

# INFLUENCE OF FOLIAR ORGANIC FERTILIZER DERIVED FROM SPENT SUBSTRATE OF *CORDYCEPS MILITARIS* ON THE GROWTH AND YIELD OF BOK CHOY (*Brassica rapa* subsp. *chinensis*)

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**Abstract** – This study aimed to evaluate the potential of *Cordyceps* spent substrate-derived foliar organic fertilizer on the growth and yield of bok choy under field conditions. The experiment consisted of five treatments, including a negative control and a positive control, arranged in a randomized complete block design with three replications. The results indicated that growth parameters, including plant height, number of leaves, and canopy diameter, did not differ significantly ( $p > 0.05$ ) among treatments. However, significant differences ( $p < 0.05$ ) were observed in the average individual weight and the yield per 2 m<sup>2</sup> plot. Compared with the negative control, plot yields in treatments with the *Cordyceps* spent substrate-derived foliar organic fertilizer increased by 11.4% at the lowest dose and up to 49.0% at the highest dose. Notably, the individual weight of bok choy plants and the yield per 2 m<sup>2</sup> plot obtained from the high foliar organic fertilizer doses (20 and 40 mL L<sup>-1</sup>) were comparable to those obtained with the commercial fish fertilizer treatment. These findings suggest that *Cordyceps* spent substrate-derived foliar organic fertilizer has strong potential as a foliar biofertilizer for improving the yield performance of leafy vegetables.

**Keywords:** biofertilizer, bok choy, *Brassica rapa* subsp. *chinensis*, *Cordyceps* spent substrate, foliar organic fertilizer.

## I. INTRODUCTION

Bok choy (*Brassica rapa* subsp. *chinensis*) is a commercially significant leafy vegetable, widely cultivated in Asia and other regions worldwide for its nutritional value and culinary versatility, particularly in countries such as China, the Philippines, Malaysia, Indonesia, Thailand, as well as in North America, Australia, and Europe [1]. In Vietnam, bok choy is widely cultivated and consumed as part of the daily diet [2].

In vegetable cultivation, including bok choy, foliar organic fertilizer application is widely recognized as an efficient strategy for increasing crop productivity, as it facilitates rapid nutrient absorption and promotes plant growth [3, 4]. Mushroom cultivation by-products and other agricultural by-products, such as manure compost and plant residues, have been utilized for the production of liquid organic fertilizers [5, 6]. These foliar liquid organic fertilizers enhance nutrient uptake and plant physiological performance, thereby increasing crop yield, while exerting indirect benefits on soil biological activity in sustainable farming systems [4].

*Cordyceps* spent substrate (CSS) is a by-product of *Cordyceps militaris* production on solid media, which is mainly composed of brown rice supplemented with egg yolk, coconut water, glucose, yeast extract, potato extract, peptone, vitamins, and minerals [7]. After harvesting the fruiting bodies, the remaining solid substrate still contains high levels of nutrients [8]; therefore, it has been used as animal feed in livestock and aquaculture to enhance productivity and product quality [8, 9]. However, limited research has yet been conducted to investigate the potential use

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Received date: 24 February 2026; Revised date: 28 March 2026; Accepted date: 31 March 2026

of CSS in the production of foliar liquid organic fertilizer for sustainable agricultural cultivation.

Accordingly, the present study examined the influence of using *Cordyceps* spent substrate-derived organic fertilizer on the growth and yield of bok choy. The findings of this study highlight the potential to enhance the efficiency of recycled organic materials by improving crop productivity and supporting sustainable agricultural production.

## II. LITERATURE REVIEW

Organic fertilizer is a material derived from natural sources such as livestock manure, green manure, coffee grounds, vermicompost, spent mushroom substrates, and plant residues. In addition to providing balanced macro-, secondary-, and micronutrients, organic fertilizers also add organic matter and beneficial microorganisms to the soil [10, 11]. As a result, soil fertility is improved, the efficiency of inorganic fertilizer use is enhanced, and the toxicity of heavy metals affecting crops is reduced. Adding organic fertilizer to the soil helps increase water-holding capacity, reduce leaching, soil erosion, and nutrient loss from the soil [10–12]. Therefore, managers, scientists, and farmers are all interested in seeking organic and sustainable alternatives to traditional agricultural chemicals.

The methods of organic fertilizer application in agricultural production also vary widely; among these, liquid fertilizer extracts derived from organic matter are considered particularly effective. Vegetable farmers have long utilized organic fertilizer extracts, such as aqueous extracts of spent mushroom substrate or compost tea, to maximize the benefits of relatively small quantities of material across large cultivation areas, thereby helping to reduce production costs [13].

Spent mushroom substrates (SMS), including *Cordyceps militaris* spent substrate, are the solid residual biomass of edible/medicinal mushroom production after harvesting fruiting bodies. Hence, these materials contain high contents of organic matter, macro elements (N, P, and K), and micro elements [14]. A liquid fertilizer extract

made from SMS by mixing it with clean water and allowing it to ferment anaerobically for a certain period [15]. Bioinoculant and molasses can also be added during the extraction of the anaerobically fermented solution to enhance microbial activity and fermentation rate [5, 16]. SMS liquid extract is rich in beneficial microbes, N, P, K, and other nutrient elements, and can be used as a foliar fertilizer [5, 15]. Consequently, this foliar fertilizer has positively affected the growth and yield of crops and promoted soil biological activity [5, 15, 17]. Particularly, *Cordyceps* spent substrate (CSS) has been used in livestock and aquaculture due to its high protein content and the presence of bioactive compounds and other nutrients [8, 9]. However, increasing attention has been given to alternative uses for this by-product to enhance resource use efficiency and reduce waste in agricultural production. One promising approach is the development of organic fertilizers from *Cordyceps* by-products, which contribute to the promotion of a circular economy and add value to the production chain.

Bok choy (*Brassica rapa* subsp. *chinensis*) originated in China and is a biennial plant; however, it is commonly cultivated as an annual crop [18, 19]. Bok choy is abundant in essential nutrients, including vitamins, minerals, and dietary fiber. Bok choy has brought great benefits to human health by protecting against chronic conditions, including inflammation, cancer, Alzheimer’s disease, cardiovascular disease, and other illnesses, thanks to possessing a rich content of non-nutritive bioactive compounds such as flavonoids, total glucosinolates, anthocyanins, kaempferol, and quercetin [19].

Due to the growing interest in improving the nutritional quality and sustainable production of leafy vegetables such as bok choy, agricultural and mushroom residues are increasingly considered as bio-based materials. Previous studies have highlighted the potential of utilizing mushroom cultivation waste substrates, including *Cordyceps* by-products, for agricultural applications [8, 9]. CSS has been effectively used in livestock production and aquaculture. However, their applica-

tion in the production of foliar fertilizers for vegetable cultivation has not yet been investigated.

Therefore, this study aimed to (1) produce a foliar organic fertilizer from *Cordyceps militaris* by-products via anaerobic fermentation and (2) evaluate its effects on the growth and yield of bok choy. The findings may provide evidence that foliar fertilizer derived from these by-products can improve crop productivity while supporting more sustainable agricultural production.

### III. MATERIALS AND METHOD

The study was carried out from October to December 2025 under field conditions on sandy loam soil with a pH of 6.5 in Hung My Commune, Vinh Long Province, Vietnam.

#### A. Foliar organic fertilizer production

*Cordyceps* spent substrate (CSS) was collected from the Applied Biology Center, Tra Vinh University, Vietnam. The CSS was manually broken into small pieces and air-dried for 12 hours. The CSS was weighed and mixed directly with dechlorinated tap water at a ratio of 1.5 kg air-dried CSS to 10 L of water. All the mixture was put in a 20 L plastic container with adding 3% molasses (v/v, based on the water volume) and 1% (w/v, based on the water volume) of EMZEO bioproduct of Duc Binh Biotechnology Co., Ltd., Hanoi, Vietnam containing *Saccharomyces* sp., *Bacillus* sp., *Lactobacillus* sp., *Actinomyces* sp. (with total microbial density more than  $10^8$  CFU/g), then stirred evenly and covered airtight to ensure anaerobic condition. The mixture was fermented at room temperature (ca. 30°C) for 10 days. The anaerobic fermentation container was shaken every two days to ensure even distribution of the mixture and enhance contact between microorganisms and the fermentation substrate, thereby improving fermentation efficiency. The lid was slightly loosened for a few seconds to release accumulated gas and reduce internal pressure. After 10 days, the fermented mixture was filtered through a clean filter cloth and collected into a blue plastic 20-litre container. The filtrate was stored in a cool, well-ventilated place until use and applied as a foliar organic fertilizer.

#### B. Preparation of transplants

The bok choy TN216 seeds used in this study were from a commercial supplier (Phu Nong Seeds Co., LTD, Ho Chi Minh City, Vietnam). Seeds were initially sown on seedbeds, and seedlings at the three-true-leaf stage were transplanted into the experimental area.

#### C. Field preparation

Before transplanting, the field was plowed and leveled to prepare the soil. Ridges were then formed, each measuring 20 cm in height and 1 m in width. Furrows about 30 cm wide were established between ridges to improve drainage and support experimental management. The experimental area was then divided into plots of 2 m<sup>2</sup>. Before planting, a thin layer of straw mulch was applied to the bed surface to conserve soil moisture, reduce evaporation, and suppress weeds.

#### D. Experimental design

A single-factor experiment was conducted under field conditions to evaluate the effects of foliar organic fertilizer on the growth and yield of bok choy. The experiment was arranged in a randomized complete block design (RCBD) with five different treatments, as described below:

NC: Negative control – no fertilizer application.

PC: Positive control – commercial fish fertilizer containing 5% nitrogen (N) applied at the manufacturer's recommended dilution rate of 2 mL L<sup>-1</sup>.

CSS-FOF 1: Foliar organic fertilizer derived from the *Cordyceps militaris* spent substrate (0.25% N) applied at a dilution rate of 4 mL L<sup>-1</sup> (supplying one-tenth the N amount compared to the PC treatment).

CSS-FOF 2: Foliar organic fertilizer derived from the *Cordyceps militaris* spent substrate (0.25% N) applied at a dilution rate of 20 mL L<sup>-1</sup> (supplying half the N amount compared to the PC treatment).

CSS-FOF 3: Foliar organic fertilizer derived from the *Cordyceps militaris* spent substrate

(0.25% N) applied at a dilution rate of 40 mL L<sup>-1</sup> (supplying an equivalent amount of N to the PC treatment).

The chemical properties of the commercial fish fertilizer and *Cordyceps* spent substrate-derived foliar organic fertilizer (CSS-FOF) used in the experiment are presented in Table 1. However, the experimental treatments were designed based solely on the N content of the fertilizers, with CSS-FOF applied at N levels equivalent to one-tenth, one-half, and equal to that of the PC treatment. Treatments were replicated three times. Each replicate covered an area of 2 m<sup>2</sup> and consisted of 50 plants (with spacing among plants of 20 cm × 20 cm).

No inorganic fertilizers were applied throughout the cultivation period. Commercial liquid fish fertilizer and an organic fertilizer derived from the CSS were used as foliar sprays. The fertilizers were diluted with water according to the ratios defined for each treatment and applied at a rate of 250 mL m<sup>-2</sup>. The first application was conducted seven days after planting and subsequently repeated at 5-day intervals. Fertilization was terminated five days before harvest. Accordingly, the PC treatment corresponded to approximately 5 L ha<sup>-1</sup> of fish fertilizer, whereas CSS-FOF 1, CSS-FOF 2, and CSS-FOF 3 corresponded to approximately 10, 50, and 100 L ha<sup>-1</sup> of the organic fertilizer, respectively.

#### Crop maintenance

After planting, irrigation was applied three times daily during the early growth stage to maintain soil moisture and promote root establishment. Once the plant had grown well, watering was reduced to once every two days, applied in the early morning or late afternoon. Sprinkler irrigation was used to ensure uniform water distribution and avoid damage to the plant base.

#### Data collection

Nine of the 50 plants in each plot were randomly sampled to obtain data on plant growth and individual plant weight. All growth parameters were recorded at 7-day intervals after planting. Growth parameters, including plant height (cm), number of leaves, and canopy diameter

(cm), were measured at 7, 14, 21, and 27 days after planting. Yield was assessed at harvest (27 days after planting). The fresh weight of individual plants and the total yield per plot were recorded. Plot yield was calculated from the total weight of 50 plants harvested from each plot. Before measurement, yellow leaves and roots were carefully removed.

#### E. Data analysis

Statistical analyses were performed using SPSS software (version 22, IBM Inc.). The effects of different treatments on the growth and yield of bok choy were examined using one-way analysis of variance (ANOVA). Before conducting the ANOVA, Levene's test was applied to check the homogeneity of variances, ensuring that the variances among the treatment groups were equal. When significant differences were found, Duncan's *post hoc* test at  $p < 0.05$  was applied to identify differences among individual treatments.

## IV. RESULTS AND DISCUSSION

### A. Results

#### Effect of foliar organic fertilizer derived from the *Cordyceps* spent substrate on the plant height of the bok choy plant

There were differences in bok choy plant height among the treatments during the growing period (Table 2). The plant heights in the treatments that applied both types of foliar organic fertilizer were higher than in the negative control without using fertilizer. The treatment of PC (using commercial fish fertilizer at the recommended dose) had the highest growth rate in plant height from the 14-day stage onwards. For example, the plant height of PC treatment at 27 days after planting (harvest day) was highest with 19.63 cm, but it was only slightly higher than that observed in the CSS-FOF treatments. Consequently, the results of a one-way ANOVA analysis showed no significant differences ( $p > 0.05$ ) in plant height amongst treatments.

#### Effect of foliar organic fertilizer derived from the *Cordyceps* spent substrate on the number of leaves of the bok choy plant

Table 1: Chemical properties of the organic fertilizers used in the experiment

Fertilizer	pH	Total N	Available P (as P <sub>2</sub> O <sub>5</sub> )	Soluble K (as K <sub>2</sub> O)	Acid amin	Acid humic
Fish fertilizer	7.0	5.0%	0.25%	0.25%	100 ppm	2%
CSS-FOF	5.0	0.25%	0.09%	0.12%	NA	NA

Note: CSS-FOF: *Cordyceps spent substrate-derived foliar organic fertilizer*; NA: Not analysed.

Table 2: Effect of foliar organic fertilizer derived from the *Cordyceps* spent substrate on the plant height of bok choy

Treatments	Plant height (cm) in different growth periods			
	7 days	14 days	21 days	27 days
NC	4.07 ± 0.09	9.43 ± 0.20	16.50 ± 0.40	18.90 ± 0.60
PC	4.20 ± 0.12	11.03 ± 0.57	17.33 ± 0.54	19.63 ± 0.89
CSS-FOF 1	3.90 ± 0.06	9.90 ± 0.46	16.47 ± 0.38	18.67 ± 0.49
CSS-FOF 2	4.17 ± 0.18	9.83 ± 0.27	16.23 ± 0.44	19.40 ± 0.25
CSS-FOF 3	4.33 ± 0.09	10.57 ± 0.39	16.97 ± 0.55	19.03 ± 0.72
<i>F</i> <sub>(4,10)</sub>	2.061	2.533	0.899	0.380
Sig.	ns	ns	ns	Ns

Note: Values are mean ± S.E of three replications; ns: non-significant; NC: negative control; PC: positive control – using commercial fish fertilizer; CSS-FOF: *Cordyceps spent substrate-derived foliar organic fertilizer*.

Differences in the number of leaves on bok choy plants across different treatments were indicated in Table 3. The results of the one-way ANOVA analysis indicated no significant differences in the number of leaves per plant among the treatments during the growth periods ( $p > 0.05$ ). However, the number of leaves of bok choy from 14 days after planting onward was highest in the PC treatment (11.07 leaves plant<sup>-1</sup>), followed by the CSS-FOF 3 treatment with 10.62 leaves plant<sup>-1</sup> (fertilizer extracted from CSS applied at a dose equivalent to the nitrogen supplied in the PC treatment).

#### Effect of foliar organic fertilizer derived from the *Cordyceps* spent substrate on the canopy diameter of bok choy

Differences in the mean canopy diameter of bok choy plants were observed among treatments during the different growing periods (Table 4). Particularly, the canopy diameter at harvest (27 days after planting) varied slightly, ranging from 22.00 to 23.50 cm among treatments. Among them, the highest canopy diameter (23.50 cm)

Table 3: Effect of foliar organic fertilizer derived from the *Cordyceps* spent substrate on the plant height and the number of leaves of bok choy

Treatments	The number of leaves per plant in different growth periods			
	7 days	14 days	21 days	27 days
NC	3.07 ± 0.17	6.43 ± 0.17	8.10 ± 0.40	10.10 ± 0.62
PC	3.17 ± 0.19	7.37 ± 0.28	8.40 ± 0.50	11.07 ± 0.69
CSS-FOF 1	2.97 ± 0.13	6.43 ± 0.32	7.87 ± 0.45	9.90 ± 0.66
CSS-FOF 2	3.20 ± 0.15	6.97 ± 0.28	8.17 ± 0.45	10.00 ± 0.78
CSS-FOF 3	3.36 ± 0.17	7.13 ± 0.27	8.37 ± 0.49	10.62 ± 0.82
<i>F</i> <sub>(4,10)</sub>	0.634	2.439	0.242	0.477
Sig.	ns	ns	Ns	Ns

Note: Values are mean ± S.E of three replications; ns: non-significant; NC: negative control; PC: positive control – using commercial fish fertilizer; CSS-FOF: *Cordyceps spent substrate-derived foliar organic fertilizer*.

was recorded in the fish fertilizer treatment, followed by the foliar fertilizer derived from *Cordyceps* spent substrate (CSS) at 40 mL diluted in 1 L of water (CSS-FOF 3), which reached 22.76 cm. Overall, differences in canopy diameter among treatments throughout the growing period were not noticeable; therefore, a one-way ANOVA indicated that the treatments did not differ statistically ( $p > 0.05$ ; Table 4).

Effect of foliar organic fertilizer derived from the *Cordyceps* spent substrate on the individual weight and plot yield of bok choy plants

The large differences in the individual mean plant weight and total yield of 2 m<sup>2</sup> plots of bok choy at harvest among treatments are shown in Table 5 and Figure 1. The research findings indicated that application of fish fertilizer and CSS-FOF at different doses increased the individual weight and plot yield of bok choy plants. The highest fresh weight per plant was recorded in the CSS-FOF 3 treatment (apply CSS-FOF at a dilution rate of 40 mL L<sup>-1</sup>), with a mean

Table 4: Effect of foliar organic fertilizer derived from the *Cordyceps* spent substrate on the canopy diameter of bok choy plants (cm)

Treatments	The canopy diameter of bok choy (cm) in different growth periods			
	7 days	14 days	21 days	27 days
NC	8.50 ± 0.10	13.73 ± 0.75	19.07 ± 0.86	22.27 ± 1.34
PC	8.20 ± 0.12	14.10 ± 0.46	20.43 ± 0.82	23.50 ± 1.11
CSS-FOF 1	8.67 ± 0.07	13.43 ± 0.87	19.37 ± 1.37	22.50 ± 1.95
CSS-FOF 2	8.37 ± 0.15	13.23 ± 0.52	19.47 ± 0.74	22.00 ± 1.81
CSS-FOF 3	8.23 ± 0.15	13.97 ± 0.58	19.40 ± 1.00	22.76 ± 1.59
$F_{(4,10)}$	2.675	0.304	0.277	0.130
Sig.	ns	ns	ns	Ns

Note: Values are mean ± S.E of three replications; ns: non-significant; NC: negative control; PC: positive control – using commercial fish fertilizer; CSS-FOF: *Cordyceps* spent substrate-derived foliar organic fertilizer.

value of 150.00 g, representing a 31.6% increase compared with the negative control (no fertilizer application). This was followed by the positive control treatment (fish fertilizer), which increased plant fresh weight by 28.9%. Meanwhile, the CSS-FOF 1 treatment (apply CSS-FOF at a dilution rate of 4 mL L<sup>-1</sup>) resulted in only a 5.6% increase (Table 5).

Table 5: Effect of foliar organic fertilizer derived from the *Cordyceps* spent substrate on the individual weight and plot yield of bok choy plants

Treatments	The individual weight and plot yield of bok choy (fresh weight)	
	Individual weight (g plant <sup>-1</sup> )	Plot yield (kg per 2 m <sup>2</sup> )
NC	114.00 ± 4.04 <sup>b</sup>	5.00 ± 0.29 <sup>b</sup>
PC	147.00 ± 7.02 <sup>a</sup>	6.77 ± 0.39 <sup>a</sup>
CSS-FOF 1	120.33 ± 6.17 <sup>b</sup>	5.57 ± 0.12 <sup>b</sup>
CSS-FOF 2	139.67 ± 2.19 <sup>a</sup>	6.53 ± 0.29 <sup>a</sup>
CSS-FOF 3	150.00 ± 4.62 <sup>a</sup>	7.00 ± 0.29 <sup>a</sup>
$F_{(4,10)}$	10.044	8.660
Sig.	$p < 0.01$	$p < 0.01$

Note: Values are mean ± S.E of three replications; Means with different letters are significantly different at the  $p < 0.05$  level according to Duncan's test; NC: negative control; PC: positive control – using commercial fish fertilizer; CSS-FOF: *Cordyceps* spent substrate-derived foliar organic fertilizer.

Regarding total plot yield, the highest yield

(7.00 kg per 2 m<sup>2</sup> plot) was obtained from the treatment receiving the highest rate of CSS-FOF (CSS-FOF 3), whereas the lowest yield (5.00 kg per 2 m<sup>2</sup> plot) was recorded in the negative control. Compared with the negative control, plot yield in the CSS-FOF treatments increased by 11.4% in CSS-FOF 1 and up to 49.0% in CSS-FOF 3, demonstrating the effectiveness of CSS-FOF application in improving yield (Table 5). In addition, the application of fish fertilizer increased plot yield by 35.4% relative to the negative control.

Although CSS-FOF 3 and the positive control (commercial fish fertilizer) provided the same nitrogen content, the increase in plot yield compared with the negative control was greater in CSS-FOF 3 (49.0%) than in the positive control (35.4%), corresponding to a 13.6% higher increase.

As a result, statistical analysis confirmed that there were significant differences ( $p < 0.01$ ) in the mean weight of a single plant and plot yield among treatments (Table 5). Among the treatments, individual plant weight and the yield of the 2 m<sup>2</sup> plot in PC, CSS-FOF 2, and CSS-FOF 3 were significantly higher than those in NC and CSS-FOF 1. However, no significant differences ( $p > 0.05$ ) were observed among PC, CSS-FOF 2, and CSS-FOF 3 for these parameters.



Fig. 1: Size of bok choy plants in different treatments; (A) – Negative control, (B) – Positive control, (C) – CSS-FOF 1, (D) – CSS-FOF 2, (E) – CSS-FOF 3

## B. Discussion

It is well established that liquid organic fertilizers extracted from solid organic materials such as cattle manure, vermicompost, and spent

mushroom substrate, which contain NPK, micronutrients, organic matter, and beneficial microorganisms [10, 11], have positive effects on crop growth and yield [5, 6, 15, 17]. Specifically, liquid organic fertilizer derived from spent mushroom substrate (SMS-LOF) can enhance plant growth and is considered a promising approach for the effective recycling of spent mushroom substrate [5]. Huang et al. [5] reported that the SMS-LOF significantly increased bok choy growth parameters, including plant height, weight, leaf length and width, and root length, compared with the control without SMS-LOF. As a result, SMS-LOF created an increase of approximately 30% in bok-choy production [5]. Similarly, Elsakhawy et al. [17] prepared a liquid extract by mixing dried spent mushroom substrate extract (SMSE) with tap water at a ratio of 1:10 for 48 hours. The extract was used as a biofertilizer at an application rate of 10 L per 4200 m<sup>2</sup>. The results showed that applying SMSE through foliar spraying, soil amendment, or their combination significantly promoted plant growth, including the number of branches, plant height, and flag leaf area of rice (*Oryza sativa* L.) [17]. Elsakhawy et al. [17] demonstrated that the SMSE application significantly increased rice grain yield (t ha<sup>-1</sup>) compared with the control treatment. Moreover, foliar spraying was more effective than soil amendment, while the combined application of both methods resulted in the highest rice grain yield. Consequently, liquid fertilizers derived from spent mushroom substrate, including CSS have great potential as biofertilizers in sustainable agriculture.

This present study also showed that the application of commercial fish fertilizer (positive control) and foliar organic fertilizer derived from CSS at the highest dose (CSS-FOF 3) increased plant height, number of leaves, and canopy diameter compared with the negative control (no fertilizer application); however, the differences were not statistically significant (Tables 2, 3, and 4). Notably, the application of fish fertilizer and CSS-FOF promoted the average individual weight and 2 m<sup>2</sup> plot yield of bok choy plants.

In particular, the individual weight of bok choy plants and the yield per 2 m<sup>2</sup> plot in the positive control (fish fertilizer application), CSS-FOF 2 (apply CSS-FOF at a dilution rate of 20 mL L<sup>-1</sup>), and CSS-FOF 3 (apply CSS-FOF at a dilution rate of 40 mL L<sup>-1</sup>) were significantly higher than those in the negative control and CSS-FOF 1 (apply CSS-FOF at a dilution rate of only 4 mL L<sup>-1</sup>). Interestingly, the increase in plot yield relative to the negative control was higher in CSS-FOF 3 (49.0%) than in the positive control (35.4%). However, no significant differences ( $p > 0.05$ ) were observed among the positive control, CSS-FOF 2, and CSS-FOF 3 treatments (Table 5). This may be explained by the fact that the absence of significant differences in plant height, leaf number, and canopy diameter suggests that these morphological traits were less responsive to fertilization. Previous studies have shown that organ-level traits (i.e. vegetative height, leaf area, root length, and root diameter) often exhibit weaker responses to nutrient availability, whereas whole-plant traits such as biomass may respond more strongly [20]. Improved nutrient availability may enhance photosynthetic activity and nutrient utilization, thereby increasing biomass accumulation and crop productivity [20, 21].

In general, the positive effects of CSS-FOF on the yield of bok choy in this study may be attributed to the N, P<sub>2</sub>O<sub>5</sub>, and K<sub>2</sub>O contained in the fertilizer (Table 1), as well as to other components that may be derived from the *Cordyceps* spent substrates, such as organic matter, amino acids, vitamins, and micronutrients [7–9]. In particular, the mean individual weight of the bok choy plants and 2 m<sup>2</sup> plot yield obtained from the CSS-FOF treatments with high doses (20 và 40 mL L<sup>-1</sup>) were comparable to that of the fish fertilizer treatment (Table 5), and no significant differences were observed among treatments. The highest application rate of CSS-FOF (40 mL L<sup>-1</sup>) supplied a nitrogen amount comparable to that provided by the fish fertilizer treatment (2 ml L<sup>-1</sup>), which may explain the similar yield observed between these treatments. Interestingly, the lower rate of CSS-FOF (20 mL L<sup>-1</sup>), which

supplied approximately half the nitrogen amount, still resulted in a comparable yield, suggesting that additional nutrients and other compounds derived from CSS, such as concentrations of  $P_2O_5$  and  $K_2O$ , amino acids, vitamins, plant growth-promoting substances, and micronutrients, may also contribute to plant growth [5].

Overall, the foliar organic fertilizer derived from CSS effectively improved the yield of bok choy and performed comparably to commercial liquid fish fertilizer, highlighting the potential of recycling CSS as a biofertilizer for sustainable agriculture.

## V. CONCLUSION

Although growth parameters, including plant height, number of leaves, and canopy diameter, did not differ significantly among treatments, the application of CSS-FOF at high doses (20 and 40 mL L<sup>-1</sup>) significantly increased the average individual weight of bok choy plants and 2 m<sup>2</sup> plot yield compared with the negative control (no fertilizer application). In addition, these yield parameters were not significantly different among the CSS-FOF 2 treatment (20 mL L<sup>-1</sup>), the CSS-FOF 3 treatment (40 mL L<sup>-1</sup>), and the positive control (commercial liquid fish fertilizer treatment). These findings indicate that CSS-FOF has significant potential as a foliar fertilizer for improving the yield of leafy vegetables.

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