Development of a Biofilm from Pleurotus sajor-caju Mycelium Using the Cast Film Technique to Enhance Mineral Nutrient Uptake and Growth of Brassica spp.

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Abstract

Brassica crops are economically important plants but are unable to form arbuscular mycorrhizal associations with arbuscular mycorrhizal fungi (AMF) because isothiocyanates (ITCs) inhibit fungal growth. Therefore, this study developed a *Pleurotus* mycelium biofilm to promote fungal colonization inside plant roots as a fungal endophyte. The results showed that the most suitable biofilm preparation method was scraping the surface of the mycelium with an inoculating loop and wrapping it with aluminum foil, which promoted hyphal aggregation and extracellular matrix formation. The biofilm exhibited the greatest tolerance in the presence of ITCs from cabbage, with a survival rate of 46.08 ± 6.17%, and extracellular matrix production increased as ITC concentration increased. In addition, the *Pleurotus* mycelium biofilm enhanced the uptake of nitrogen, phosphorus, and potassium (NPK) to 56.40 ± 0.44, 2.64 ± 0.02, and 31.90 ± 0.25 mg g^{-1} dry weight, respectively — approximately 1.57 ± 0.02, 1.47 ± 0.03, and 1.48 ± 0.02 times higher than the control group — and promoted plant growth at an average rate of 0.76 ± 0.05 cm day^{-1}, compared to 0.22 ± 0.02 cm day^{-1} in untreated plants. These findings suggest that the developed biofilm has potential for application as a biological product to improve nutrient uptake efficiency and promote plant growth.

keywords : Brassica crops, Pleurotus, fungal endophyte, biofilm

Introduction

Thailand is a fertile country highly suitable for agriculture, with a vegetable cultivation area of over 1.13 million rai and a total yield of approximately 1.8 million ton (Department of Agricultural Extension, 2024). This reflects the vital role of the agricultural sector in the country's economy. Within this sector, plants in the genus Brassica are widely grown economiccrops. However, they face a major limitation: they cannot form mycorrhizal relationships with arbuscular mycorrhizal fungi (AMF). This is because Brassica roots produce isothiocyanates (ITCs), which inhibit fungal growth, resulting in lower nutrient absorption

efficiency compared to plants that can form mycorrhizal associations.

To address this limitation, this study aims to develop a biofilm from Pleurotus fungal mycelium using the cast film method. This biofilm is designed to support the colonization of Pleurotus mycelium around plant roots. Once the mycelium penetrates the root tissues, it can establish itself as an endophytic fungus. This approach is expected to enhance nutrient absorption and promote the growth of Brassica plants without relying on mycorrhizal relationships

Methodology

The research was systematically designed and divided into three main parts: (1) selecting the most suitable method for fabricating a *Pleurotus* mycelium biofilm; (2) evaluating the compatibility between the biofilm and isothiocyanates (ITCs) extracted from *Brassica* plants; and (3) assessing the effectiveness of the mycelium biofilm as a plant growth-promoting material by measuring nutrient uptake and the growth of *Brassica* plants

1) Preparation of the Pleurotus Mycelium Biofilm

Pure Pleurotus cultures were grown on Potato Dextrose Agar (PDA) at 25°C for 21 days. The mycelium was then scraped from the agar surface using an inoculating loop and transferred to Potato Dextrose Broth (PDB).
The culture was incubated at 25°C for 14 days to allow the mycelia to aggregate and form a biofilm for subsequent experiments

2) Evaluation of the Compatibility Between the Biofilm and Isothiocyanates (ITCs) from Brassica Plants

The biofilm was exposed to ITCs extracted from different Brassica species at concentrations of 0.01, 0.1, and 1 mM. Optical density (OD) was measured using a spectrophotometer to calculate the survival percentage of the biofilm. The biofilm diameter and changes in its physical appearance were also recorded after exposure to the ITCs for 24 and 48 hours to evaluate its tolerance to the compounds.

3) Evaluation of the Performance of the Pleurotus Mycelium Biofilm

The alginate film was fabricated using the Cast Film method. A 3% (w/v) sodium alginate solution (500 mL) was prepared and allowed to stand for 24 hours. Then, 200 mL of the solution was mixed with 5% (v/v) glycerol, poured onto a plate, and dried to form a biofilm. The mechanical properties of the biofilm were then evaluated using a tensile testing machine.
The biofilm was applied to the Brassica species that showed the highest compatibility with the biofilm. Two experimental groups were established: (1) plants grown without the biofilm (control) and (2) plants grown with the Pleurotus mycelium biofilm. Plant growth was monitored for 30 days by measuring plant height and observing leaf and root characteristics.
Nutrient uptake by the plants was evaluated using leaf samples collected from each experimental group. The leaves were washed,

dried at 65°C, ground into a fine powder, and digested with concentrated acid under heat. The resulting solution was analyzed using a spectrophotometer to determine the concentrations of nitrogen, phosphorus, and potassium (NPK) in the plant tissues. Nutrient uptake was calculated using the equation Nutrient Uptake = C × DW, where C is the nutrient concentration in the plant tissue and DW is the dry weight of the sample.

Result and Discussion

1) Selection of a Suitable Method for Mycelium Biofilm Preparation

The biofilm formation results demonstrated its potential for use as a protective matrix that maintains the viability of fungal mycelium.

Table 1. Results of Biofilm Formation and Mycelium Cultivation

Method	Result
Pure mushroom culture wrapped with aluminum foil.	
The surface mycelium was gently scraped using an inoculating loop.	

According to Table 1, the biofilm prepared from mycelium collected from the surface of a pure fungal culture using an inoculating loop showed dense and uniform hyphal aggregation. A continuous biofilm matrix was formed, resulting in a strong and stable biofilm structure with high structural integrity. Therefore, the biofilm was suitable for use in subsequent experiments.

2) Selection of Suitable *Brassica* Species for Maintaining Biofilm Stability and Survival

When the biofilm was exposed to isothiocyanates (ITCs) from different *Brassica* species at various concentrations, clear differences in biofilm structure and survival were observed. The biofilm responded differently depending on the plant species and the concentration of ITCs.

Table 2. Biofilm Survival Percentage under Different ITC Treatments

Brassica Species	Biofilm Survival (%)
Cabbage	46.08 ± 6.17
Radish	29.81 ± 4.39
Chinese kale	40.97 ± 6.04
Broccoli	40.89 ± 5.28

According to Table 2, the highest biofilm survival percentage was observed in the cabbage ITC treatment, reaching 46.08 ± 6.17%. This result indicates that the biofilm maintained higher survival under exposure to cabbage-derived isothiocyanates (ITCs) than under ITCs from the other *Brassica* species tested.

Table 3. Changes in Biofilm Diameter under ITC Treatment

Plant Species	ITC Concentration (mM)	Change in Biofilm Diameter (mm)
Cabbage	0.01	0.84 ± 0.05 (–)
	0.10	3.14 ± 0.05 (+)
	1.00	6.67 ± 0.08 (+)

Note: (+) indicates an increase in biofilm diameter, while (–) indicates a decrease.

According to Table 3, the biofilm exposed to cabbage-derived isothiocyanates (ITCs) showed an increase in biofilm diameter as the ITC concentration increased. The biofilm diameter increased from 0.84 ± 0.05 mm at 0.01 mM to 6.67 ± 0.08 mm at 1.00 mM. These results suggest that the biofilm maintained its structure and exhibited structural adaptation under cabbage ITC treatment.

3) Performance of the *Pleurotus* Biofilm Prepared by the Cast Film Method

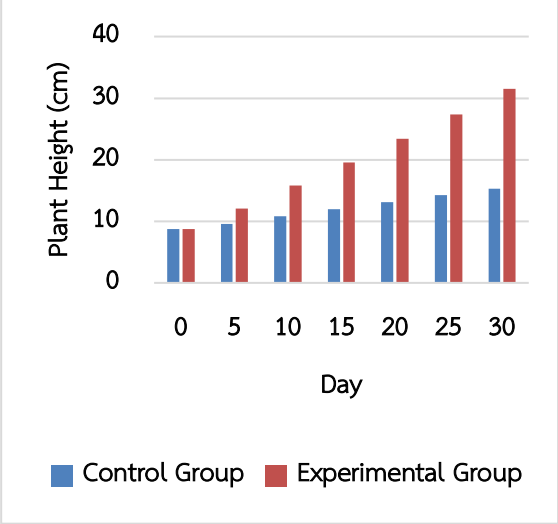
The *Pleurotus* biofilm prepared using the Cast Film method enhanced mineral absorption and promoted the growth of *Brassica* plants. The biofilm also supported fungal colonization within plant roots as a fungal endophyte.

Table 4. Mineral Absorption Capacity of the *Pleurotus* Biofilm

Treatment	Plant N (mg g^{-1} dry weight)	Plant P (mg g^{-1} dry weight)	Plant K (mg g^{-1} dry weight)
Pleurotus biofilm	56.40 ± 0.44	2.64 ± 0.02	31.90 ± 0.25

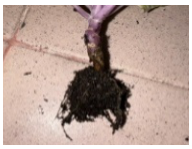
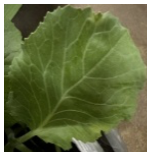
เคาะ According to Table 4, the *Pleurotus* biofilm treatment enhanced plant nutrient uptake, with nitrogen, phosphorus, and potassium uptake values of 56.40 ± 0.44 mg g^{-1} dry weight, 2.64 ± 0.02 mg g^{-1} dry weight, and 31.90 ± 0.25 mg g^{-1} dry weight, respectively. These results indicate the potential role of *Pleurotus* biofilm in improving plant access to and absorption of essential nutrients required for growth.

Figure 1. Plant Growth under Different Treatment Conditions



According to Figure 1, the treatment with *Pleurotus* biofilm showed the highest improvement in plant growth. The plants exhibited an average height increase of 0.76 ± 0.05 cm per day. These results demonstrate the potential of *Pleurotus* biofilm in promoting plant growth and improving plant development.

Table 5. Root and Leaf Characteristics of Plants Treated with *Pleurotus* Biofilm

Time	Root Characteristics	Leaf Characteristics
Day 1		
Day 30		

According to Table 5, plants treated with *Pleurotus* biofilm showed the best root development. The roots exhibited extensive branching, a dense root system, and good distribution throughout the growth medium. The leaves appeared dark green and showed healthy development. These characteristics were consistent with improved nutrient uptake and plant growth performance.

Conclusions

This study successfully developed a *Pleurotus* mycelium biofilm using the Cast Film method. The biofilm served as a supportive matrix that maintained fungal structure and enabled the interaction between fungal mycelium and *Brassica* plants under exposure to isothiocyanates (ITCs). The results showed that applying *Pleurotus* biofilm with cabbage improved plant nutrient uptake, including nitrogen, phosphorus, and potassium, with values of 56.40 ± 0.44 mg, 2.64 ± 0.02 mg, and 31.90 ± 0.25 mg, respectively. Plants treated with the biofilm exhibited healthy leaves with uniform green coloration and no visible nutrient deficiency symptoms, reflecting improved plant growth performance. The average growth rate reached 0.76 ± 0.05 cm per day. These findings demonstrate the potential of *Pleurotus* biofilm as a biological support material for enhancing nutrient availability and promoting plant growth in *Brassica* species

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