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Optimization bio-organic fertilizer production from watermelon rind with *Trichoderma spp.* for improved *Brassica juncea* growth

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ABSTRACT

This study evaluates the effectiveness of Trichoderma spp. in decomposing watermelon rind to produce bio-organic fertilizer. Response Surface Methodology (RSM) with Central Composite Design (CCD) was employed to optimize the cultivation conditions for Brassica juncea using the composted fertilizer. Results demonstrated that Trichoderma spp. significantly accelerated decomposition and improved fertilizer quality. Among the tested parameters, composting duration and fertilization rate significantly influenced plant growth, particularly leaf length and width, while microbial inoculant concentration had no notable effect. Microbial analysis confirmed that Coliform, E. coli, and Salmonella levels were below regulatory limits (ND 108/2017/ND-CP), with none detected in fertilizers produced with Trichoderma spp.. RSM optimization identified the optimal composting conditions as 18 days, 4 wt.% Trichoderma spp., and 50 v/v% fertilizer volume fraction. A high-accuracy mathematical model was developed to predict plant growth responses, effectively forecasting the impact of composting parameters on crop development. These findings highlight the potential of Trichoderma-based bio-organic fertilizers in sustainable agriculture.

1. INTRODUCTION

Bio-organic fertilizers are environmentally friendly alternatives that provide essential nutrients to plants and help prevent soil degradation (Mahish et al., 2024). Recently, bio-organic fertilizers have emerged as a viable solution to manage agricultural by-products, particularly in the agricultural regions of Vietnam. By converting substantial amounts of agricultural by-products into green fertilizers, the production and use of bio-organic fertilizers offer a dual benefit for environmental sustainability (Karthiga et al., 2022; Mahish et al., 2024; Zhang et al., 2024).

Watermelon is one of the most widely consumed fruits in Southeast Vietnam, with over 87.9 tons consumed daily in Ho Chi Minh City as of 2021. In Vietnam, watermelon is used both as a fresh fruit and as an ingredient in juice, candy, and cake production (Dung et al., 2011). This extensive use results in a significant quantity of watermelon rinds being discarded into the environment daily (Nguyen & Tran, 2008). Comprising about one-third of the total fruit weight, watermelon rinds are rich in moisture, protein, fiber, sodium, potassium, phosphorus, and various metallic ions (Feizy et al., 2020). Due to their high organic content, watermelon rinds have the potential to be repurposed as a valuable organic material for producing bio-organic fertilizers, especially in the Mekong Delta region. In addition to organic materials, microbes play a crucial role in the production of bio-organic fertilizers. The choice of bacteria for composting organic compounds is influenced by environmental conditions. *Trichoderma spp*., a genus of fungi in the family Hypocreaceae, is known for its rapid decomposition of organic matter in high-temperature and high-moisture environments (Harman et al., 2004), making it well-suited for the tropical monsoon climate of Can Tho City.

While research on watermelon has explored various applications, including adsorption, medical uses, material science, and food science, recent studies have also highlighted the potential of watermelon rind extracts to enhance the physicochemical properties of packaging films and binary-composite materials (Molina et al., 2023; Wang et al., 2023; Gathiru et al., 2024; Kouniba et al., 2024; Vijayan et al., 2024). However, research on the use of watermelon rind for fertilizer production remains limited, despite the promising potential for creating high-quality bio-organic fertilizers from this by-product. The urgency of utilizing agricultural waste and by-products for fertilizer production is underscored by the significant environmental impact of excess by-products (Ling Wen Xia et al., 2024; Phiri et al., 2024; Wang et al., 2024). Over time, research has evolved from using natural decomposition agents such as pig manure and human excreta to employing isolated microorganisms like Bacillus spp., Rhizobacteria, and biochar (Babcock-Jackson et al., 2023; Upadhyay et al., 2024). More recently, the use of mixed microbial cultures in composting has shown promise due to their efficiency and ability to suppress harmful bacteria (Gagliardi et al., 2024; Xu et al., 2024).

This study aims to evaluate the effectiveness of using *Trichoderma spp*. to decompose watermelon rind and produce bio-organic fertilizer. Response Surface Methodology (RSM) with Central Composite Design (CCD) was employed to optimize the conditions for growing *Brassica juncea* using the bio-organic fertilizer derived from watermelon rind composted by *Trichoderma spp*.

2. MATERIALS AND METHOD

2.1. Materials

Watermelon rinds were colleted form from juice shops in Can Tho City. *Trichoderma sp* were obtained from Biocont Vietnam Company.

2.2. Bio-organic composting design

After collecting, watermelon rinds were cut into 2×2 cm pieces and weighed to accurately determine the initial dry weight. Then, *Trichoderma sp* fungus was mixed carefully with watermelon rinds at different ratios of and placed in designed containers with a capacity of 10 L. Tighten the solids in the container then close the lid. The liquid created during the composting process was collected through the liquid valve at the bottom of the containers and sprinkled back on the solids.

Figure 1. Container design for composting

The experiment was designed in two containers for further comparison (Figure 1). Container 1 is naturally decomposed watermelon rinds without using microbial inoculants. Container 2 is decomposed watermelon rinds by commercial *Trichoderma sp* (10^8 CFU/g) microbial inoculants. The environmental conditions were within the temperature range of 28-32 ºC.

Decomposition rate of watermelon rind: The biological efficiency of watermelon rind decomposition was assessed by measuring dry weight loss during the composting process. The mass is weighed directly using a digital scale.

The composting of watermelon rinds was conducted over a 21-day period. Throughout this process, daily measurements of temperature, pH, volume loss, dry weight, and CO_2 concentration were obtained using a sensor (MOTHERTOOL GCH-2018). Upon completion of the composting period, the total organic matter and total nitrogen content were quantified in accordance with Vietnamese standards.

Table 1. Factor levels of independent variables for the crop fertilization process with 3 factors

Independent factors	- α (Axial)	Low level	Medium level	High level	+α (Axial)
	–1.68	**–1**	**0**	**+1**	**+1.68**
Composting time (day) X_1	–1.68	–1	0	+1	+1.68
Microbial inoculant content (wt.%) X_2	2.23	7	14	21	25.77
Fertilizer volume fraction (v/v %) X_3	1.32	2	3	4	4.68

The chemical equations for the organic matter degradation process have been added to the revised manuscript. The products formed during incubation are presented as follows. In the aerobic biodegradation process, bacteria decompose organic matter according to the following reactions:

$$\text{Organic matter} + O_2 + \text{microorganisms} \rightarrow CO_2 + H_2O + Q \quad (1)$$

$$\text{Organic matter} + O_2 + \text{microorganisms} \rightarrow CO_2 + H_2O + \text{new cells} \quad (2)$$

$$\text{Cells} + O_2 \rightarrow CO_2 + H_2O + NH_3 \quad (3)$$

The primary components of watermelon rind waste are cellulose, starch, and protein. In nature, bacteria and fungi can break down cellulose, starch, and protein into basic inorganic compounds such as CO_2, H_2O, and NH_3. Additionally, the intermediate products provide a carbon source. Nitrogen-fixing bacteria and carbon sources are suitable for use in bio-organic fertilizers.

2.3. Chemical and biological properties of organic fertilizer

After decomposition, the organic fertilizer was analyzed for its chemical and biological properties. Total organic carbon was quantified using the Walkley-Black method (TCVN 9294:2012), while nitrate content was determined by the Kjeldahl method (TCVN 10682:2015). Salmonella presence was detected using Salmonella Shigella Agar Medium, and coliforms and E. coli were quantified using the Most Probable Number (MPN) method (Nguyen et al., 2013).

2.4. Optimization of fertilization parameters for Brassica Juncea cultivation with *Trichoderma spp*. bio-organic fertilizer

RSM combined with CCD was utilized to determine the optimal fertilization parameters for maximizing crop productivity. The study focused on three independent variables: composting time (X_1), microbial inoculant content (X_2), and fertilizer volume fraction (X_3), with the goal of optimizing leaf length (Y_1) and leaf width (Y_2). By conducting experiments with successive variation in each parameter, we selected the central values and their deviations at ±1. The independent variables were coded as ±1 for factorial points, 0 for the center points, and ±α for the axial points ($\alpha = (2^k)^{1/4} = 1.68$). The CCD included a full factorial design (2^3) with six replicates at the center points, resulting in a total of 20 experiments (n = 3) with three variables. The experimental design comprised 8 factorial points, 6 axial points, and 6 center points. In Central Composite Design (CCD), center points are used to create a balance between the linear and quadratic factors. Typically, 3 to 6 center points are recommended. The number of center points is usually selected based on the objectives of the experiment and the required accuracy in estimating variance. Therefore, we selected 6 center points. The coded levels of the independent variables used in the RSM design for bio-organic fertilizer with *Trichoderma spp.* are detailed in Table 1. Statistical analysis of the model was performed using ANOVA.

3. RESULTS AND DISCUSSION

3.1. Results of bio-organic composting

3.1.1. Decomposition rate of watermelon rind by microbial inoculants

Figure 2 illustrates the decomposition rates across two different treatments. Watermelon rinds subjected to natural decomposition exhibited a 43% decomposition rate 5 days after composting, which was the lowest among the treatments. In contrast, watermelon rinds inoculated with *Trichoderma spp.* achieved a 68% decomposition rate at the same time point. This indicates that the presence of microbial inoculants significantly enhances the rate of organic matter decomposition. Microbial activity was notably high in the initial days of the composting process. Consequently, the decomposition rate increased rapidly from day 1 to day 7. However, from day 7 to day 14, the rate of decomposition with microbial inoculants only increased by approximately 6–8%. As shown in Figure 2, watermelon rinds inoculated with *Trichoderma spp.* were mostly decomposed after 10 days of composting, whereas natural decomposition required a longer period of 3 to 4 weeks.

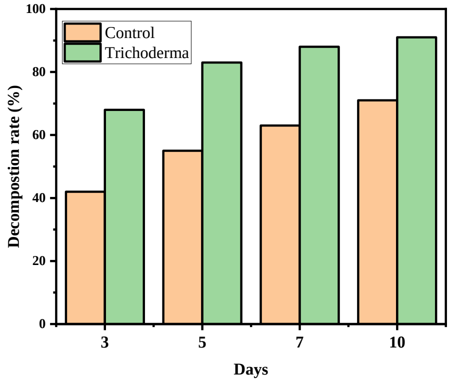

Figure 2. Decomposition rate of watermelon rind without and with microbial inoculants (a 4 wt.% *Trichoderma spp.* content)

3.1.2. Changes in pH during the composting of watermelon rinds

pH values were recorded daily using a pH meter throughout the composting process of watermelon rinds. The results are presented in Figure 3. A pH range of 7.0 to 8.0 is optimal for short-term aerobic digestion of waste sludge (Zhou et al., 2019). However, achieving this pH range in the composting of watermelon rinds is challenging due to their high content of weak organic acids. Initially, watermelon rinds had a pH of approximately 4.2. Adding *Trichoderma spp.* raised the initial pH to 4.35, attributed to the alkalinity of the microorganisms. During the early stages of composting, pH values decreased due to the production of organic acids (Wong et al., 2017). As composting progressed, pH values increased significantly, primarily due to the rising moisture content in the watermelon rinds. At the end of the composting process, the pH values for both treatments without microbial inoculants and with *Trichoderma spp.* ranged from 4.7 to 4.9. This can be explained that the weak organic acids in the watermelon rind had already decomposed, rendering the impact of *Trichoderma spp.* on the pH of the process insignificant. These pH values meet the standards outlined in Regulation 108/2017/NĐ-CP for organic fertilizers.

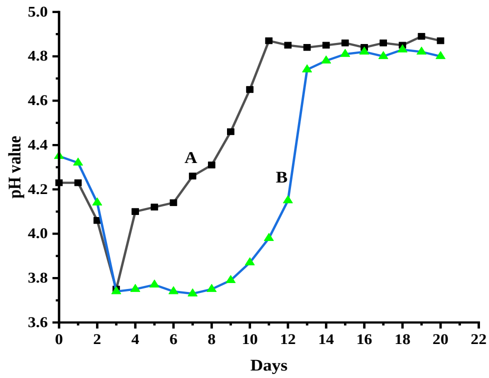

Figure 3. Changes in pH during the composting of watermelon rinds: (A) without microbial inoculants, (B) with *Trichoderma spp* (a 4 wt.% Trichoderma spp. content).

3.1.3. Temperature

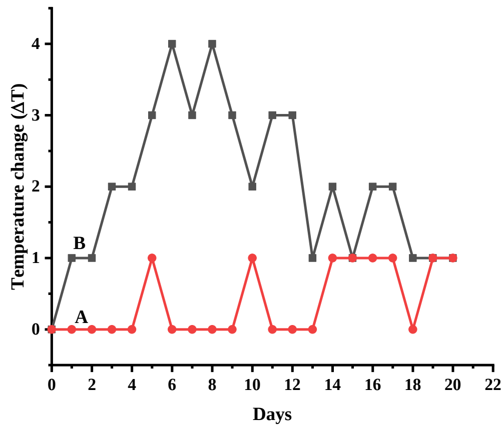

Figure 4. Changes in temperature during the composting of watermelon rinds: (A) without microbial inoculants, (B) with *Trichoderma spp* (using 4 wt.% *Trichoderma spp* content).

The temperature of the composting process is an important parameter used to indicate the microorganisms' activities. Changes in temperature during the composting of watermelon rinds are shown in Figure 4. The initial temperature of all conditions is about 28 °C (ΔT = 4 °C). For composting with *Trichoderma sp*, the temperature rapidly increased to maximum (ΔT = 4 °C) after 6 days. This could be explained by the increase in microbial population and higher degradation rate during the compost (Czekała et al., 2016). The decrease in temperature change from 4 °C to 0 °C (6–15 days) was due to insufficient oxygen supply for microorganisms, which led to low decomposition rate. After day 15, the temperature dropped to the ambient by the depletion of readily degradable organic compounds in the compost mixture. The temperature of the natural composting sample remained stable during the composting period indicates that there was little microorganisms' activity. The slight increase at day 13 corresponds with the CO_2 emission evolution trend, which confirmed the increase of the microbial population.

Table 2. Actual value of independent factors with responses for fertilizer with *Trichoderma spp*

STD	Run	Composting time (day) X_1	Microbial inoculant content (wt.%) X_2	Fertilizer volume fraction (v/v%) X_3	Leaf length (mm) Y_1	Leaf width (mm) Y_2
1	5	7	2	25	94	46
2	9	21	2	25	102	51
3	14	7	4	25	92	61
4	7	21	4	25	95	54
5	2	7	2	50	94	65
6	4	21	2	50	101	57
7	12	7	4	50	98	60
8	6	21	4	50	110	58
9	16	3	3	37.5	91	64
10	11	25	3	37.5	107	50
11	3	14	1.4	37.5	96	57
12	18	14	4.6	37.5	103	53
13	8	14	3	16.5	102	55
14	13	14	3	58.5	109	52
15	17	14	3	37.5	101	68
16	10	14	3	37.5	104	55
17	1	14	3	37.5	102	50
18	19	14	3	37.5	99	50
19	15	14	3	37.5	102	50
20	20	14	3	37.5	105	48

3.1.4. CO_2 emission

CO_2 emissions were monitored daily using a CO_2 detector throughout the composting process of watermelon rinds. As shown in Figure 5, CO_2 evolution was used as an indicator of composting efficiency. The maximum CO_2 emissions recorded were 94 ppm for condition A and 977 ppm for condition B on days 15 and 7, respectively. These results suggest that the inclusion of *Trichoderma spp.* significantly enhances the efficiency of composting watermelon rinds. In contrast, natural composting conditions exhibited lower CO_2 emissions, likely due to a lower microbial density. The observed increase in CO_2 emissions on days 11 and 12 may be attributed to the introduction of additional microbes from the environment. Overall, the CO_2 emission trends observed across all conditions are consistent with the temperature patterns shown in Figure 5, supporting the effectiveness of microbial inoculants in improving the composting efficiency of watermelon rinds.

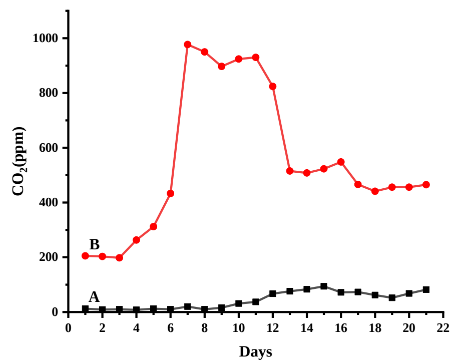

Figure 5. CO_2 emission trends during watermelon rind composting: (A) without microbial inoculants, (B) with *Trichoderma spp*.

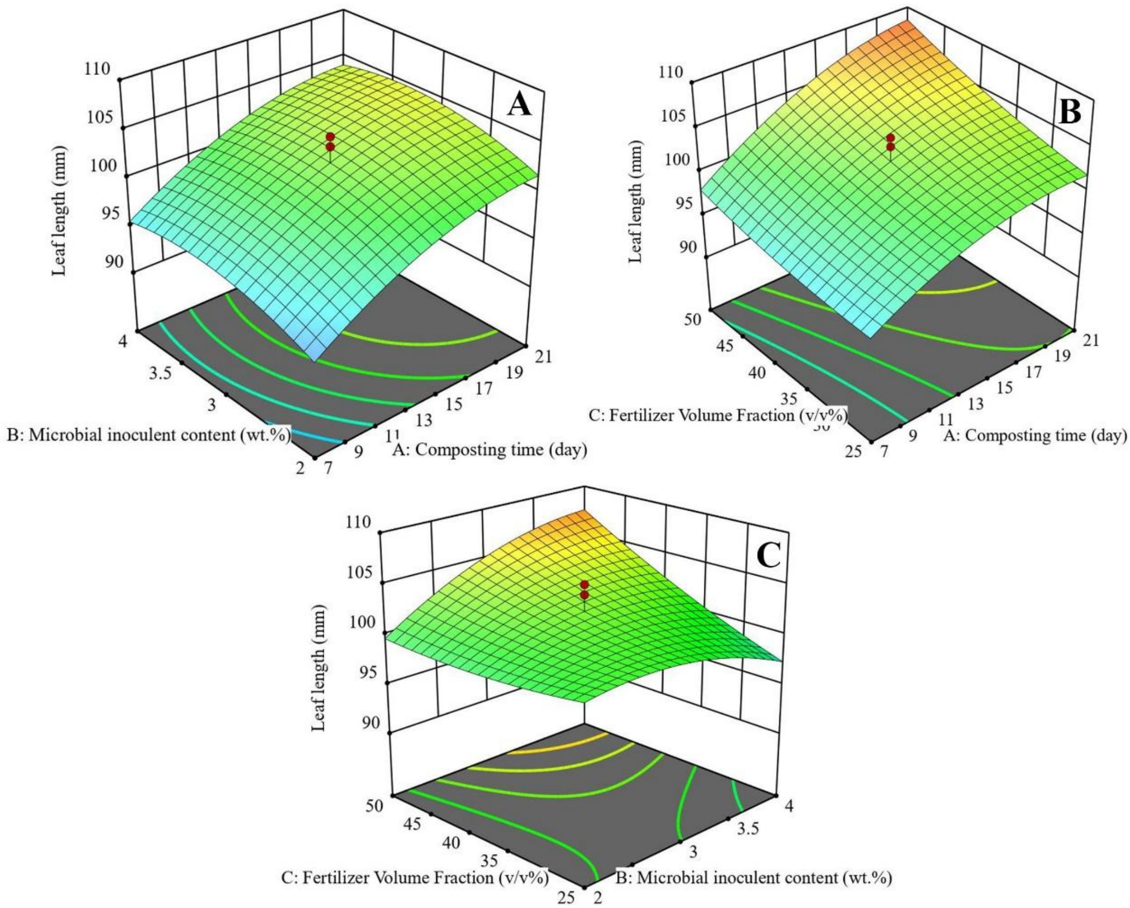

Figure 6. 3D response surface plot showing the relationship between independent variables of fertilizer with *Trichoderma spp.* on leaf length: (A) composting time and microbial content; (B) composting time and fertilizer volume fraction; (C) microbial content and fertilizer volume fraction

3.2. Characterization of bio-organic fertilizer rom watermelon rinds

3.2.1. C/N ratio

The carbon-to-nitrogen (C/N) ratio of a fertilizer indicates the rate of decomposition and nutrient release when the organic material is incorporated into the soil. A high C/N ratio signifies insufficient nitrogen, which hinders microbial activity and, consequently, reduces composting efficiency. Table 3 shows that the C/N ratio of the bio-organic fertilizer composted without microbial inoculants is higher (approximately 13.6) compared to those with microbial inoculants (approximately 6.3–7.5). This demonstrates that microbial inoculants significantly enhance both the decomposition efficiency and the quality of the bio-organic fertilizer produced from watermelon rinds. The sample with *Trichoderma spp.* exhibited the highest C/N ratio, which aligns with the CO_2 emission and temperature changes observed. The C/N ratio of bio-organic fertilizers derived from watermelon rinds using microbial inoculants is comparable to that of bio-organic fertilizers from banana fusarium (4.5–7.1) but is still lower than the optimal C/N ratio for composting (Shen et al., 2013).

Table 3. Results of total carbon and total nitrogen contents in bio-organic fertilizers

Bio-organic fertilizer	Total organic carbon content (%)	Total nitrogen content (%)	C/N ratio
Without microbial inoculants	0.380	0.028	13.571
Trichoderma spp.	0.545	0.073	7.466

3.2.2. Harmful microorganism population

According to ND 108/2017/ND-CP, bio-organic fertilizers must have limited levels of harmful bacteria, including E. coli, Salmonella, and Coliform, to ensure they are safe for use. Table 4 presents the results for harmful bacteria in bio-organic fertilizers. The data indicate that all bio-organic fertilizers composted from watermelon rinds meet the safety limits for harmful bacteria and are thus suitable for soil application. The fertilizers composted with *Trichoderma spp.* do not contain harmful bacteria, which can be attributed to the antimicrobial properties of *Trichoderma spp. (Tyśkiewicz et al., 2022)*.

Table 4. Results of harmful bacteria contents in bio-organic fertilizers

Bio-organic fertilizer sample	*E.coli* (MNP/mL)	*Coliform* (MNP/mL)	*Salmonella* (cfu/mL)
Without microbial inoculants	1.9	1.9	ND
Trichoderma spp.	ND	ND	ND
Limitation*	<3000	$<1.10\times10^3$	ND

*ND: not detected; *ND 108/2017/ND-CP*

3.3. Optimization of bio-organic fertilizer with *Trichoderma spp*. on vegetable growth

3.3.1. Experimental results investigating the influence of independent factors on responses.

The results of the experiment, designed as shown in Table 1, are summarized in Table 4. A probabilistic model was developed using regression analysis to describe the relationship between dependent and independent variables. RSM combined with CCD was employed to optimize the process, with experiments detailed in Table 4. The study investigated the effects of composting time (X_1), microbial inoculant content (X_2), and fertilizer volume fraction (X_3) on leaf length (Y_1) and leaf width (Y_2).

3.3.2. Optimization of bio-organic fertilizer with Trichoderma spp. on leaf length

3D response surface plots, generated by the models, were utilized to illustrate the effects of composting duration, microbial inoculant content, and fertilizer volume fraction on the growth of *Brassica juncea* at day 14. These graphs are presented in Figure 6.

The 3D response surface analysis indicates that composting duration significantly impacts the growth of *Brassica juncea*, more so than fertilizer volume fraction. The optimal composting duration for maximizing bio-organic fertilizer efficacy is between 15 and 17 days, during which there is a marked increase in leaf length. Beyond 17 days, the increase in leaf length is minimal. Fertilizer volume fraction has a minor effect on plant growth, with an optimal volume fraction of 40%. Microbial inoculant content does not significantly influence leaf length.

The significance of these factors was assessed using ANOVA (Table 5). The results revealed that composting duration and fertilizer volume fraction significantly affect plant growth ($p < 0.005$), while microbial inoculant content does not ($p > 0.005$). Additionally, an interaction between microbial inoculant content and fertilizer volume fraction was observed. The second-order polynomial equation derived from these results is:

$$Y_1 = 102.36 + 4.26X_1 + 1.16X_2 + 2.33X_3 + 0.000X_1X_2 + 1X_1X_3 + 2{,}75X_2X_3 - 2.01X_1^2 - 1.72X_2^2 + 0.6131X_3^2$$

Multivariate regression analysis with a second-order polynomial equation was carried out on data to yield an equation that was used to optimize the product responses.

Leaf length = 96.08 + 1.33 × (composting time) + 3.25 × (microbial inoculant content) – 0.93 × (fertilizer volume fraction) + 0.000 × (composting time × microbial inoculant content) + 0.011 × (composting time × fertilizer volume fraction) + 0.22× (microbial inoculant content × fertilizer volume fraction) – 0.04 × (composting time)^2 – 1.72 × (microbial inoculant content)^2 + 0.004 × (fertilizer volume fraction)^2

3.3.3. Effects of bio-organic fertilizer with Trichoderma spp. on leaf width

The influence of composting duration, microbial inoculant content, and fertilizer volume fraction on the leaf width of *Brassica juncea* at day 14 is illustrated using 3D response surface plots generated by the models. These plots are shown in Figure 7.

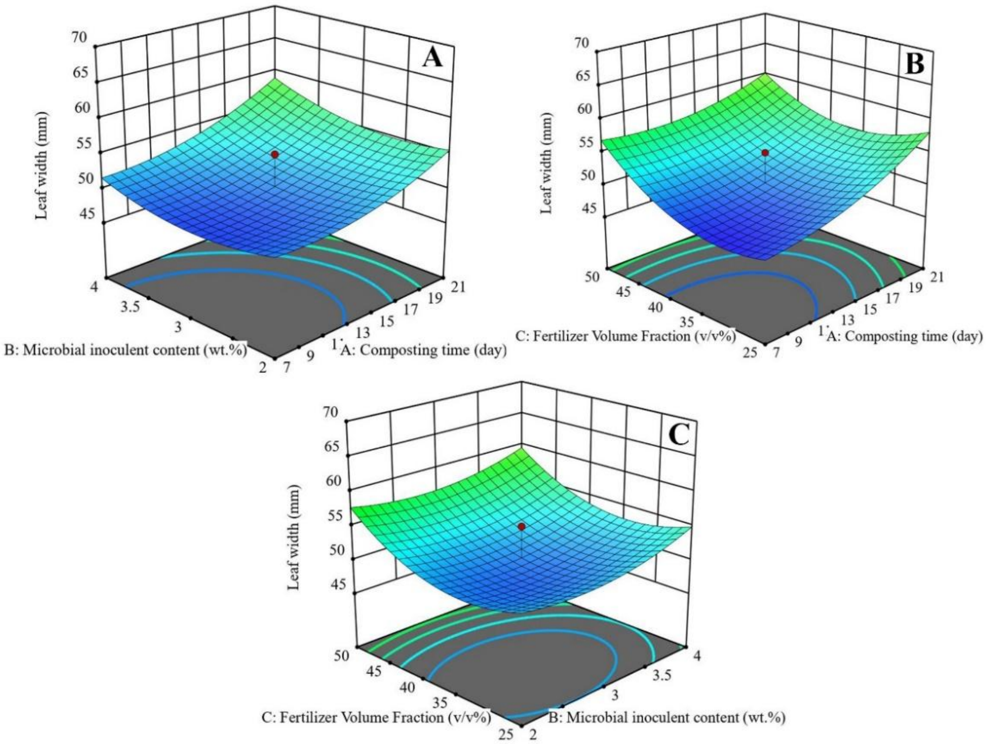

Figure 7. 3D response surface plot showing the relationship between independent variables of fertilizer with *Trichoderma spp.* on leaf width: (A) composting time and microbial content; (B) composting time and fertilizer volume fraction; (C) microbial content and fertilizer volume fraction

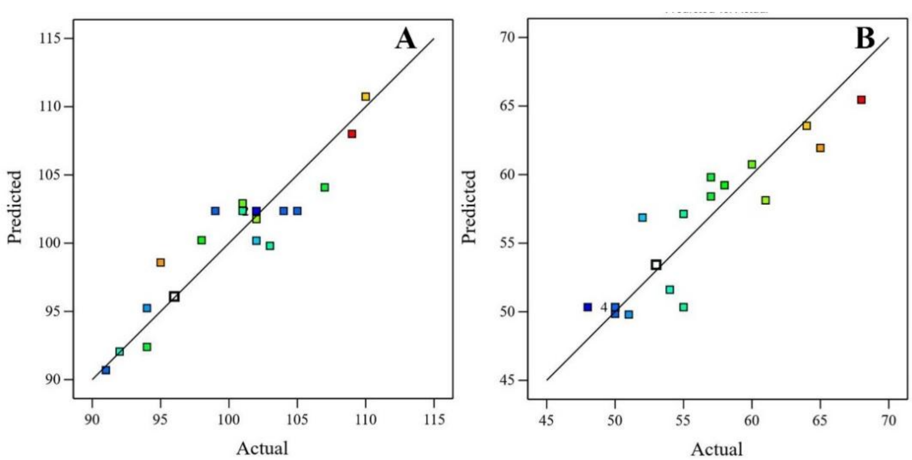

Figure 8. Comparison of predicted and experimental values for leaf length (A) and leaf width (B) in plants treated with *Trichoderma spp*. bio-organic fertilizer

The 3D response surface plots demonstrate that both composting duration and fertilizer volume fraction significantly affect leaf width, while microbial inoculant content does not. However, the impact of composting time and fertilizer volume fraction on leaf width is less pronounced compared to their effect on leaf length. The optimal conditions for leaf width are a composting duration of 17 days and a fertilizer volume fraction of 45%.

Table 5. ANOVA test of the relationship between independent variables (composting time, microbial inoculant content and fertilizer volume fraction) of fertilizer with *Trichoderma spp* on leaf length

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	486.27	9	54.03	7.47	0.0021	significant
A-Composting time	235.01	1	235.01	32.51	0.0002	significant
B-Microbial content	17.61	1	17.61	2.44	0.1496	not significant
C-Fertilizer volume	73.93	1	73.93	10.23	0.0095	significant
AB	0.0000	1	0.0000	0.0000	1.0000	not significant
AC	8.00	1	8.00	1.11	0.3175	not significant
BC	60.50	1	60.50	8.37	0.0160	significant
A^2	47.62	1	47.62	6.59	0.0280	significant
B^2	36.90	1	36.90	5.10	0.0474	significant
C^2	5.45	1	5.45	0.7535	0.4057	not significant
Residual	72.28	10	7.23			
Lack of Fit	49.45	5	9.89	2.17	0.2082	not significant
Pure Error	22.83	5	4.57			
Cor Total	558.55	19				

Table 6. ANOVA test of the relationship between independent variables (composting time, microbial inoculant content and fertilizer volume fraction) of fertilizer with *Trichoderma spp* on leaf width

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	529.34	9	58.82	5.91	0.0052	significant
A-Composting time	129.92	1	129.92	13.06	0.0047	significant
B-Microbial content	17.61	1	17.61	1.77	0.2129	not significant
C-Fertilizer Volume	89.16	1	89.16	8.97	0.0135	significant
AB	2.00	1	2.00	0.2011	0.6634	not significant
AC	18.00	1	18.00	1.81	0.2082	not significant
BC	0.5000	1	0.5000	0.0503	0.8271	not significant
A^2	38.98	1	38.98	3.92	0.0759	not significant
B^2	46.41	1	46.41	4.67	0.0561	significant
C^2	213.32	1	213.32	21.45	0.0009	significant
Residual	99.46	10	9.95			
Lack of Fit	71.96	5	14.39	2.62	0.1573	not significant
Pure Error	27.50	5	5.50			
Cor Total	628.80	19				

The significance of these factors was evaluated using an ANOVA test (Table 6). The results indicate that composting duration and fertilizer volume fraction have a significant effect on leaf width ($p < 0.005$), whereas microbial inoculant content does not ($p > 0.005$). The second-order polynomial equation derived from these results is:

$$Y_2 = 50.34 + 3.17X_1 + 1.16X_2 + 2.56X_3 + 0.5X_1X_2 - 1.5X_1X_3 - 0.25X_2X_3 + 1.82X_1^2 + 1.93X_2^2 + 3.84X_3^2$$

Multivariate regression analysis using this second-order polynomial equation provided the following optimized equation for leaf width:

Leaf width = 83.82 – 0.15 × (composting time) – 10.69 × (microbial inoculant content) – 1.34 × (fertilizer volume fraction) + 0.07 × (composting time × microbial inoculant content) – 0.017 × (composting time × fertilizer volume fraction) – 0.02 × (microbial inoculant content × fertilizer volume fraction) + 0.04 × (composting time)^2 + 1.93 × (microbial inoculant content)^2 + 0.025 × (fertilizer volume fraction)^2

3.3.4. Accuracy of the model

To verify the accuracy of the model compared to experiments, experiments to verify the model's suitability were conducted at several points in the plot. The actual values are compared in Table 7.

To assess the accuracy of the model, two plots comparing actual and predicted values were created based on the data from Table 7. These plots help evaluate how well the model's predictions align with experimental results. Figure 8 presents a comparative analysis of predicted and experimental leaf length (Figure 8A) and width (Figure 8B) values for Brassica juncea cultivated with *Trichoderma spp*. bio-organic fertilizer. Figure 8A illustrates the model's capacity to predict leaf length based on composting time, microbial inoculant content, and fertilizer volume fraction by comparing experimental and predicted values. Similarly, Figure 8B evaluates the model's efficacy in forecasting leaf width in relation to the same independent variables. Collectively, these plots offer a visual assessment of the model's accuracy in predicting leaf dimensions.

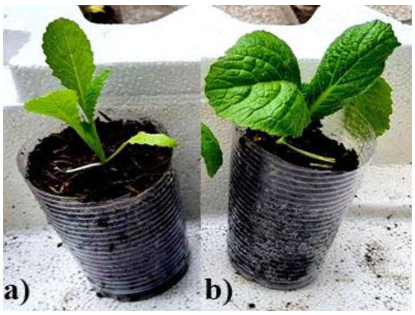

Figure 9. Images of Brassica juncea grown under: a) natural conditions; b) organic bio-fertilizer made from watermelon rind under optimal conditions

The results show that Brassica juncea grown with bio-fertilizer from watermelon rind exhibited better growth compared to those grown under natural conditions (Figure 9).

With R^2=0.87 for the leaf length model and R^2=0.8418 for the leaf width model, it can be concluded that both models demonstrate a high level of accuracy. The R^2 values indicate that the models explain 87% and 84.18% of the variance in leaf length and leaf width, respectively. Therefore, these models are effective tools for evaluating and predicting the effects of composting time, microbial inoculant content, and fertilizer volume fraction on the growth of *Brassica juncea*.

Table 7. Responses with predicted values and actual value of plant growth for bio-organic fertilizer by *Trichoderma spp.*

STD	Actual leaf length (mm)	Predicted leaf length (mm)	Actual leaf width (mm)	Predicted leaf width (mm)
1	94	95	46	50
2	102	102	51	60
3	92	92	61	52
4	95	99	54	64
5	94	92	65	60
6	101	103	57	63
7	98	100	60	60
8	110	111	58	66
9	91	91	64	50
10	107	104	50	62
11	96	96	57	55
12	103	100	53	58
13	102	100	55	58
14	109	108	52	68
15	101	102	68	52
16	104	102	55	52
17	102	102	50	52
18	99	102	50	52
19	102	102	50	52
20	105	102	48	52

4. CONCLUSIONS

This study demonstrates the successful production of bio-organic fertilizers from watermelon rinds using *Trichoderma spp*. Composting with *Trichoderma spp.* resulted in bio-organic fertilizers with a significantly lower C/N ratio (7.48) compared to those produced without microbial inoculants (13.57). The pH of the fertilizers ranged from 4.7 to 5.1. Microbial analysis confirmed that the levels of Coliform, E. coli, and Salmonella in all treatments met the Vietnamese regulatory standards of ND 108/2017/ND-CP, with none detected in the *Trichoderma spp.* composted fertilizers. RSM and CCD were employed to assess the impact of composting time, microbial inoculant content, and fertilizer volume fraction on the growth of *Brassica juncea*. The results indicate that the bio-organic fertilizers produced using *Trichoderma spp.* are highly effective, free of harmful bacteria, and beneficial for enhancing the growth of *Brassica juncea* and potentially other vegetables.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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