay pellets are popular planting material for those who love to grow plants in pots. The calcined clay pellets consist of clay (C), phosphate rock (PR), and rice husk ash (RHA). [(1-*x*)(50C–50PR)-*x*RHA], *x*(RHA) = 0, 0.25, 0.30, 0.35, 0.40, 0.45, 0.50, 0.55, 0.60, and 0.65 wt% were prepared by a conventional solid-state reaction method. The samples were made into a spherical shape with a diameter of 10 mm and fired at 600°C to 1000°C. The effect of *x* contents on phase formation, microstructure, and chemical properties of [(1-*x*)(50C–50PR)-*x*RHA] was studied. X-ray diffraction revealed the typical assemblages with quartz, illite, and kaolinite in all the samples. SEM images of samples showed irregular packing and a highly porous microstructure. The addition of *x*(RHA) contents results in porous microstructure in all the samples. The surface area and pore volume of samples increased from 8.83 $m^2 \cdot g^{-1}$ to 14.71 $m^2 \cdot g^{-1}$ and 0.938 $cm^3 \cdot g^{-1}$ to 0.942 $cm^3 \cdot g^{-1}$, respectively, with the increase of *x*(RHA). The density of the samples slightly decreased from 2.45±0.06 $g \cdot cm^{-3}$ to 1.94±0.05 $g \cdot cm^{-3}$, with an increase in *x*(RHA) contents. The capability of calcined clay pellets to immobilize plant growth-promoting fungi was then studied. The results showed that orchid endophytes, as plant growth-promoting fungi, grow well on the calcined clay pellets saturated with potato dextrose broth (PDB). Besides, all fungi can live on calcined clay pellets and stay viable for at least 35 days after inoculation. These results suggested that the calcined clay pellets could serve as planting material that enhances plant growth (via its nutrients and growth-promoting fungi) simultaneously.

1. Introduction

Nowadays, people grow plants in pots for their hobbies or leisure activities. The calcined clay pellet is a good answer for a new generation of plant lovers/gardeners because it can provide several benefits besides decorating the pots. The calcined clay pellet also helps speed up plant growth by providing plenty of aeration to the root zones and simultaneously keeps moisture that lasts long in the pots, reducing the frequency of watering required [1,2]. The main component of a calcined clay pellet is clay. Generally, clay has a fine particle size, thus making it easier to mold. However, there are few ventilation openings; it is low in nutrients and burning the clay at high temperatures will cause the essential elements to be lost [3]. The problem of improper physical properties of clay can be improved by adding of waste materials and adding minerals beneficial to plants. Phosphate rock is the primary phosphate fertilizer source, which is known for keeping plants healthy and enhancing new growth. It does this by adding phosphorus to soil, making other nutrients accessible to the plants [4]. To solve the problem of the porosity of clay, mixing clay with agricultural waste materials such as rice husk, rice husk ash, bagasse, and bagasse ash results in good drainage.

Rice husk ash (RHA) is the major agricultural waste in Thailand. It contains approximately 90% silica, which is a highly porous structure and lightweight, with a high specific surface area [5-7]. Therefore, attempts to do co-modified clay with phosphate rock and rice husk ash are expected to improve the porous structure and add essential nutrients to clay pellets, which finally will be available for plant uptake. Another main factor for enhancing plant growth and yield is the interaction between plants and microbes. Aside from some microbes reported as plant pathogens, there are many microbes, including soil bacteria, soil fungi, endophytic fungi, and mycorrhizal fungi, that were reported as plant growth promoters. These plant growth-promoting microbes can provide more water, assist with several essential minerals' absorption, produce some plant growth hormones, and prevent root plants from some plant pathogenic infections [8-10]. The roles of these microorganisms have been known as symbiosis associations. These symbiosis associations occur between plants and microbes in their natural habitat. For example,

the endophytic fungi were studied, and it was concluded that plants' association with endophytic fungi could increase their fitness compared with uncolonized plants [11], and the mycorrhizal fungi are found in the root of many plant species. These fungi gave the abovementioned advantages to the host plant.

Several studies have concluded that mycorrhizal fungi could promote plant growth by regulating nutritional and hormonal balance by solubilizing nutrients and producing plant growth regulators. Moreover, some microbes show antimicrobial activity by destroying other microbes [8-10,12]. Most plants can grow and survive in a natural habitat using these advantages to reduce and resist stress conditions, including biotic and abiotic stresses. Therefore, these microbes have been studied and widely used as an inoculum made from potting media, sand, and vermiculite to enhance plant growth and yield, especially for plants grown in pots or crops that lack microbial sources [13-16]. However, several microbial inoculums have a limitation in terms of their viability or shelf life. To improve the efficacy of microbial inoculum viability and to extend the shelf life, the immobilization of microbial cells onto appropriate substrates has been carried out, resulting in easy recovery and reuse of the microbes [17]. The advantages of immobilization also include enhancing the operational stability of cells and their protection from the effects of biotic and abiotic factors, *viz* extreme pH, and toxic compounds, and the reduction of the risk of contamination of cell cultures [18].

Therefore, in this work, the effect of *x*(RHA) contents on phase formation, microstructure, and porosity of [(1-*x*)(50C-50PR)-*x*RHA] with *x*(RHA)=0, 0.25, 0.30, 0.35, 0.40, 0.45, 0.50, 0.55, 0.60, and 0.65 wt% was studied. In addition, the capability of the calcined clay pellet as immobilization substrate for growth-enhancing endophytic fungi was also investigated.

2. Materials and method

2.1 Preparation and characterization of calcined clay pellets

The [(1-*x*)(50C-50PR)-*x*RHA], *x*(RHA)=0, 0.25, 0.30, 0.35, 0.40, 0.45, 0.50, 0.55, 0.60, and 0.65 wt%, were prepared by conventional solid-state reaction method. The raw materials such as clay (C), phosphate rock (PR) and rice husk ash (RHA) were weighed and ball-milled for 24 h in ethanol using zirconia balls as milling media. Drying was done at 120°C for 6 h. The resulting powder was then crushed in a mortar and run through a stainless-steel sieve. The powders were spherical with a diameter of about 10 mm. The samples were burned between 600°C and 1000°C for 2 h in air at a heating/cooling rate of 5°C·min^{-1}.

The crystal structure of the calcined powders was analyzed using X-ray diffraction in the 2θ range of 10° to 90° (Philips PW 3040/60 X'Pert Pro). The microcrystalline structure was evaluated by studying the surface integrity and morphology microstructure using a field emission scanning electron microscope (FESEM, Apreo, Thermo Fisher Scientific, USA). The theoretical density for each composition was calculated using Archimedes' method. Surface areas were determined through the BET technique using a surface area analyzer (BET, Micromeritics, TriStar II 3020).

2.2 Fungal immobilization on calcined clay pellets

2.2.1 Fungal material

All endophytic fungi (fungal isolate SPGT005, SPGT006 and SPGT007) have been received from a previous study [19]. The endophytic fungi were isolated from the root of a terrestrial orchid, *Spathoglottis* sp., which presented a peloton structure of orchid mycorrhiza in its root cortex. Each fungal isolate was cultivated on potato dextrose agar (PDA) to activate fungal growth before immobilization.

2.2.2 Preparation of calcined clay pellets for fungal immobilization

The calcined clay pellets were prepared in 2 treatments. For treatment1, the calcined clay pellets were soaked with PDB for 12 h and contained in a 4 oz tissue culture bottle. For treatment2, which is used as a negative control, the calcined clay pellets were soaked with distilled water for 12 h and contained in a 4 oz tissue culture bottle. Then, all treatments were autoclaved at 121°C pressure 15 lb·in^{-2} for 15 min for sterilization. All treatments were kept and allowed to cool down to room temperature (or about 25°C to 30°C) before use.

2.2.3 Fungal immobilization and determination of fungal growth and viability on immobilized clay pellets

Hyphal tips of each fungal isolate grown on PDA culture media were cut into 0.5 cm^2 × 0.5 cm^2 and inoculated onto calcined clay pellets of treatment1 and treatment2 (three replicates for each fungal isolate/treatment). Then, the inoculated calcined clay pellets were incubated at room temperature for 35 days. Fungal mycelium growth and viability on calcined clay pellets of all treatments were determined by collecting three inoculated calcined pellets of each treatment from tissue culture bottles. Then they were placed on PDA culture media to observe the viability and growth of fungal hypha from the calcined clay pellets. The determination was done every 7 days after inoculation for 35 days.

3. Results and discussion

3.1 Preparation and characterization of calcined clay pellets

The XRD diffraction pattern of 50C-50PR, with different calcination temperatures between 600°C and 1000°C, is shown in Figure 1. For the calcination temperature of 600°C to 800°C, the diffraction peaks associated with quartz with corresponding ICDD file no. 00-001-0649 [20] at 2θ range of 20.9°, 26.6°, 36.5°, 39.5°, 42.4°, 45.7°, 50.2°, 54.8°, 59.7°, 64.9°, and 68.2° and also presented kaolinite (ICDD 00-001-0527) [21] at 2θ around 24.3°. It can also be seen that the diffraction peak of kaolinite gradually decreased and disappeared at higher than 800°C of the calcination temperature. In contrast, the illite (ICDD 00-002-0042) [22] at 2θ around 19.5°, 27.9°, 31.3°, 33.3°, and 62.6° appeared after thermal treatment as higher than 800°C. It implied that the kaolinite was decomposed

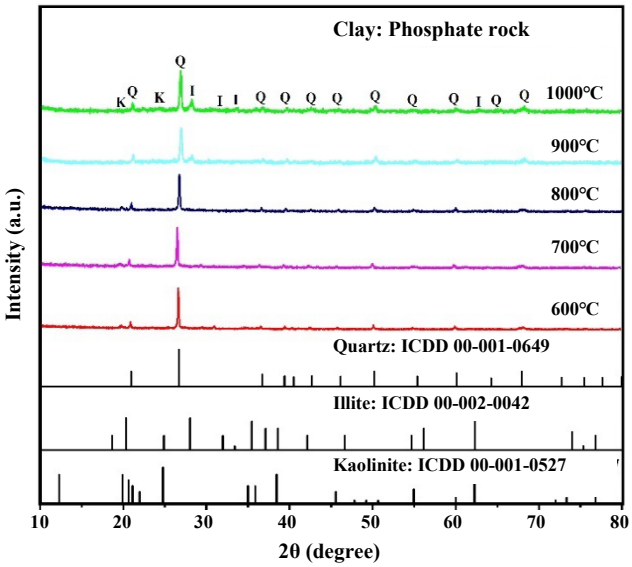

Figure 1. XRD diffraction pattern of (50C-50PR) at the difference of calcination temperature between 600°C and 1000°C.

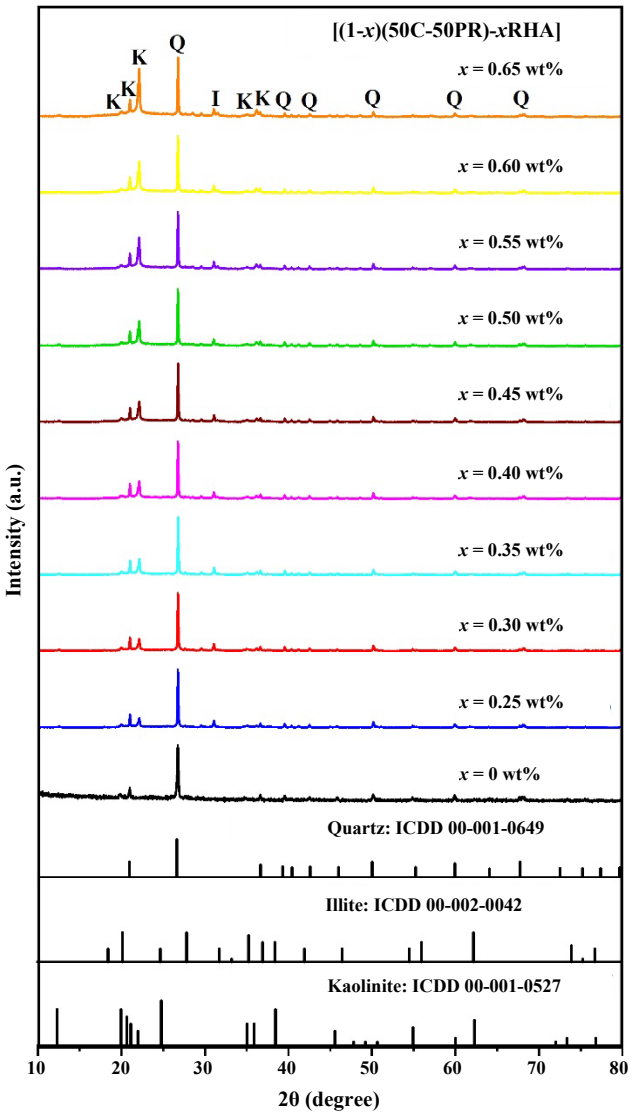

Figure 2. XRD diffraction pattern of [(1-*x*)(50C-50PR)-*x*RHA] with difference of *x*(RHA) contents.

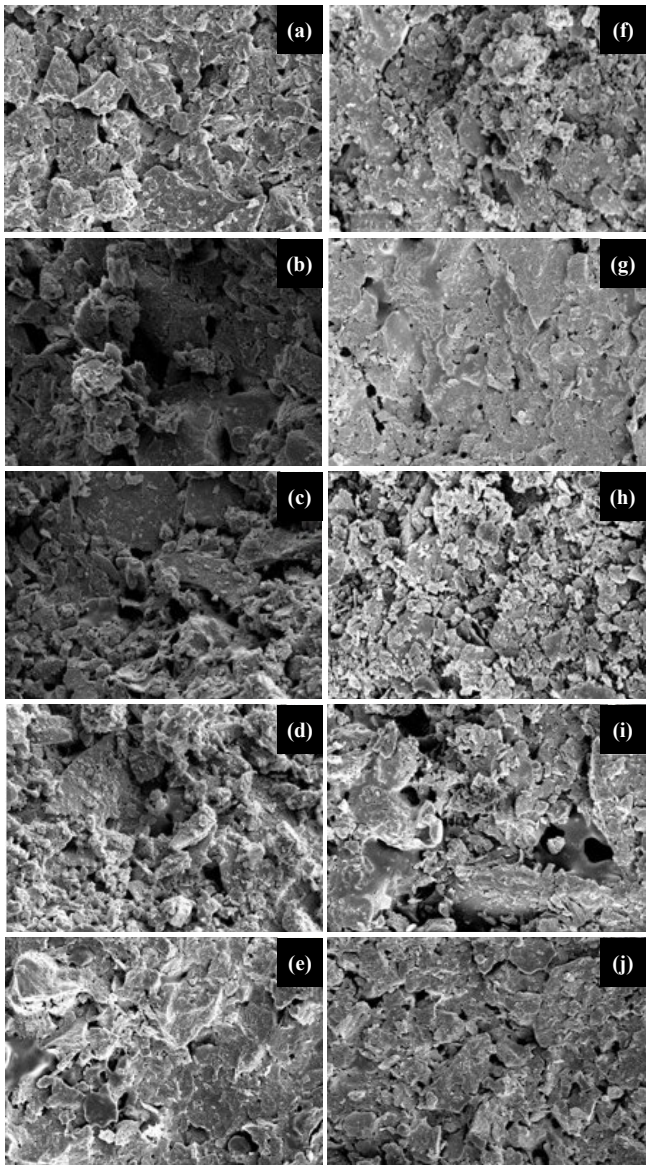

Figure 3. Surface morphology of [(1-*x*)(50C-50PR)-*x*RHA] at difference of *x*(RHA) contents: (a) *x*(RHA) = 0 wt%, (b) *x*(RHA) = 0.25 wt%, (c) *x*(RHA) = 0.30 wt%, (d) *x*(RHA) = 0.35 wt%, (e) *x*(RHA) = 0.40 wt%, (f) *x*(RHA) = 0.45 wt%, (g) *x*(RHA) = 0.50 wt%, (h) *x*(RHA) = 0.55 wt%, (i) *x*(RHA) = 0.60 wt% and (j) *x*(RHA) = 0.65 wt%.

and transferred to illite at a temperature higher than 800°C. The XRD diffraction pattern of (1-*x*)(50C-50PR)-*x*RHA with *x*(RHA) = 0, 0.25, 0.30, 0.35, 0.40, 0.45, 0.50, 0.55, 0.60, and 0.65 wt%, which calcined at 800°C are presented in Figure 2. For the samples *x*(RHA) > 0.65 wt%, molding was found to be difficult (unable to mold). At *x*(RHA) = 0 wt%, the main phase of the samples is quartz at 2θ range of 26.6°, 39.7°, 42.5°, 50.1°, 60.1°, and 68.2°. A small amount of kaolinite is at 2θ range of 19.7°, 20.7°, 35.0°, and 36.2°. With an increase in *x*(RHA) contents, the peak of kaolinite at 2θ around 19.7°, 20.7°, 35.0°, and 36.2° slightly increased and also present a small peak of illite at 2θ around 31.0° and 36.1°. It means that an increase in *x*(RHA) content effect to increase in kaolinite phase. Generally, kaolinite has influenced on mechanical properties and absorbing water particles which contributes to the increase of samples' weight [23].

The surface morphology of [(1-*x*)(50C-50PR)-*x*RHA] at different *x*(RHA) contents is shown in Figure 3. The surface morphology was studied for the compositions with 0 wt% to 0.65 wt% *x*(RHA). The particle size of all the samples exhibited irregular shape and agglomeration. Moreover, it had a porous insert in the structure because of the amount of decomposition of RHA during burning [5,24]. The density of the samples slightly decreased from 2.45±0.09 g·cm^{-3} to 1.94±0.12 g·cm^{-3}, with an increasing in *x*(RHA) contents from 0 wt% to 0.65 wt% (Figure 4). The RHA particles are fine, resulting in many evenly distributed small pores and decreased densities [5,25].

Figure 5 shows BET surface area and pore volumes of (50C-50PR-*x*RHA) at different *x*(RHA) contents. All the samples showed irregular packing of mineral grains, and a higher amount of micro and macro-porosity in the *x*(RHA) contributed to the high surface area, pore volume, and pore size. The pore volume of samples dramatically increased from 0.938 cm^3·g^{-1} to 0.942 cm^3·g^{-1} with increased *x*(RHA). The specific BET surface area shows the same trend. Normally, pure natural clay showed rough surface morphology with a relatively smaller surface area (99.37 m^2·g^{-1}) due to the tight accumulation of clay minerals [26]. It is noticed that the BET surface at *x*(RHA) = 0 wt% was 8.83 m^2·g^{-1}. With an increasing in *x*(RHA) contents, it showed a considerable increase in surface area from 8.83 m^2·g^{-1} to 14.71 m^2·g^{-1}, which could be attributed to the introduction of *x*(RHA) with small particles into the (50C-50PR) system, along with active ingredients and its structural transformation into the matrix under high-temperature conditions. The BET results corresponding with the densities results are shown in Figure 5.

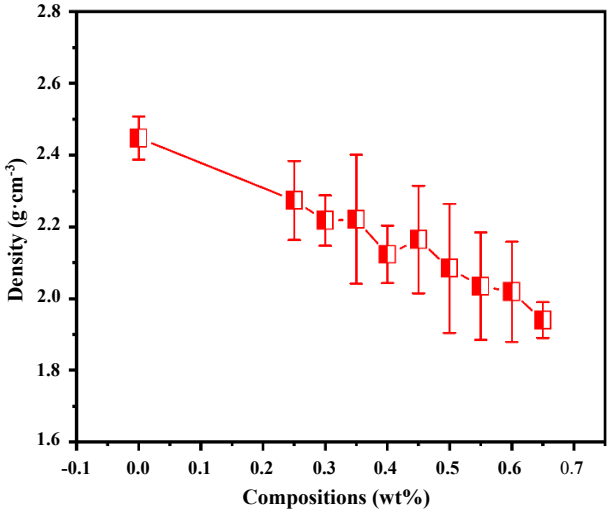

Figure 4. The densities of [(1-*x*)(50C-50PR)-*x*RHA] at difference of *x*(RHA) contents.

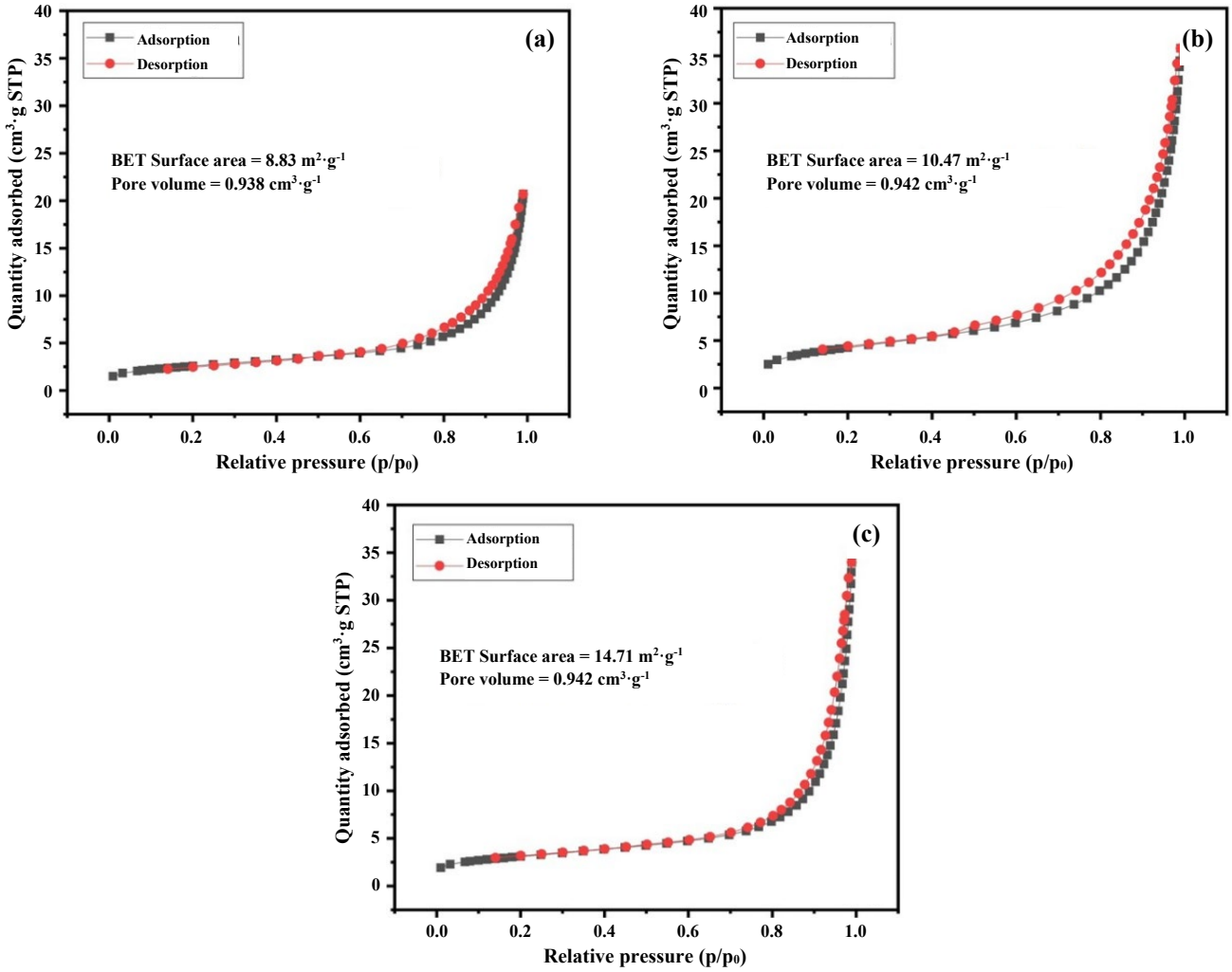

Figure 5. BET surface area and pore volumes of (50C-50PR-*x*RHA) samples at (a) *x*(RHA) = 0 wt%, (b) *x*(RHA) = 0.35 wt%, and (c) *x*(RHA) = 0.65 wt%.

3.2 Fungal immobilization and determination of fungal growth and viability on immobilized clay pellets

The optimum sample with *x*(RHA) = 0.65 wt%, which has high porosity but retains its shape, was selected to study the fungal effect. After fungal inoculation, the mycelium growth on calcined clay pellets soaked with PDB (treatment1) was found. In comparison, calcined clay pellets soaked with sterile distilled water (treatment2) did not have mycelium growth (Figure 6). The fungal mycelium of three isolates was grown and covered all calcined clay pellets treatment1 in the tissue culture bottle within 12 days to 16 days after inoculation (Table 1). These results indicated that culture and immobilizing orchid endophytic fungi with calcined clay pellets required the culture media to be used as a food source for those fungi. Then, the immobilization could occur after fungi could grow, germinate hypha, and produce spores on the calcined clay pellets. The results from several studies also supported and showed how to perform fungal immobilization or produce fungal inoculum from planting materials such as vermiculite, peat moss, and chopped coconut husk [27,28]. In a recent report, the production of mycorrhizal inoculum was studied. It revealed that the suitable potting media for fungal inoculum is chopped coconut husk soaked with PDB for 12 h [14]. Furthermore, the maximum spore production of mycorrhizal fungi using mixed sand, vermiculite, and organic source (vermicompost) was reported. The recent studies and the results indicated that the culture medium or organic source was necessary for fungal growth in potting material.

The viability of all immobilized fungi on calcined clay pellets was investigated by observing the growth of fungal hypha germinated from the calcined clay pellets on a PDA plate after inoculation. It was found that all fungi immobilized with clay pellet treatment1 had viability. The immobilized fungi could germinate and become hypha from the calcined clay pellets on a PDA plate after inoculation for 7, 14, 21, 28, and 35 days (Figure 7(a-c)). This study revealed that the calcined clay pellets could immobilize fungi. All fungi could be cultured and immobilized on calcined clay pellets and remain viable for at least 35 days after inoculation. As a result, this study reported an advantage function of the planting material, calcined clay pellets, which can be used as a potting media and source of plant growth-enhancing fungi at the same time. For further study, the shelf life and plant growth-promoting stability of immobilized fungi on calcined clay pellets will be conducted, and a prototype of the commercial product will be developed.

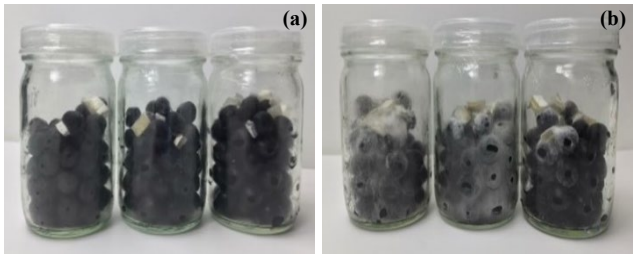

Figure 6. Growth of fungal isolate SPGT006 on the calcined clay pellets soaked with distilled sterile water (a) and the calcined clay pellets soaked with potato dextrose broth (b). Note: 12 days after inoculation.

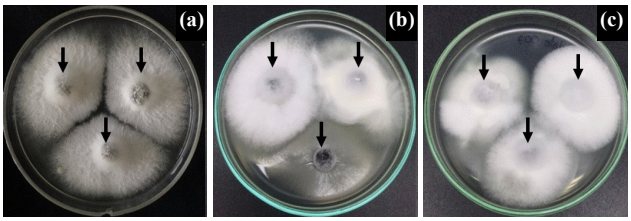

Figure 7. Hyphal germination of fungal isolate SPGT005 (a), SPGT006 (b), and SPGT007 (c) from the calcined clay pellets (pointed arrow) on potato dextrose agar plate after inoculation. Note: 35 days after inoculation.

Table 1. The time needed for the fungal mycelium to fully colonized on all calcined clay pellets in tissue culture bottles.

Fungal isolate	Time (day)
SPGT 005	16±3.00
SPGT 006	12±2.80
SPGT 007	13±0.58

The results showed **mean±SD (n = 3)**

4. Conclusions

The calcined clay pellets (50C-50PR-*x*RHA) with *x*(RHA) = 0 wt% to 0.65 wt% were successfully synthesized by using the solid-state reaction method using the calcination temperature of 800°C. The addition of *x*(RHA) resulted in an evenly distributed porous microstructure. The highest BET surface area and pore volume were found in the composition with *x*(RHA) = 0.65 wt%, which makes it a potential candidate for fungal immobilization. This study revealed an advantage function of the calcined clay pellets that had the ability to immobilize endophytic fungi. Therefore, the calcined clay pellets in this study could be applied and used as a potting media for plant growth-enhancing fungi at the same time.

Acknowledgements

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