Enhancing Agricultural Biodiversity through EFB Biochar -*Trichoderma* Synergies: Effect on Growth and Nutrient Content of *Capsicum annuum*

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Abstract

Rising demand for palm oil increases waste production, mainly empty fruit bunches (EFB), which causes environmental pollution. However, EFB can be converted to biochar as a soil amendment. This research investigates how the integration of EFB biochar (EFBB) and Trichoderma biofertilizer (TBF) impacts the agronomic performance of *C. annuum*. While individual benefits of EFBB and TBF have been studied, their combined effects on C. annuum have yet to be investigated. Randomized Complete Block Design (RCBD) with six treatments and four replications was employed. The treatment includes T1= Control (Chemical Fertilizer), T2= 100% EFBB, T3= 100% TBF, T4= 75% EFBB + 25% TBF, T5= 50% EFBB + 50% TBF, and T6= 25% EFBB + 75% TBF. The data collection included plant growth, yield, and nutrient analysis. Analytical procedures included SPAD readings, biomass quantification, Kjeldahl and ICP-OES analyses. *C. annuum* was cultivated in a polybag filled with topsoil under net house conditions and drip irrigation with a timer system for 120 days. The study revealed the positive impacts of combining EFBB and TBF on the agronomic performance of C. annuum, with 25% EFBB + 75% TBF treatment (T6) yields the best overall performance in terms of plant height (52.52 cm), fruit weight per plant (97.95 g), and number of harvests per cycle (2.90), indicating a strong synergistic effect. The findings provide valuable insights into EFBB and TBF application as a soil amendment to reduce the use of chemical fertilizers. However, further studies are essential to confirm the persistence and scalability of the observed effects before making recommendations for commercial settings.

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1 Introduction

To reach the estimated food demands for 2050 in decreasingly suiting climates, current agricultural techniques must be complemented by sustainably intensified practices. Malaysia plays a significant role in the global palm oil industry, accounting for nearly 28% of global palm oil production and 33% of global palm oil exports, contributing RM36.9 billion or 37.1% to the agricultural value added (Begum et al., 2019; Abubakar et al., 2021; Mahmud & Chong, 2021; Mohamed & Yusup, 2021; Siraj et al., 2021). The rapid expansion of oil palm plantations in Malaysia has increased agricultural waste, mainly EFB, which comprises the fibrous outer layer surrounding the palm fruit. In Malaysia, the industry produces 26 to 30 million tons of EFB waste annually, either dumped in the mill area or burned, causing environmental issues like air pollution and land degradation (Tiemann et al., 2018; Zainuddin et al., 2018).

There is considerable interest in converting EFB into beneficial soil amendments, such as biochar, derived from the pyrolysis of organic materials under anaerobic conditions (He et al., 2021; Zhou et al., 2021). Numerous studies have shown the positive impact of EFBB on soil physicochemical properties, such as enhanced water and nutrient retention, carbon sequestration, and reduced nutrient leaching, making it a sustainable option for improving soil fertility and mitigating climate change (Gruss et al., 2019; Wijitkosum & Jiwnok et al., 2019; Siedt et al., 2020; Wang et al., 2021).

Complementary to biochar, organic inputs such as *Trichoderma*-based biofertilizers (TBF) further support sustainable agriculture by reducing chemical fertilizer dependence. *Trichoderma* is a naturally occurring fungus that benefits agriculture, including biocontrol against pathogens, promotion of plant growth, nutrient solubilization, organic waste decomposition, and forming beneficial associations with plants by colonizing root systems (Zin et al., 2020; Poveda, 2021; Syam et al., 2021; Yahya et al., 2021; Kubheka & Ziana, 2022). These interactions between *Trichoderma* and plants enhance nutrient availability, improving plant growth, vigor, and overall yield, thus making it an attractive option for sustainable crop production (Liu et al., 2022).

Developing sustainable agricultural practices that ensure adequate food production without compromising finite resources, such as soil, is challenging for modern agriculture (Rombel et al., 2022; Garbowski et al., 2023; Lateef et al., 2023). This challenge searches for environmental alternatives, like soil amendments and biofertilizers, which contribute to preserving soil, ecosystems, and human health (Maitra et al., 2020; Etminani et al., 2021). These organic inputs significantly improve soil fertility by enhancing soil properties, increasing soil organic matter, balancing soil nutrients, and improving crop availability.

While numerous studies have been conducted on both EFBB and TBF individually, their combined effects have yet to be investigated. Therefore, this study was conducted to determine the effect of EFBB and TBF on the agronomic performance of *C. annuum*, including growth, yield, plant nutrient, and soil analysis. By evaluating the impact of the EFBB and TBF combined on *C. annuum*, valuable insights can be gained regarding its potential for broader agricultural applications.

2 Materials and Methods

2.1 Site Descriptions and Planting Materials

The research was conducted at the Farm Unit, UiTM Sarawak Branch, Samarahan Campus, for 120 days from April 2022 to August 2022 under a net house condition (9m × 9m) equipped with a drip irrigation system. The plant material used in this study was the Capsicum annuum L. Var. Kulai (F-1 Hybrid SJ2-461). It was cultivated in 40cm × 40cm polybags filled with topsoil with a planting distance of 60cm × 60cm. The chemical fertilizer and TBF used in this study were purchased from a local shop in Samarahan, Sarawak, Malaysia.

2.2 EFB Biochar Preparation

The raw biomass of EFB was collected from smallholders in Samarahan, Sarawak, Malaysia. The EFB was thoroughly washed with tap water to remove any impurities and then cut into small pieces not exceeding 2 to 3 cm in length. Subsequently, the samples were sun-dried for approximately 4-5 days, followed by further drying in an oven at 105°C until a constant weight was achieved (Chan et al., 2021). Once dried, the EFB samples were crushed and ground into a fine powder using a heavy-duty grinder. The powdered EFB was placed into folded aluminium foil and weighed using analytical balance to determine the precise amount of biomass before being carbonized at 400°C for 2 hours (Abel et al., 2021). This carbonization process, known as pyrolysis, transformed the EFB biomass into biochar. After the carbonization process, the biochar was allowed to cool down to room temperature. It was then weighed to determine the yield of biochar obtained from the initial amount of EFB biomass. The biochar was then ground to pass a 1 mm sieve and kept at room temperature before analysis (Bohari et al., 2020; Siti et al., 2021).

2.3 Experimental Designs and Treatments

The research used RCBD with six treatments, four replications, and five plants per treatment, giving 120 experimental units. The nutrient amount applied to *C. annuum* seedlings was modified based on findings from Rivitra et al. (2021). The treatment was conducted on the 2nd, 7th, and 10th week after treatment (WAT). The details of the treatments are shown in Table 1. The fruit was harvested from the 8th to 12th WAT; the destructive sampling was carried out on the 13th WAT. The plants were partitioned into two parts: root and shoot. Nursery maintenance practices, such as polyethylene bag weeding, pests, and disease control, were regularly and closely monitored. The plant was watered twice daily using drip irrigation with a timer system.

Table 1. Compositions of treatments

Treatments	Percentage	Quantity
T1	Control (Commercial chemical fertilizer)	NPK 12:12:17:2+TE 5g- standard recommendation
T2	100% EFBB	100g
T3	100% TBF	100g
T4	75% EFBB + 25% TBF	75g + 25g
T5	50% EFBB + 50% TBF	50g + 50g
T6	25% EFBB + 75% TBF	25g + 75g

*Note: EFBB = Empty Fruit Bunch Biochar; TBF = *Trichoderma* biofertilizer

2.4 Data Collection

The data recorded include plant growth, plant yield, soil, and plant analysis. The parameters collected for plant growth were relative chlorophyll content, plant height, stem diameter, and plant biomass. Relative chlorophyll content, plant height, and stem diameter were collected biweekly, while plant biomass was collected at harvest. The relative chlorophyll content was measured by a portable chlorophyll meter (SPAD-502, Konica Minolta Japan), plant height was measured with measuring tape, and the stem diameter was measured with a digital caliper. The plant biomass was recorded after destructive sampling. The plant was uprooted and washed under tap water to remove the soil and unwanted particles. The plant was weighed before being dried at 65°for for three days until it achieved constant weight. The yield data included the number of fruits per plant, fruit weight per plant, and the number of harvests per cycle. The data were recorded for four weeks of the harvesting period. Soil analysis included pH, EC, and selected nutrient content such as nitrogen (N), phosphorus (P), and potassium (K). N, P, and K were also analyzed for plant analysis.

2.5 Soil and Plant Analysis

The soil sample was weighed and oven-dried at 65°C until it achieved constant weight. The pH was measured using a digital pH meter (HI198103, Hanna Instruments), while EC was determined using an EC meter (Mettler-Toledo AG 8603 Schwerzenbach, Switzerland) (Hassan et al., 2007; Siti et al., 2021). The Kjeldahl Digestion and Distillation Method analyzed N for both soil and plant, while the wet digestion method was carried out to determine P and K and analyzed using Agilent 5800 Series Inductively Coupled Plasma – Optical Emission Spectrometer (ICP-OES) (Gupta, 2007).

2.6 Statistical Analysis

The differences in all parameters between treatments were computed using the Statistical Analysis System (SAS, 2013) and PROC ANOVA. Duncan's Multiple Range Test (DMRT; $p<0.05$) was used to identify significant treatment groupings.

3 Results and Discussion

3.1 Growth Parameters

The results for growth parameters show no significant difference ($p<0.05$) among all the treatments, as presented in Table 2. However, it can be concluded that treatment T6 achieved the highest mean values of plant height (52.52cm) and stem diameter (0.91cm) as compared to control. Treatment T2 gave a higher plant height after treatment T6. This result supports the previous finding that biochar can improve plant growth. Meanwhile, treatment T3 has the lowest mean values of plant height (47.19cm) and stem diameter (0.85cm), respectively.

The results indicated that the combination of EFBB and TBF positively impacts the plant height and stem diameter of *C. annuum* because, when combined, they provide positive benefits that enhance plant growth. According to several resources, EFBB improves soil structure by enhancing soil aeration, water holding capacity (WHC), and drainage (Duan et al., 2021; Kamali et al., 2021). This advantage allows the roots to acquire oxygen and water more efficiently. Besides, Aziz (2021) also stated that EFBB enhances nutrient retention because it has a high CEC, which allows EFBB to retain and slowly release the nutrients in the soil, thus avoiding nutrient leaching (Ghorbani et al., 2019).

Furthermore, TBF enhances nutrient solubilization and availability to plants because the enzymes and organic acids produced by *Trichoderma* sp. help break down the organic and inorganic compounds, thus helping release the essential elements like N, P, K, and micronutrients (Sood et al., 2020). Syam (2021) states that applying *Trichoderma* can only increase plant growth when combined with foliar fertilizer application. The statement explains why treatment T3 (100% TBF) has the lowest mean value for almost all parameters but the highest when combined with 25% EFBB.

Treatment T4 has the highest mean value for the relative chlorophyll content, 65.57. This result suggests that the combination of 75% EFBB and TBF enhanced chlorophyll production. The favorable impact of this treatment on chlorophyll content may be due to the combined positive effects of EFBB and TBF on nutrient availability and uptake, resulting in improved photosynthetic capacity. There was no significant difference among all the treatments for plant biomass. However, treatment T2 has the highest mean value (23.84g), followed by T6 (23.80g), T4 (22.38g) and T5 (22.30g). This finding indicates the positive influence of EFBB and TBF on the growth of *C. annuum*.

3.2 Yield Parameters

Number of fruits per plant

Table 2 shows the result for yield parameters. Treatment T1 has the highest mean number of fruits per plant (6.95). This result could suggest that the specific conditions or factors associated with treatment T1 are favorable for fruit production. The fact that it has a significantly higher number of fruits than treatments T3 and T5 indicates that these conditions might be particularly conducive to fruit development. This supports that chemical fertilizer alone might have positively influenced fruit set and development in the *C. annuum*. Treatment T6 has a relatively high mean number of fruits per plant, similar to treatment T1. The result suggests that 25% EFB + 75% TBF could give similar results to treatment T1.

Treatment T3 has the lowest mean number of fruits per plant (2.40). This result could imply that TBF is less conducive to fruit development. It is expected that the use of TBF alone is not sufficient for the growth and yield of plants. Moreover, treatment T5, which includes 50% EFBB and 50% TBF, had the lowest mean value for the number of fruits per plant after T3. The result suggests that combining EFBB and TBF in equal proportions may not have resulted in optimal fruit production and yield conditions. It is possible that the ratio used in treatment T5 did not provide the necessary nutrient balance or growth-promoting factors required for substantial fruit development. Treatment T2 has a lower mean number of fruits per plant than treatment T1 but is still relatively higher than treatment T3 and T5. This result indicates that the nutrient content of EFBB alone may not be sufficient to meet the growth and yield requirements of the *C. annuum*. According to Adu (2022), combining EFBB with other materials, such as mineral fertilizers, will increase crop growth and yield by 16.4%.

Weight of fruit per plant

Treatment T6 has the highest mean fruit weight per plant (97.95g), as shown in Table 2. This indicates that treatment T6 is highly conducive to promoting larger fruit development. The significant difference between treatment T6 and T3 reinforces that treatment T6 provides more favorable conditions for achieving heavier fruits. The combination of EFBB and TBF in this ratio could enhance fruit growth and increase the yield of *C. annuum*. EFBB may have improved soil fertility, nutrient availability, and water retention, promoting fruit development and weight (Abdulrahman et al., 2016). Conversely, TBF could have enhanced nutrient solubilization and uptake, improving fruit quality and yield. Interestingly, treatment T3, which consisted of 100% TBF, had the lowest mean value for fruit weight per plant (49.44g), suggesting that TBF alone might not provide sufficient nutrients for growth-promoting factors for optimal fruit development. This finding aligns with expectations that TBF may work more effectively with other nutrient sources, such as organic fertilizers (Islam et al., 2023).

Number of Harvests per Cycle

Treatment T6 showed the highest mean number of harvests per cycle (2.90), significantly different from treatments T2, T3, and T5. The result suggests that the combination of 25% EFBB and 75% TBF creates suitable conditions for achieving a high number of harvests within the same cycle. Treatment T1 showed a moderate mean number of harvests per cycle (2.45). The result suggests that the conditions provided by chemical fertilizer are suitable for achieving a reasonable number of harvests within the same cycle. However, it is important to note that treatment T1 is not significantly different from treatments T4 and T6, which indicates that the combination of EFBB and TBF

(T4 and T6) can provide similarly favorable conditions for multiple harvests. Treatments T2 and T3 displayed a lower mean number of harvests per cycle (1.50 and 1.30, respectively), which suggests that using 100% EFBB and 100% TBF as the sole amendment may not provide suitable conditions for frequent harvesting within the same cycle.

Table 2. Effect of EFBB and TBF on the growth and yield performance

Treatment	Growth				Yield		
	Plant Height (cm)	Stem Diameter (cm)	Chlorophyll Content	Total Dried Plant Biomass(g)	Number of Fruits per plant	Fruit Weight per plant (g)	Number of harvests per cycle
T1	51.72 [a]	0.87 [ab]	65.54 [a]	18.10 [ab]	6.95 [a]	86.50 [ab]	2.45 [ab]
T2	51.75 [a]	0.86 [ab]	63.46 [a]	23.84 [a]	3.65 [ab]	50.91 [bc]	1.50 [bc]
T3	47.19 [a]	0.85 [ab]	62.58 [a]	17.63 [b]	2.40 [b]	34.61 [c]	1.30 [c]
T4	48.73 [a]	0.86 [ab]	65.57 [a]	22.38 [ab]	4.25 [ab]	63.06 [abc]	2.05 [abc]
T5	48.42 [a]	0.87 [ab]	64.02 [a]	22.30 [ab]	2.50 [b]	42.28 [bc]	1.35 [c]
T6	52.52 [a]	0.91 [a]	64.72 [a]	23.80 [a]	6.10 [ab]	97.95 [a]	2.90 [a]

Remarks: Means with the same letter at a given parameter are not significantly different according to Duncan's Multiple Range Test (DMRT) at $\alpha = 0.05$; values are the means of four replicates. T1= Control (Chemical Fertilizer), T2= 100% EFBB, T3= 100% TBF, T4= 75% EFBB + 25% TBF, T5= 50% EFBB + 50% TBF, T6= 25% EFBB + 75% TBF.

3.3 Soil and Plant Analysis

Soil pH and EC

Table 3 shows the effect of EFBB and TBF on soil. There is a significant difference between T0, T1, and other treatments. This suggests that the EFBB does raise the soil pH, which impacts the nutrient availability to the plants. T1 has the highest mean EC and has a significant difference from other treatments but not significant to the T0. Table 4 shows a relationship between soil pH and EC because pH affects the solubility of salts in the soil, which affects EC. As pH increases, the solubility of salts in the soil decreases, which leads to a decrease in EC. Conversely, as pH decreases, the solubility of salts in the soil increases, which leads to an increase in EC (Jeyaseeli et al., 2021).

Soil N, P, and K

In treatments with relatively low N content, such as T6 (0.27%), the *C. annuum* in this treatment may have already taken up a significant amount of N from the soil for their growth and development. As a result, the soil's N content appears lower (Xianliang et al., 2022). This aligns with the growth and yield of T6. Conversely, treatments with higher N content, such as T2 (0.42%) and T5 (0.47%), could suggest that the *C. annuum* has not yet absorbed as much N from the soil, leading to a relatively higher N concentration remaining in the soil. The higher nutrient values in treatments T3, T4, T5, and T6 can be attributed to the synergistic effects of EFBB and TBF. EFBB, known for its high surface area and porosity, acts as a nutrient reservoir, preventing their leaching and making them readily available for plant uptake (Ghorbani et al., 2019). TBF further enhances this, facilitating nutrient solubilization and enhancing plant nutrient absorption. The lower nutrient content observed in T1 can be attributed to the sole reliance on chemical fertilizers. While chemical fertilizers can provide quick nutrient availability, they often lack the organic matter and microbial activity necessary for sustained nutrient retention and plant health. Applying chemical fertilizers might lead to potential nutrient leaching, where nutrients are washed away from the root zone due to excessive watering or rainfall, resulting in reduced soil nutrient content.

Table 3. Effect of EFBB and TBF on soil

Treatment	Soil pH	Soil EC (dS/m)	N (%)	P (%)	K (%)
T0	6.50^{d}	2.33^{a}	0.11^{e}	0.52^{d}	6.74^{a}
T1	6.70^{cd}	2.55^{a}	0.03^{f}	1.35^{b}	2.48^{d}
T2	7.13^{ab}	1.57^{ab}	0.42^{a}	1.13^{c}	4.76^{b}
T3	6.90^{bc}	1.88^{ab}	0.33^{b}	1.35^{b}	2.31^{d}
T4	7.23^{a}	1.22^{b}	0.20^{d}	1.05^{c}	6.32^{a}
T5	7.33^{a}	1.33^{b}	0.47^{a}	1.13^{c}	5.01^{b}
T6	7.33^{a}	1.55^{c}	0.27^{c}	1.53^{a}	4.01^{c}

Note: Means with the same letter at a given parameter are not significantly different according to Duncan's Multiple Range Test (DMRT) at $\alpha = 0.05$; values are the means of three replicates. T0= Before treatment, T1= Control (Chemical Fertilizer), T2= 100% EFBB, T3= 100% TBF, T4= 75% EFBB + 25% TBF, T5= 50% EFBB + 50% TBF, T6= 25% EFBB + 75% TBF.

Plant Nutrient Analysis

Based on the findings in Table 4, there are no significant differences in the N and P2O5 content of *C. annuum*. However, *C. annuum*, treated with a combination of 25% EFB and 75% TBF (T6), exhibited the highest levels of N (3.89%) and P2O5 (1.04%). This indicates that the uptake of nutrients by the plants was significantly enhanced, leading to improved plant growth. EFBB has a high surface area and porosity, which can adsorb and retain nutrients in the soil. Besides, the EFBB can improve soil structure, creating a favorable environment for root growth (Chia et al., 2022). According to Marra (2021), TBF can solubilize and liberate nutrients from organic matter, thereby augmenting their accessibility to plants. Consequently, the plant can access and utilize nutrients more efficiently, improving overall growth and development. The concentration of K2O was higher in the roots of plants treated with 100% TBF (T3), which is 5.39%. TBF can solubilize potassium (K) from the soil and release it in a form accessible to plant roots (Nosheen et al., 2021). As a result, the roots of the plants treated with TBF could take up more K, leading to higher levels of K2O in the root tissues.

Table 4. Plant Nutrient Content

TREATMENT	N (%)	P2O5(%)	K2O (%)
T1	3.60 [a]	0.61 [a]	3.23 [b]
T2	3.36 [a]	0.67 [a]	4.39 [ab]
T3	3.51 [a]	0.59 [a]	5.39 [a]
T4	3.64 [a]	0.66 [a]	3.04 [b]
T5	3.24 [a]	0.95 [a]	4.20 [ab]
T6	3.89 [a]	1.04 [a]	4.85 [ab]

Remarks: Means with the same letter at a given parameter are not significantly different according to Duncan's Multiple Range Test (DMRT) at $\alpha = 0.05$; values are the means of four replicates. T1= Control (Chemical Fertilizer), T2= 100% EFBB, T3= 100% TBF, T4= 75% EFBB + 25% TBF, T5= 50% EFBB + 50% TBF, T6= 25% EFBB +75% TBF

The combination of EFBB and TBF provides not only short-term benefits for crop growth but also long-term improvements in soil health and ecosystem balance. Over time, EFBB helps increase the amount of organic carbon in the soil because of its stable structure, which breaks down very slowly (Adu et al., 2022). This stability allows the soil to store more carbon, reduce greenhouse gas emissions, and maintain good physical qualities such as porosity, structure, and water-holding ability. These properties are especially important for soils, where organic matter usually decomposes quickly and nutrients are easily lost.

At the same time, *Trichoderma*-based biofertilizers enrich the soil around plant roots with helpful microorganisms that can survive for many growing seasons (Pang et al., 2017). These beneficial microbes help release nutrients from organic matter, improve root development, and protect plants from harmful pathogens by competing with them and producing natural antifungal compounds. Over continued use, *Trichoderma* can build a more balanced and resilient soil microbial community, improving the biological fertility of the soil (Woo et al., 2023).

Together, the chemical and physical stability of EFBB and the biological activity of TBF create a healthy, self-sustaining soil system that supports efficient nutrient cycling. The improved cation exchange capacity and reduced nutrient loss provided by EFBB ensure that important nutrients stay available for plant uptake. Overall, using EFBB and TBF together is a promising long-term soil management approach that can help prevent soil degradation, reduce the need for chemical fertilizers, and promote sustainable farming. Future research should focus on field trials to measure how long these benefits last, especially regarding changes in soil microbes, enzyme activity, and carbon storage capacity.

4 Conclusion

In conclusion, the application of EFBB in combination with TBF has positively affected the agronomic performance of *C. annuum*. Findings suggest that the synergistic effects of EFBB and TBF have the potential to serve as an alternative organic fertilizer, reducing the dependency on chemical fertilizers in agricultural systems. However, it is crucial to consider specific soil conditions, environmental factors, and crop requirements to maximize the benefits of this combination. Further research is needed to explore the effects of EFBB and TBF on other crops, optimize the combination rates, and investigate the long-term effects on soil fertility, crop productivity, and environmental sustainability. This will provide a more comprehensive understanding of the interactions between these organic inputs and their potential applications in diverse agricultural systems. This research highlights the transformative potential of converting agricultural waste into biochar to enhance tropical soil biodiversity and lock away atmospheric carbon. While promising, its success hinges on standardized production methods, long-term field validation, and integrated policies that support its adoption at plantation scale.

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