

Characterization and Amendment of Biochar Derived from Oil Palm Fronds for Enhanced Growth and Phytometabolites of Indica Rice

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ABSTRACT

This research examines the biochar produced from Oil Palm Fronds (OPF) and its effect on the growth and phytometabolites of indica rice. OPF biochar was characterized using Scanning Electron Microscope coupled with an Energy Dispersive X-ray Spectrometer (SEM-EDS) and an X-ray Diffractometer (XRD). The elemental composition analysis revealed that the main elements were carbon (C) at 72.17% and oxygen (O) at 20.38%. Other elements included silicon (Si), aluminum (Al), calcium (Ca), magnesium (Mg), chlorine (Cl), iron (Fe), potassium (K), sodium (Na), copper (Cu), and titanium (Ti). Moreover, the XRD pattern indicated the presence of quartz (SiO₂) and sylvite (KCl), which exhibited beneficial effects on plants. The effects of the OPF biochar on the rice growth (plant height, fresh weight, and dry weight), photosynthetic pigment contents (carotenoid, chlorophyll A and B), and antioxidant enzyme activity (peroxidase and catalase) were evaluated. Rice seedlings were cultivated in culture media treated with OPF biochar at several doses (0, 200, 400, and 800 mg/L). The growth and overall content of phytometabolites increased at each concentration of biochar, reaching a maximum at 400 mg/L, while the 800 mg/L showed

adverse effects. These findings demonstrated that OPF biochar has significant potential for improving the indica rice growth and phytometabolite propagation.

Keywords-agricultural waste; biochar; palm leaves; secondary metabolites; antioxidant enzyme; photosynthetic pigments

I. INTRODUCTION

Thailand is a major agricultural product exporter worldwide, especially of rice, sugarcane, oil palm, and cassava [1, 2]. Oil palm is the most important economic crop in southern Thailand, with approximately 1.01 million Ha under cultivation [3]. The expansion of oil palm plantation has led to a significant increase in waste products, such as empty fruit bunches, pulp, shells, pressed fibers, and leaves, [3, 4]. These by-products represent an excellent source of lignocellulosic biomass. Specifically, OPFs, generate several tons of waste per Ha and can be converted into multipurpose activated biochar [5].

Biochar is a carbon-rich material used in many agricultural and environmental applications, like soil fertilizer, improved seedling growth, increased biomass of soil microorganisms, heavy metal adsorption, wastewater treatment, and climate change mitigation [6-8]. It has been shown that biochar enhances the biological and physicochemical properties of soils including texture, bulk density, porosity, buffering, and Cation Exchange Capacity (CEC) [9]. According to [10], biochar can improve the microbial interactions in wheat roots and boost the rhizosphere microbial diversity. These advantages have resulted in increased agricultural productivity and growth.

Besides the soil improvement, biochar has also been reported to influence phytometabolites, such as enzymes, carotenoids, flavonoids, and chlorophyll [11]. These metabolites play a key role in plant physiological processes including photosynthesis and antioxidant activity in plants, which encourage the growth and help plants to withstand stressful and unsuitable environmental conditions. Furthermore, increasing the amount of such phytometabolites can contribute to a rise in the nutritional quality of crops. In plant in vitro culture, biochar derived from agricultural waste has been used as culture medium supplements for growing plants. Studies indicate that culture media amended with biochar affect the plant growth, although the outcomes vary depending on the type of agricultural waste used and the concentration of biochar applied [6]. Thus, it is necessary to investigate the characteristics and concentration of biochar for application use.

Rice (*Oryza sativa* Linn.) is a staple food for more than half of the global population, with Thailand remaining one of the world's largest rice exporters [12]. Producing sufficient rice for domestic consumption and export is, therefore, very important. Improving the rice yield requires suitable cultivation practices, and desirable traits, such as stay-green leaves, deep root traits, and high ability of photosynthesis, which have been identified as indicators of high productivity [13].

The aim of this research was to investigate the physicochemical properties of biochar derived from OPF and its effect on the growth and metabolite production of indica rice. This work demonstrated the potential of applying biochar

in agricultural biotechnology within the framework of a circular bioeconomy.

II. MATERIAL AND METHODOLOGY

A. Preparation and Characterization of Biochar

OPF wastes were collected from a local plantation area in Nakhon Si Thammarat province, southern Thailand. The wastes were dried under the sunlight for two days before being placed into a 200 L charcoal kiln. Biochar was produced through a slow pyrolysis process conducted under anaerobic conditions at 450-500 °C for 5 h, with the temperature raised at a rate of 10 °C min⁻¹ [14-16]. After pyrolysis, the kiln was cooled to room temperature. The obtained biochar was collected, stored under dry conditions, and used for physicochemical analyses.

The surface morphological characteristics and elemental analysis were examined with a Scanning Electron Microscope equipped with an Energy Dispersive X-ray Spectrometer (SEM-EDS; JEOL JSM-6610LV, Oxford X-Max 50). The SEM analysis was conducted under low vacuum at an accelerating voltage of 25 kV. A pH meter (Mettler Toledo, SevenCompact) was utilized to determine the pH values of the produced OPF biochar. Moreover, the chemical composition present in the biochar was performed with XRD, Rigaku, SmartLab operated at 40 kV and 30mA, with a scanning range of $2\theta = 5-80^\circ$ [16-18].

B. Plant Materials and Cultivation

Indica rice seeds (*Oryza sativa* Linn. *Indica*) were obtained from Phatumthani Rice Research Center and the Seed Center, Roiet, Office of Grain Rice Department, Ministry of Agriculture and Cooperatives, Thailand. For the surface sterilization, the rice seeds were dehusked and sterilized using 70% ethanol for 3 min, 5% (v/v) commercial bleach solution (5.25% NaClO) for 40 min, and 30% (v/v) commercial bleach solution for 30 min. The seeds were then put on sterile cellulose filter papers in a Petri dish after being repeatedly rinsed with double-sterilized distilled water [19].

The sterilized seeds were cultured on NB basal medium containing 2 mg/L of 2,4-D, 500 mg/L proline, 500 mg/L glutamine, 30 g/L sucrose, and 8 g/L agar. The medium pH was adjusted to 5.6-5.8 prior to sterilization. To investigate the effect of biochar, the culture medium was supplemented with different concentrations of OPF-derived biochar (0, 200, 400, and 800 mg/L). The rice plants were grown for four weeks at 25 ± 2 °C under a 16/8 h light/dark photoperiod and a 1000 lux of Light-Emitting Diodes (LEDs) [19, 20].

C. Evaluation of Rice Growth and Phytometabolites

After the cultivation period, the seedling growth was evaluated by measuring the fresh weight, dry weight, and plant height. The dry weight was determined by drying seedling in a hot-air oven at 70 °C for 48 h, and then stored in a desiccator until the weight was stable [20]. To assess the effects of OPF

biochar on rice phytometabolites, the following parameters were measured: photosynthetic pigments (chlorophyll A, chlorophyll B and total carotenoids), antioxidant enzymes (peroxidase and catalase), and total flavonoids. The total flavonoid content was examined using the method described in [21]. Chlorophyll A, chlorophyll B, and the total carotenoid contents were calculated according to the equations used in [22, 23].

In order to determine the antioxidant enzymes, fresh seedling leaves were crushed in liquid nitrogen and then mixed with phosphate buffer (pH = 7). The leaves' extract was centrifuged at 4500 rpm (4 °C, 1 h), and the supernatant was used for the analysis of the antioxidant enzymes' activities. Peroxidase and catalase activities were determined following the protocol of [24].

D. Statistical Analysis

All statistical analyses were performed using SPSS software version 15.0 (IBM SPSS Statistics, Chicago, IL, USA). One-way Analysis of Variance (ANOVA) was performed on data obtained from three replicates ($n = 3$), and the results were expressed as means \pm Standard Deviations (SD). To find the significant differences between the treatment means at a significance threshold of $P < 0.05$, the Duncan Multiple Range Test (DMRT) was employed.

III. RESULTS AND DISCUSSION

A. Physical and Chemical Characteristics of OPF Biochar

The surface morphology of OPF biochar was examined using SEM at a magnification of 6,000, as illustrated in Figure 1. The SEM image revealed the/a non-definite shape and porous structure. Figure 2 presented the elemental composition of OPF biochar. The analysis using SEM-EDS exhibited that carbon (C) and oxygen (O) were the major elements at the weight percentage of 72.17 and 20.38, respectively. The minor elements included aluminum (Al, 1.63%), silicon (Si, 1.50%), calcium (Ca, 1.33%), magnesium (Mg, 0.86%), chlorine (Cl, 0.83%), iron (Fe, 0.56%), potassium (K, 0.36%), sodium (Na, 0.23%), copper (Cu, 0.07%), and titanium (Ti, 0.07%).

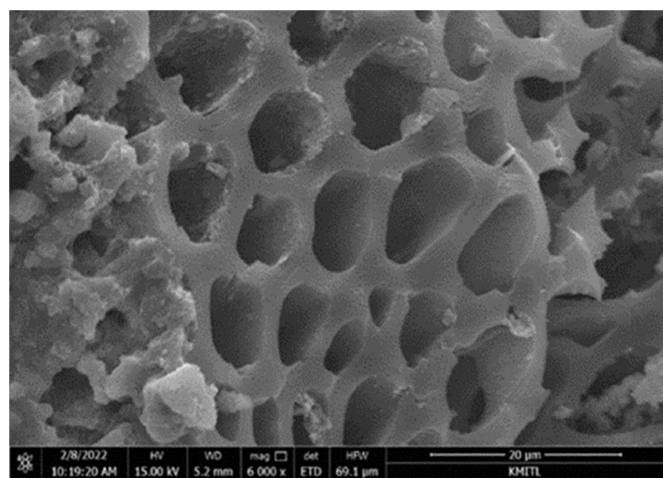


Fig. 1. Scanning electron micrograph of OPF biochar at 6,000 magnifications, Scale bars: 20 μ m.

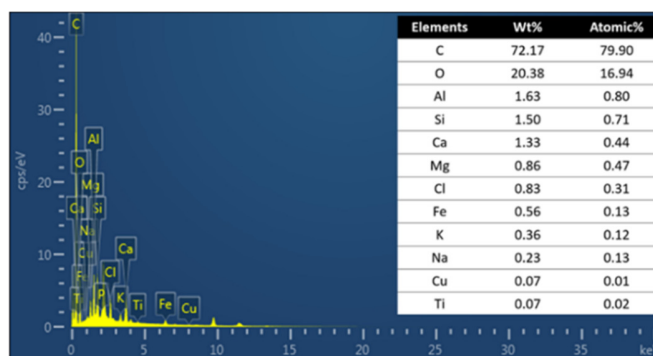


Fig. 2. EDS spectrum and elemental composition of OPF biochar.

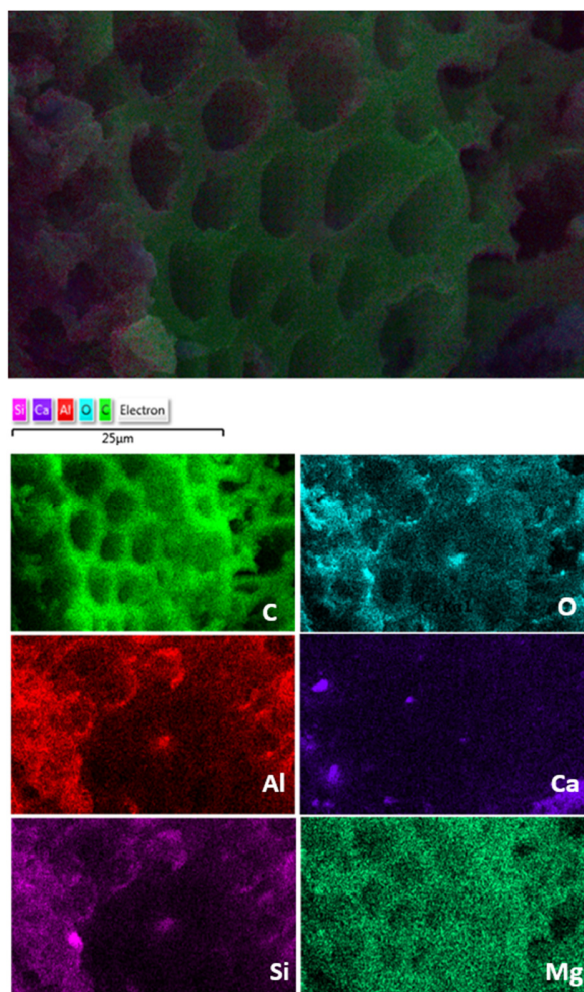


Fig. 3. EDS elemental mapping obtained from OPF biochar, Scale bars: 25 μ m.

Element mapping confirmed the distribution of five key elements within the OPF biochar structure (Figure 3). C (green) and O (cyan) were major distributed in the porous structure along with other minor elements, such as Mg, Al, and Si. The pH of OPF biochar was strongly alkaline at 10.15. The chemical composition was further examined by the XRD profile as depicted in Figure 4. The XRD pattern revealed a mostly amorphous carbon rich material with a few crystalline

phases including quartz and sylvite. A prominent silica or quartz (SiO₂) peak was visible in the X-ray diffractogram at 2θ = 28°, while additional peaks indicated the presence of sylvite (KCl).

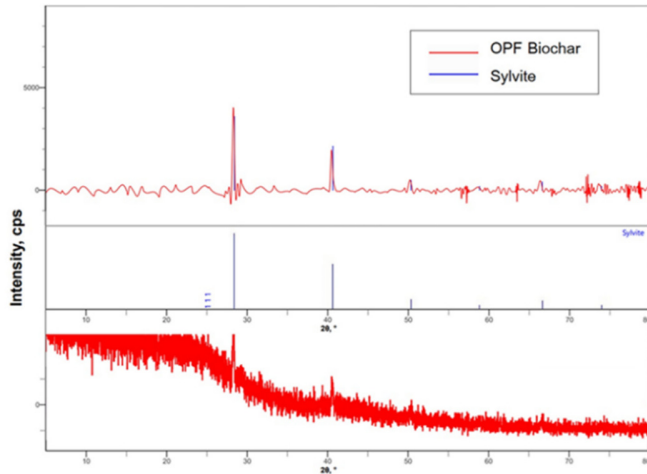


Fig. 4. X-ray diffraction patterns of biochar produced by oil palm frond.

B. Evaluation of Indica Rice Growth in Various Concentrations of OPF Biochar

The effects of OPF biochar supplementation on indica rice seedling growth were evaluated by measuring the growth performance of the seedlings in terms of the rice plant height, fresh weight, and dry weight. Table I presents the growth response of indica rice seedlings at various concentrations of OPF biochar. The greatest increase was observed at 400 mg/L, where the rice seedlings exhibited a higher plant height compared to both the control and other treatment concentrations.

The biomass accumulation, expressed as fresh and dry weight, followed a similar trend. An increase in the biomass of rice seedlings was observed at the end of the cultivation period, with the concentration of 400 mg/L showing the highest biomass production reaching 0.24 g (fresh weight) and 0.03 g (dry weight). However, a decrease in the formation of rice seedling biomass was observed at 800 mg/L. The growth of rice seedlings in different concentrations of OPF biochar is displayed in Figure 5.

TABLE I. THE GROWTH RESPONSE OF INDICA RICE SEEDLINGS IN VARIOUS CONCENTRATIONS OF OPF BIOCHAR

Time (weeks)	Biochar (mg/L)	Height (cm)	Fresh weight (g)	Dry weight (g)
1	0	12.86 ± 0.15	0.04 ± 0.24	0.0057 ± 0.81
	200	14.72 ± 0.44	0.05 ± 0.17	0.0079 ± 1.26
	400	16.61 ± 0.25	0.07 ± 0.39	0.0126 ± 0.75
	800	12.68 ± 0.36	0.05 ± 0.19	0.0059 ± 0.18
2	0	14.62 ± 0.29	0.10 ± 0.41	0.0125 ± 0.65
	200	15.15 ± 0.74	0.13 ± 0.32	0.0149 ± 0.72
	400	17.55 ± 0.16	0.15 ± 0.27	0.0182 ± 0.74
	800	13.06 ± 0.24	0.13 ± 0.52	0.0156 ± 0.66
3	0	19.55 ± 0.11	0.14 ± 0.63	0.0198 ± 0.50
	200	19.60 ± 0.69	0.14 ± 0.72	0.0223 ± 0.84
	400	21.27 ± 0.35	0.17 ± 0.26	0.0316 ± 0.71
	800	19.59 ± 1.14	0.14 ± 0.81	0.0300 ± 0.55
4	0	21.35 ± 0.67	0.14 ± 0.30	0.0234 ± 1.46
	200	21.48 ± 0.22	0.19 ± 0.12	0.0224 ± 0.87
	400	23.47 ± 0.61	0.24 ± 0.29	0.0357 ± 0.56
	800	21.33 ± 0.46	0.20 ± 0.50	0.0270 ± 0.49

TABLE II. PHYTOMETABOLITES PRODUCED BY INDICA RICE SEEDLINGS IN VARIOUS CONCENTRATIONS OF OPF BIOCHAR

Time (weeks)	Biochar (mg/L)	Chlorophyll A (mg/g)	Chlorophyll B (mg/g)	Carotenoids (mg/g)	Peroxidase (unit/g protein)	Catalase (unit/g protein)
1	0	0.89 ± 0.56	1.66 ± 0.33	322.02 ± 2.55	0.84 ± 0.81	1.91 ± 1.36
	200	1.28 ± 2.36	1.78 ± 0.27	330.64 ± 1.84	1.00 ± 0.56	2.14 ± 0.68
	400	1.35 ± 1.21	1.89 ± 1.71	344.36 ± 2.94	1.11 ± 1.62	2.23 ± 0.46
	800	1.24 ± 0.54	1.67 ± 1.26	334.20 ± 1.43	0.84 ± 0.83	1.91 ± 1.24
2	0	1.98 ± 0.97	1.87 ± 0.88	313.07 ± 1.51	1.28 ± 0.42	2.09 ± 2.03
	200	2.54 ± 0.42	2.32 ± 0.16	365.90 ± 0.85	1.33 ± 0.66	2.21 ± 1.47
	400	2.80 ± 0.28	2.56 ± 0.77	374.89 ± 0.39	1.45 ± 1.76	2.32 ± 0.58
	800	1.97 ± 1.19	1.88 ± 0.68	310.02 ± 0.07	1.24 ± 1.32	2.00 ± 1.67
3	0	2.90 ± 0.08	1.65 ± 0.94	335.36 ± 1.49	1.37 ± 0.69	2.26 ± 2.41
	200	3.12 ± 0.74	1.96 ± 1.52	358.58 ± 0.77	1.41 ± 0.37	2.44 ± 1.44
	400	3.36 ± 1.14	2.22 ± 1.41	368.65 ± 1.19	1.60 ± 0.09	2.52 ± 0.53
	800	3.06 ± 1.59	1.96 ± 0.57	356.82 ± 1.46	1.37 ± 1.26	2.25 ± 1.50
4	0	3.37 ± 0.87	2.69 ± 0.14	360.09 ± 0.55	1.42 ± 1.83	2.73 ± 1.74
	200	3.52 ± 0.77	3.14 ± 1.63	367.53 ± 2.12	1.52 ± 0.45	3.23 ± 1.09
	400	3.84 ± 0.55	3.24 ± 1.71	382.00 ± 2.41	1.73 ± 1.96	3.50 ± 0.49
	800	3.30 ± 1.34	2.75 ± 0.98	368.91 ± 1.74	1.48 ± 1.66	3.08 ± 1.31

Note for Tables I and II: The values in the same column demonstrated a significant difference (p ≤ 0.05), as shown by the characters, and are expressed as mean ± SD (n = 3).



Fig. 5. The indica rice seedlings growth in different concentrations of OPF biochar: 0 mg/L (A), 200 mg/L (B), 400 mg/L (C) and 800 mg/L (D).

C. Evaluation of Phytometabolites Produced by Indica Rice Plants in Various Concentrations of OPF Biochar

The effects of OPF biochar on the physiological responses of the rice seedlings were evaluated by analyzing the photosynthetic pigments (chlorophyll A, chlorophyll B, and carotenoid) and antioxidant enzyme activities (peroxidase and catalase). The results are summarized in Table II.

After four weeks of cultivation, the highest levels of chlorophyll A, chlorophyll B, and carotenoid were found in the concentration of 400 mg/L, at 3.84 mg/g, 3.24 mg/g, and 382 mg/g, respectively. However, at 800 mg/L, there was a significant reduction in all photosynthesis pigments. Similarly, the activities of peroxidase and catalase were significantly enhanced at 200 and 400 mg/L of OPF biochar with the maximum activities of 1.73 and 3.50 units/g protein, respectively. At 800 mg/L, the enzyme activity decreased. As major antioxidant enzymes, peroxidase and catalase protect the plants from oxidative stress; thus, the observed increases at moderate biochar concentrations suggest an improved stress tolerance.

The morphological structure of biochar is an important factor in enhancing the structure of the planting materials. The ability of biochar to hold water and adsorb nutrients is typically associated to its surface characteristics, such as the pore size and pore distribution. Carbon, the dominant element, is produced during pyrolysis, while alkaline metals (Na, K) and alkaline earth metals (Ca, Mg) were also detected by the EDS analysis [25-28]. The XRD analysis further showed the lack of the cellulose peak because of the combustion and formation of

new phase, such as quartz (SiO_2) and sylvite (KCl). This finding is similar with those in [29], where the elemental composition of the palm kernel shell was C, O, K, Ca, Cl, Al and Mg. These elements were also components of inorganic compounds, such as quartz (Si and O), sylvite (K and Cl), and feldspar (K, Na, Al, Si and Ca). Furthermore, previous studies of biochar from plant biomass and feedstock revealed that the biochar was enriched with inorganic compounds, such as silicates, calcite, and sylvite [30-32]. For instance, the inorganic mineral sylvite is an important source of potassium (K), one of the three major macronutrients for plant growth and metabolism [33-35]. Similarly, silicon (Si) has been reported to have beneficial effects on the growth of many crops, including cucumber, barley, wheat, and rice [36]. The plant physiological role of Si is related to the ability of plants to absorb macronutrients and improve the plant erection [37]. Additionally, previous studies indicated that biochar might stimulate the production of plant hormones, such as brassinosteroid, auxin, gibberellic acid, and abscisic acid, which contributes to the plant growth performance [38, 39].

Although OPF biochar promoted the growth and biomass of the rice seedlings at moderate levels, at high levels this could not have happened. Several studies have shown that the reduced plant growth at high biochar concentrations was associated with increased pH and Electrical Conductivity (EC), which can lead to a lack of plant nutrients [25, 40].

In this research, the contents of chlorophyll A, chlorophyll B, and carotenoids significantly increased with increasing OPF biochar concentrations, reaching a maximum at 400 mg/L. These results agreed with the findings of [41], where the effect of the biochar combination with N fertilizer on photosynthetic pigments of rice was reported. It was found that the average photosynthetic rate and concentrations of chlorophyll A and B were higher in biochar-treated plants than in biochar-free controls. Supplementing with biochar, enhanced the absorption of magnesium ions (Mg^{2+}), which were essential for the synthesis of chlorophyll [39]. However, these photosynthetic pigments were significantly decreased at high concentrations of OPF biochar (800 mg/L). The reduction of chlorophyll content at high levels of biochar could be associated with the high EC of the culture medium, which might be resulting in a salinity stress of the rice seedlings [25].

The impact of biochar on the metabolome and microbiome of the wheat rhizosphere was investigated in [10]. It was discovered that adding 0.25% of biochar improved the production of flavonoids and anthocyanins, among other primary and secondary metabolites. The type and composition of biochar, concentrations, planting material circumstances, and crop species were some of the variables that may have affected the levels of carotenoid and chlorophyll [38, 39].

Regarding the antioxidant enzymes, peroxidase and catalase are essential for protecting the plants from a variety of stressful situations [42]. In this study, their activities were clearly enhanced, suggesting that the rice seedlings experienced a reduced oxidative stress. The ability of biochar to scavenge excess Reactive Oxygen Species (ROS) in plants contributed to stress resistance [43]. Biochar not only has the potential to increase the antioxidant activity, but also significantly increase

the crop yield even under stress conditions [44, 45]. It is an effective adsorbent and is, therefore, essential for eliminating different wastes from plant tissue culture media.

IV. CONCLUSION

This research investigated the biochar derived from Oil Palm Fronds (OPFs) and its ability to enhance the growth and phytometabolites, such as chlorophyll, carotenoids, and the antioxidant enzyme activity, of indica rice. The obtained OPF biochar was rich in carbon (72.17%) and oxygen (20.38%) with minor elements, such as aluminum (Al), silicon (Si), calcium (Ca), magnesium (Mg), iron (Fe), chlorine (Cl), potassium (K), sodium (Na), copper (Cu), and titanium (Ti). The X-ray Diffraction (XRD) pattern confirmed the presence of inorganic compounds, such as quartz (SiO₂) and sylvite (KCl), which are major sources of essential elements for the rice plant growth.

A moderate concentration of OPF biochar (400 mg/L) significantly enhanced the rice plant growth, photosynthetic pigment content, and antioxidant enzyme activity, whereas a high concentration (800 mg/L) negatively affected the growth and metabolite accumulation. These findings suggest that the biochar derived from OPFs can provide an effective approach for agricultural waste management and may be applied to improve indica rice propagation. Although the results obtained at the laboratory scale are encouraging, further investigation is needed to evaluate the effects of OPF biochar on the growth, phytometabolite production, and yield of indica rice under field conditions.

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