


## Article

# Effects of Amendment with Various Vermicomposts on the Soil Fertility, Growth of *Brassica chinensis* L., and Resistance of *Spodoptera litura* Fabricius larvae

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**Abstract:** Amendments with vermicomposts can reduce the incidence of pests. In this study, earthworms were fed different foods to produce four vermicomposts. A pot experiment was then conducted to assess different vermicomposts' effects on soil fertility, and the secondary metabolite content and antioxidant capacity of *Brassica chinensis* L., and on the growth of *Spodoptera litura* larvae. The results showed that the characteristics of vermicomposts are mainly affected by food supplements, and that the application of vermicomposts can improve soil fertility, whereas increasing the soil and leaf sulfur content can decrease the relative growth rate of *S. litura* larvae. However, there were no significant differences in the total phenolic content (TPC), total flavonoid content (TFC), nor the DPPH free radical scavenging ability under the different treatments.

**Keywords:** antioxidant capacity; secondary metabolite; soil fertility; *Spodoptera litura* Fabricius larvae; vermicompost



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## 1. Introduction

Large amounts of agricultural waste, including rick husk, straw, mushroom sawdust, and poultry litter, are produced from agricultural activities. Such agricultural waste can be converted into compost through suitable fermentation processes, which can be applied to land to increase soil fertility [1]. Rotting thermophilic compost typically undergoes a three-stage process involving a mesophilic stage, a thermophilic stage, and a curing stage [2–4]. Unlike typical composting, vermicomposting takes place at ambient temperatures through interactions between earthworms and microorganisms. Vermicompost (VC) generally has large populations and a high diversity of microorganisms resulting from the lower temperature during vermicomposting compared with composting. The application of VC has been shown to increase soil quality and the growth of plants [5–8] and decrease pests [9–11]. Secondary metabolites produced by plants under VC amendment provably decrease the incidence of pests [9,12].

Earthworms enrich biomass in terrestrial ecosystems and have an important influence on soil structure [13]. Epigeic, anecic, and endogeic are three major types of earthworms, of which the epigeic type is the most suitable for producing VC [14]. *Eisenia andrei*, *Eisenia foetida*, and *Perionyx excavates* are epigeic earthworms commonly found in soil [14], and vermicomposting is a degradation process conducted by the interaction of earthworms and microorganisms, which can convert organic waste into VC. During vermicomposting, most organic waste is converted into VC, and only 5–10% is metabolized by earthworms [14].

Many previous studies have demonstrated that VC application can enhance aggregate stability [13,15,16], improve soil quality, and promote plant growth [5–7]. Domínguez et al. [17] demonstrated that populations of microorganisms in VC were richer than in raw material, and these microorganisms could include nitrogen-fixing bacteria, actinomycetales, and mycorrhizal fungi [8]. The nutrients released during VC's mineralization, and plant hormones (e.g., auxin, gibberellin, and cytokinin) in the VC, can improve the growth of plants [5,8]. Because VC is enriched with populations of microorganisms, it can be applied to biologically control plant diseases [18]. The application of VC has been demonstrated to decrease the harmfulness of *Myzus persicae*, *Pseudococcus spp.* and *Pieris brassicae* [9]. Similar results were obtained by Yardim et al. [19], who reported that the harmfulness of *Manduca quinquemaculata*, *Acalymma vittatum*, and *Diabrotica undecimpunctata* for plants was reduced under VC amendment. An increase in the total phenolic content (TPC) of tomato plant leaves under VC amendment produced a higher resistance of the plants to *Bemisia tabaci* [20].

A chemical-based defense, producing secondary metabolites, might occur in plants under stress, affecting the enzymes involved in digestion and metabolism, and minimizing harm to herbivores [21–24]. Moreover, a hypersensitive response also causes the accumulation of large amounts of reactive oxygen species (ROS) to combat insect infestations. Under biological or nonbiological stress, plants must synthesize secondary metabolites to alleviate the negative influence of ROS on cells [22,23]. Increases in secondary metabolites (e.g., phenolic compounds and flavonoids) in plants have been shown to protect against the consumption/destruction of plants by insects [24–26]. Infestations of *Arachis hypogaea* L. with *Amsacta albistriga* W., *Approaerema modicella* D., and *Spilosoma Obliqua* W. larvae have been shown to increase the TPC of plants [26,27]. Under chemical-based treatments, different plant species synthesize different secondary metabolites (for example, Brassicaceae synthesize glucosinolate) by utilizing sulfur (S) from the uptake of sulfate ( $\text{SO}_4^{2-}$ ) by their roots [28,29]. The toxic effects of glucosinolate on various insects have been demonstrated by Ahuja et al. [28], Falk et al. [29], and Schoonhoven et al. [24]. Because of S's low mobility, mature leaves have a higher S content [29].

In this study, four types of VCs were produced by feeding earthworms different kinds of agricultural waste, and the VCs were then applied to soil samples to grow pak choi. The pak choi was infested with *S. litura* larvae to assess the different VCs' effects on the growth of the larvae. Moreover, the effects of larvae infestation on TPC, total flavonoid content (TFC), and the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging ability of the pak choi was also assessed. The objectives of this study included (1) comparing the characteristics of the four produced VCs; (2) assessing the VCs' effects on soil fertility and the growth of pak choi; and (3) assessing the influences of VC on the secondary metabolite content, antioxidant capacity, and resistance to *S. litura* larvae of the pak choi.

## 2. Materials and Methods

### 2.1. VCs, Crop, Soil, and Larvae

A combination of two species of earthworms—red wigglers (*E. andrei* or *E. foetida*) and Indian blue worms (*P. excavates*)—were used in this study, and were fed four kinds of organic waste separately to produce four VCs. Used shiitake mushroom sawdust was used as the primary medium for the growth of the earthworms, which were fed with four food supplements: (1) VM—only mushroom sawdust and no other organic waste, (2) VRM—mushroom sawdust combined with rice bran, (3) VPM—mushroom sawdust combined with pig manure, and (4) VCM—mushroom sawdust combined with cabbage. In total, 5.0 kg of mushroom sawdust was placed into an opaque rectangular polypropylene box (L 47 cm × W 33 cm × H 18 cm), the moisture content was adjusted to 70–75%, and 0.5 kg of earthworms were added. The top of each box was covered with a 32-mesh nylon net to reduce the evaporation of water, prevent the escape of the earthworms, and avoid their predation by animals. After one week of incubation, 50 g of the four different food supplements were added every 2 d, and residual foods were removed if necessary. The

moisture content during the experiment was adjusted to levels of 70–75% every 2–3 d by weighing and adding deionized water (DI water). Feeding and DI water supply were stopped on the 53rd d, and the four produced VCs were collected 7 d thereafter. The VCs were then air dried and used in the pot experiment, as described in Section 2.2.

A leafy vegetable (pak choi; *Brassica chinensis* L. var. *Chinensis*), commonly found in Taiwan's markets, was used as the study crop. Pak choi leaves are usually consumed by tobacco cutworm (*Spodoptera litura* Fabricius) larvae, and pak choi was therefore selected to test its capacity to resist insects under different VC treatments. The surface layer (0–30 cm) of an important farmland soil (Erhlin soil series from central Taiwan) was selected as the soil for the study. Soil samples were air dried, ground, and then sieved with a 5-mesh stainless steel sieve. The sieved soil samples were then used in the pot experiment, as described in Section 2.2. The larvae of second-instar tobacco cutworms were bought from the Taiwan Agricultural Chemicals and Toxic Substances Institute, and third-instar larvae were used in the infestation experiment. The pot experiment was conducted, as explained in Section 2.2, using recommended amounts (RAs) of nitrogen (N), phosphoric oxide ( $P_2O_5$ ), and potassium oxide ( $K_2O$ ) as specified by the Agriculture and Food Agency of the Council of Agriculture (AFA of COA) in Taiwan at 250, 150, and 180 kg ha<sup>-1</sup>, respectively.

## 2.2. Pot Experiment

The four kinds of VCs described in Section 2.1, produced by feeding different food supplements, were applied to the soil samples, and the crops were then planted. Six treatments with four replicates were used in this experiment to assess the effects of applying different VCs on growth, nutritional content, antioxidant capacity, secondary metabolite content, and the DPPH free radical scavenging ability of pak choi. The six treatments were (1) CK—control without applying any chemical fertilizers and VCs; (2) CF—urea ( $CON_2H_4$ ), calcium superphosphate ( $Ca(H_2PO_4)_2 \cdot H_2O$ ), and potassium chloride (KCl), applied according to their RAs; (3) VM; (4) VRM; (5) VPM; and (6) VCM. Because VC has to be mineralized to release nutrients, the amounts of VC in treatments (3), (4), and (5) were applied at five times the RAs of N based on the N content of different VCs.

Sieved soil samples, prepared as described in Section 2.1, were homogeneously mixed with different CFs or VCs, and 1.0 kg of the mixture was then added to each pot. The pot experiment was conducted in a growth chamber (14 h lighting, temperature  $25.16 \pm 1.66$  °C, relative humidity  $60.83 \pm 17.17\%$ ), and 30 seeds of pak choi were sown in each pot. The soil moisture in each pot during the pot experiment was controlled at 50–70% of water-holding capacity (WHC) by weighing and adding DI water every 2–3 d. Only 10 seedlings with similar shoot heights were left at the 7th d after germination. After growing for six weeks, two replicates of each treatment were randomly selected, and each pot was infested with eight third-instar *S. litura* larvae for one week (Figure 1). The initial fresh weights of larvae were determined and recorded before infestation.

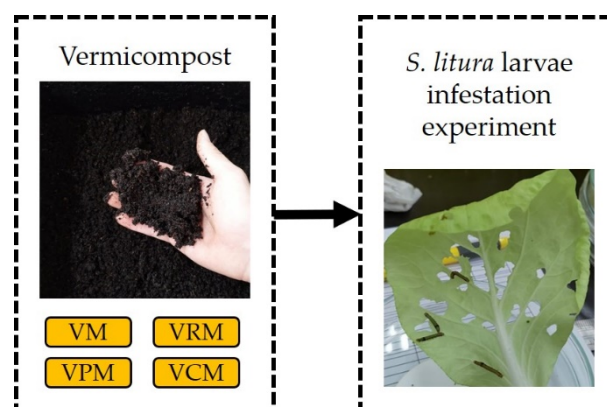


Figure 1. Photos of vermicompost and *S. litura* larva infestation experiment.

After growing for seven weeks, the shoots grown under different treatments were harvested, washed first with tap and then DI water, and measured their shoot heights and fresh weights. The final fresh weights of the larvae were determined and recorded and the relative chlorophyll content (i.e., SPAD reading) of the most extended leaf of each replicate grown under a different treatment was determined with a chlorophyll meter (SPAD-502, Konica Minolta, Osaka, Japan). Plant tissues were oven-dried at 70 °C for 72 h or freeze-dried for 48 h according to the property being analyzed, as described in the next section.

### 2.3. VC, Soil, Plant, and Larvae Analyses

The moisture content, pH [30], electrical conductivity (EC) [31], total nitrogen content (TN) [32], and organic matter content (OM) [33] in the VCs were analyzed. Additionally, the VCs were digested with nitric acid and perchloric acid ( $v/v = 4:1$ ) [34]; the concentrations of P in the digestants were determined according to Murphy and Riley [35]; and the concentrations of K and S in the digestants were determined with a flame photometer (Sherwood 410, Sherwood Scientific Ltd., Cambridge, UK) and ion chromatography (930 Compact IC Flex, Metrohm, Herisau, Switzerland), respectively. The concentrations of calcium (Ca) and magnesium (Mg) in the digestants were determined with an atomic absorption spectrometer (Z-2000, Hitachi, Tokyo, Japan), and the cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn) in the digestants were determined with an inductively coupled plasma atomic emission spectrometry (ICP-OES Avio 200, Perkin Elmer, MA, USA).

The soil samples were collected after the pot experiment, ground, and passed through 10-mesh or 80-mesh stainless steel sieves according to the property analyzed. The soil properties (i.e., pH, EC, and OM) were analyzed using the same methods as those for the VCs described in the above paragraph. Other properties analyzed included concentrations of available N [36], available P [37], and available S [38]; exchangeable concentrations of K, Ca, and Mg [39]; and wet aggregate stability (WAS) [40].

The oven-dried plant tissue was ground and digested with nitric acid and perchloric acid ( $v/v = 4:1$ ) [34], and the concentrations of N, P, K, Ca, Mg, and S were then determined using the same method as described for the VCs. The freeze-dried tissue was used to determine the antioxidant capacity and secondary metabolite content. The DPPH free radical scavenging ability, TPC, and TFC were determined according to Hatano et al. [41]. The relative growth rate (RGR) of *S. litura* larvae was calculated using Equation (1).

$$\text{Relative growth rate (RGR)} = \frac{\text{Initial fresh weight of larvae/final fresh weight of larvae}}{\text{Period of experiment}} \quad (1)$$

### 2.4. Statistical Analysis

Statistical analysis was performed using the Statistical Analysis System (SAS) v9.4 software. One-way analysis of variance (ANOVA) was performed using a generalized linear model (GLM) across treatments. Fisher's protected least significant difference (LSD) test was used to identify significant differences between the means, and a value of  $p < 0.05$  denoted statistical significance.

## 3. Results and Discussion

### 3.1. VC Properties

The VCM had the highest pH of the four produced VCs, which possibly resulted from the difference in the food supplement and the mineralization rate of the OM (Table 1). During the degradation of OM, the fulvic acid and humic acid produced can decrease the pH of VC [42]; however, the pH of VRM increases when the salts released during the degradation of OM accumulate, or the releasing  $\text{NH}_3$  reacts with water [43]. The VRM and VPM treatments had higher EC than the VM and VCM treatments, possibly due to the release of N, P, and K ions during the degradation of OM [44]. Previous research reported that more leachate was produced by earthworms when their foods had a high

water content [5]. The lower EC of VCM possibly resulted from the high water content of cabbage compared with other food supplements; more soluble salts might have leached out of the VCM than from the other VCs. The four VCs had more than 64% OM, and the C/N ratio was at levels of 16.4–23.4, which was still within the regulated range (10.0–25.0) announced by the AFA of COA. Immobilization is preferred for a high C/N ratio, and a N depression period may occur during the utilization of organic waste [43]. The C/N ratio was regressed as an important index to assess the maturation and quality of compost [45].

The degradation and mineralization of OM occurred through interactions between earthworms and microorganisms, and the content of N, P, K, and Ca in the VCs thus increased [43]. The content of different nutrients in the VCs was determined by the food supplement. Relative to VCM and VM, VRM and VPM had a higher total N and P<sub>2</sub>O<sub>5</sub> content. The highest K<sub>2</sub>O, calcium oxide, and magnesium oxide content was observed in the VRM, VM, and VRM, respectively. The experimental results were in agreement with Fahey et al. [46], who reported that the family Brassicaceae is the primary occurrence of glucosinolate in vegetables, thus, the VCM had the highest S content of the four VCs. The S content of VCM and the other three VCs was 0.51% and 0.18–0.33%, respectively. Except for Cr and Zn, trace metals in the four VCs were not detectable.

### 3.2. Pot Experiment

#### 3.2.1. VCs' Effects on Soil Properties

Table 2 shows the soil properties after the pot experiment. For the same treatment, there was no significant effect of the *S. litura* larvae infestation on the soil properties analyzed in general. Whether infested with *S. litura* larvae or not, the CF treatment had lower pH values of the six treatments, and there was no significant difference between the CK treatment and the other four VC treatments. Urea was used as the N source in the CF treatment, and ammonium (NH<sub>4</sub><sup>+</sup>) was released during urea hydrolysis [47]. The secretion of hydrogen ions (H<sup>+</sup>) during the uptake of NH<sub>4</sub><sup>+</sup> or other cations by the roots could acidify the soil pH of the rhizosphere, and the H<sup>+</sup> released during the nitrification of NH<sub>4</sub><sup>+</sup> could also decrease the soil pH. Under different VC treatments, soil EC was determined by the concentrations of soluble salts released during the mineralization of VC [44]. The experimental results showed that the application of CF and various VCs significantly ( $p < 0.05$ ) increased the EC from 5.3–6.0 mS m<sup>-1</sup> in the CK to levels of 6.3–18.4 mS m<sup>-1</sup>, especially for CF. Relative to CK and CF, amendment with different VCs significantly ( $p < 0.05$ ) increased the soil OM content from 2.6–3.0% to levels of 3.9–4.9%. Soil OM is an index of soil quality that plays an important role in enhancing the formation of aggregates, fertility, water retention, and the richness of microorganisms [48]. Many previous studies have demonstrated that VC application can increase WAS [13,15,16]; however, there was no statistical difference between the CK and VC treatments, which possibly resulted from the shorter duration of the pot experiment.



**Table 1.** The basic properties of the four vermicomposts produced.

| Treatment | Water Content | pH        | EC                    | OM         | C/N Ratio | TN        | P <sub>2</sub> O <sub>5</sub> | K <sub>2</sub> O | CaO       | MgO       | S           | Cd | Cr    | Cu                  | Ni | Pb | Zn     |
|-----------|---------------|-----------|-----------------------|------------|-----------|-----------|-------------------------------|------------------|-----------|-----------|-------------|----|-------|---------------------|----|----|--------|
|           | (%)           |           | (dS m <sup>-1</sup> ) | (%)        |           |           |                               |                  |           |           |             |    |       | mg kg <sup>-1</sup> |    |    |        |
| VM        | 45.9 ± 0.6    | 8.0 ± 0.1 | 3.1 ± 0.3             | 64.4 ± 4.5 | 21.5      | 1.7 ± 0.1 | 1.5 ± 0.0                     | 1.2 ± 0.0        | 5.4 ± 0.5 | 0.9 ± 0.0 | 0.18 ± 0.01 | ND | 1 ± 1 | ND                  | ND | ND | 9 ± 2  |
| VRM       | 45.8 ± 0.0    | 6.8 ± 0.1 | 5.4 ± 0.3             | 65.5 ± 2.0 | 16.4      | 2.3 ± 0.1 | 2.8 ± 0.0                     | 2.0 ± 0.0        | 3.2 ± 0.3 | 1.6 ± 0.0 | 0.23 ± 0.02 | ND | 1 ± 0 | ND                  | ND | ND | 8 ± 1  |
| VPM       | 46.8 ± 0.4    | 6.9 ± 0.2 | 5.2 ± 0.6             | 68.3 ± 7.4 | 16.8      | 2.4 ± 0.2 | 2.5 ± 0.1                     | 1.2 ± 0.0        | 4.3 ± 0.2 | 1.0 ± 0.0 | 0.33 ± 0.01 | ND | 1 ± 1 | ND                  | ND | ND | 10 ± 3 |
| VCM       | 34.0 ± 0.4    | 8.5 ± 0.0 | 2.1 ± 0.0             | 70.2 ± 2.2 | 23.4      | 1.7 ± 0.1 | 1.4 ± 0.0                     | 1.3 ± 0.0        | 4.5 ± 0.1 | 0.9 ± 0.0 | 0.51 ± 0.33 | ND | ND    | ND                  | ND | ND | 12 ± 9 |

Note: EC: electrical conductivity; OM: organic matter content; C/N ratio: carbon to nitrogen ratio; TN: total nitrogen content. VM: vermicomposted mushroom sawdust waste; VRM: vermicomposted rice bran; VPM: vermicomposted pig manure; VCM: vermicomposted cabbage. Mean ± standard deviation; ND: Not detectable.

**Table 2.** The basic soil properties of different treatments.

| Treatment | pH              | EC                    | OM             | WAS           | Avail. N                              | Avail. P            | Ex. K          | Avail. S        | Ex. Ca                | Ex. Mg                 | Ca/Mg          |
|-----------|-----------------|-----------------------|----------------|---------------|---------------------------------------|---------------------|----------------|-----------------|-----------------------|------------------------|----------------|
|           |                 | (mS m <sup>-1</sup> ) | %              |               |                                       | mg kg <sup>-1</sup> |                |                 | (g kg <sup>-1</sup> ) | (mg kg <sup>-1</sup> ) | (Mole Ratio)   |
|           |                 |                       |                |               | Without <i>S. litura</i> larvae       |                     |                |                 |                       |                        |                |
| CK        | 8.21 ± 0.18 ab  | 5.35 ± 0.45 f         | 2.53 ± 0.05 c  | 63.7 ± 2.0 a  | 6.75 ± 0.00 b                         | 6.99 ± 0.04 g       | 41.1 ± 3.1 c   | 2.40 ± 0.44 def | 3.34 ± 0.05 d         | 216 ± 7 c              | 9.27 ± 0.16 a  |
| CF        | 8.04 ± 0.09 bc  | 6.33 ± 0.07 fe        | 2.94 ± 0.02 c  | 55.8 ± 4.5 a  | 6.72 ± 0.00 b                         | 15.5 ± 0.2 f        | 78.5 ± 32.0 bc | 2.00 ± 0.00 f   | 3.41 ± 0.00 d         | 219 ± 2 c              | 9.34 ± 0.08 a  |
| VM        | 8.21 ± 0.03 ab  | 8.29 ± 0.13 cd        | 4.19 ± 0.19 ab | 57.7 ± 4.5 a  | 10.1 ± 3.4 ab                         | 27.0 ± 2.8 de       | 86.6 ± 12.0 bc | 2.36 ± 0.35 ef  | 3.70 ± 0.06 abc       | 361 ± 11 b             | 6.15 ± 0.08 c  |
| VRM       | 7.90 ± 0.00 cd  | 7.64 ± 0.02 cde       | 4.78 ± 0.53 a  | 55.3 ± 4.5 a  | 13.5 ± 0.0 a                          | 88.9 ± 6.1 a        | 77.8 ± 1.0 bc  | 3.01 ± 0.16 def | 3.62 ± 0.02 c         | 454 ± 20 a             | 4.80 ± 0.24 d  |
| VPM       | 8.13 ± 0.05 abc | 6.80 ± 0.15 def       | 4.21 ± 0.13 ab | 61.2 ± 5.2 a  | 10.1 ± 3.4 ab                         | 38.8 ± 4.6 b        | 52.3 ± 4.4 bc  | 3.23 ± 0.57 cde | 3.79 ± 0.08 abc       | 374 ± 23 b             | 6.09 ± 0.25 c  |
| VCM       | 8.35 ± 0.01 a   | 7.88 ± 0.23 cde       | 4.55 ± 0.07 ab | 50.1 ± 6.6 a  | 13.5 ± 6.7 b                          | 32.9 ± 1.7 bcd      | 198 ± 6 a      | 4.07 ± 0.07 bc  | 3.86 ± 0.02 a         | 368 ± 11 b             | 6.31 ± 0.15 c  |
|           |                 |                       |                |               | Infested with <i>S. litura</i> larvae |                     |                |                 |                       |                        |                |
| CK        | 8.14 ± 0.13 abc | 5.92 ± 0.36 f         | 2.60 ± 0.18 c  | 64.7 ± 4.7 a  | 6.73 ± 0.01 b                         | 6.87 ± 0.50 g       | 41.1 ± 3.0 c   | 3.31 ± 0.14 cde | 3.36 ± 0.10 d         | 239 ± 8 c              | 8.47 ± 0.53 b  |
| CF        | 7.69 ± 0.17 d   | 18.4 ± 0.7 a          | 2.91 ± 0.05 c  | 50.7 ± 13.0 a | 10.1 ± 3.4 ab                         | 21.8 ± 1.7 ef       | 57.0 ± 6.8 bc  | 2.87 ± 0.65 def | 3.23 ± 0.13 d         | 219 ± 12 c             | 8.87 ± 0.16 ab |
| VM        | 8.15 ± 0.14 abc | 8.68 ± 0.85 bc        | 3.99 ± 0.18 b  | 62.0 ± 1.3 a  | 13.5 ± 0.0 a                          | 29.1 ± 1.0 cde      | 185 ± 10 a     | 3.40 ± 0.25 bcd | 3.69 ± 0.01 abc       | 353 ± 4 b              | 6.29 ± 0.05 c  |
| VRM       | 8.14 ± 0.00 abc | 8.81 ± 0.37 bc        | 4.83 ± 0.28 a  | 64.5 ± 1.7 a  | 6.72 ± 0.00 b                         | 92.2 ± 3.1 a        | 153 ± 16 a     | 4.04 ± 0.28 bc  | 3.66 ± 0.03 bc        | 463 ± 7 a              | 4.75 ± 0.11 d  |
| VPM       | 8.11 ± 0.07 abc | 8.44 ± 0.84 c         | 4.24 ± 0.14 ab | 60.5 ± 1.0 a  | 13.5 ± 0.0 a                          | 37.4 ± 0.2 b        | 96.1 ± 21.0 b  | 4.41 ± 0.08 b   | 3.76 ± 0.01 abc       | 366 ± 5 b              | 6.17 ± 0.11 c  |
| VCM       | 8.09 ± 0.00 abc | 10.2 ± 0.90 b         | 4.49 ± 0.26 ab | 54.8 ± 9.0 a  | 13.5 ± 6.7 a                          | 35.4 ± 1.9 bc       | 199 ± 28 a     | 5.93 ± 0.25 a   | 3.80 ± 0.01 ab        | 367 ± 14 b             | 6.22 ± 0.22 c  |

Note: EC: electrical conductivity; OM: organic matter content; WAS: wet aggregate stability; Avail.: available; Ex.: exchangeable. Mean ± standard deviation. Means within a column followed by the same letters are not significantly different,  $p < 0.05$ , according to Fisher's protected least significant difference (LSD) test. CK: control; CF: chemical fertilizer; VM: vermicomposted mushroom sawdust waste; VRM: vermicomposted rice bran; VPM: vermicomposted pig manure; VCM: vermicomposted cabbage.

N mainly exists in an organic form in soil and has to be mineralized into inorganic forms before its uptake by plants [35]. Although the application of different VCs did not significantly affect the concentrations of available N in general, the concentrations of available P, exchangeable K, available S, exchangeable Ca, and exchangeable Mg under VC treatments significantly increased compared with CK ( $p < 0.05$ ). Of the four VCs used, the VRM treatment produced the highest concentrations of available P and exchangeable Mg; moreover, the highest concentrations of exchangeable K and available S were found under the VCM treatment. Kuo [37] revealed that the concentration of available P suitable for the growth of crops, determined using the Olsen method, is  $10 \text{ mg kg}^{-1}$ ; however, the concentration of available P was only  $6.8\text{--}7.0 \text{ mg kg}^{-1}$  under the CK treatment, which was insufficient. Because the soil used in this study had moderate alkalinity, the P ions possibly formed precipitates with Ca and Mg ions and, thus, decreased the availability of P [49]. The application of different VCs increased the available P content to  $27\text{--}93 \text{ mg kg}^{-1}$ , which is sufficient for growing plants. The acidic materials (i.e., carbonic acid, nitric acid, and sulfuric acid) produced by the earthworms and microorganisms could have increased the release of K in an insoluble form and increased the concentration of soluble K [44]; thus, the concentrations of exchangeable K significantly increased from  $41.1 \text{ mg kg}^{-1}$  under the CK treatment to levels of  $52\text{--}200 \text{ mg kg}^{-1}$  under VC treatments ( $p < 0.05$ ), whether the pak choi were infested with *S. litura* larvae or not.

Relative to CK, the VPM and VCM treatments had significantly higher concentrations of available S ( $p < 0.05$ ), at levels of  $3.2\text{--}6.0 \text{ mg kg}^{-1}$ . The VCM treatment had the highest available S content because of the higher S content in the VCM compared with the other VCs (Table 1). In agreement with the finding of Rini et al. [50], the concentrations of exchangeable Ca and exchangeable Mg significantly increased to levels of  $3.6\text{--}3.9 \text{ g kg}^{-1}$  and  $350\text{--}470 \text{ mg kg}^{-1}$ , respectively, under various VC treatments compared with CK ( $p < 0.05$ ). Also, the mole ratios of exchangeable Ca to exchangeable Mg under VM, VPM, and VCM treatments were close to the recommended value of six [51,52].

### 3.2.2. VCs' Effects on *B. chinensis*

The infestation of *S. litura* larvae decreased the SPAD reading and the fresh weight of pak choi grown in the same treatment in general (Table 3). The plants grown under the CF treatment had the highest SPAD readings, shoot heights, and fresh weights of the six treatments used. Of the four VCs used, the three growth exhibitions under the VM, VRM, and VPM treatments were higher than those under the VCM treatment. The food supplement was found to affect the populations of microorganisms, the mineralization of the VC, and the concentrations of different nutrients in the soils and crops, which changed accordingly [53]. The experimental results showed that the application of VCs either slightly or significantly increased ( $p < 0.05$ ) the concentrations of N, P, K, and Mg in the pak choi in general (Table 4). Of the four VCs used, the highest concentrations of P, K, and Mg were found under the VRM treatment; moreover, the highest S content ( $0.22\text{--}0.24\%$ ) was observed under the VCM treatment, which resulted from the higher S content compared with the other VCs. The S element is a critical constitution of glucosinolate and a secondary metabolite of Brassicaceae, and sulfate is the major form of S taken up by plants, producing amino acids including cystine, cysteine, and methionine [54].

**Table 3.** The SPAD readings, shoot heights, and fresh weights of *B. chinensis* grown in the different treatments.

| Treatment                             | SPAD Reading     | Shoot Height (cm) | Fresh Weight (g plant <sup>-1</sup> ) |
|---------------------------------------|------------------|-------------------|---------------------------------------|
| Without <i>S. litura</i> larvae       |                  |                   |                                       |
| CK                                    | 7.45 ± 0.85 def  | 19.0 ± 1.1 ef     | 3.16 ± 0.01 e                         |
| CF                                    | 16.9 ± 1.0 a     | 25.2 ± 0.8 a      | 18.1 ± 0.2 a                          |
| VM                                    | 12.1 ± 0.0 bc    | 21.2 ± 0.3 cde    | 8.86 ± 1.90 bc                        |
| VRM                                   | 11.5 ± 1.7 bc    | 23.3 ± 0.3 abc    | 11.3 ± 1.6 b                          |
| VPM                                   | 13.2 ± 2.8 ab    | 22.6 ± 0.9 bcd    | 11.2 ± 1.0 b                          |
| VCM                                   | 10.7 ± 0.5 bcde  | 18.8 ± 0.7 f      | 4.00 ± 0.49 de                        |
| Infested with <i>S. litura</i> larvae |                  |                   |                                       |
| CK                                    | 7.05 ± 0.25 ef   | 20.3 ± 0.4 def    | 3.09 ± 0.14 e                         |
| CF                                    | 10.6 ± 1.3 bcde  | 24.4 ± 1.0 ab     | 8.05 ± 0.58 c                         |
| VM                                    | 8.95 ± 0.75 cdef | 22.3 ± 1.4 bcd    | 6.86 ± 1.23 cd                        |
| VRM                                   | 10.6 ± 0.6 bcde  | 23.3 ± 0.4 abc    | 8.06 ± 0.32 c                         |
| VPM                                   | 11.1 ± 1.0 bcd   | 22.2 ± 0.6 bcd    | 8.78 ± 1.37 bc                        |
| VCM                                   | 6.40 ± 1.60 f    | 18.7 ± 0.0 f      | 4.39 ± 0.41 de                        |

Note: Mean ± standard deviation. Means within a column followed by the same letters are not significantly different,  $p < 0.05$ , according to Fisher's protected least significant difference (LSD) test. The meaning of each code is the same as Table 2.

**Table 4.** The nitrogen, phosphorous, potassium, calcium, magnesium, and sulfur content of *B. chinensis* grown in the different treatments.

| Treatment                             | N              | P                 | K              | Ca              | Mg                | S                   |
|---------------------------------------|----------------|-------------------|----------------|-----------------|-------------------|---------------------|
|                                       | %              |                   |                |                 |                   |                     |
| Without <i>S. litura</i> larvae       |                |                   |                |                 |                   |                     |
| CK                                    | 1.38 ± ±0.05 e | 0.453 ± 0.019 e   | 3.60 ± 0.27 c  | 2.56 ± 0.13 d   | 0.329 ± 0.024 f   | 0.122 ± 0.098 bcde  |
| CF                                    | 3.10 ± 0.25 bc | 0.512 ± 0.033 de  | 3.67 ± 0.24 c  | 3.47 ± 0.03 a   | 0.486 ± 0.024 bc  | 0.071 ± 0.019 e     |
| VM                                    | 1.86 ± 0.29 de | 0.807 ± 0.062 bc  | 5.91 ± 0.02 ab | 2.86 ± 0.05 bcd | 0.408 ± 0.003 de  | 0.105 ± 0.005 de    |
| VRM                                   | 2.51 ± 0.10 cd | 0.895 ± 0.022 abc | 6.33 ± 0.64 ab | 2.53 ± 0.19 d   | 0.504 ± 0.011 b   | 0.109 ± 0.016 cde   |
| VPM                                   | 2.53 ± 0.35 cd | 0.731 ± 0.138 bcd | 5.37 ± 0.27 b  | 2.37 ± 0.06 d   | 0.465 ± 0.000 bcd | 0.119 ± 0.020 bcde  |
| VCM                                   | 1.47 ± 0.37 de | 0.775 ± 0.135 bc  | 5.27 ± 0.66 b  | 2.54 ± 0.39 d   | 0.423 ± 0.040 cde | 0.221 ± 0.065 a     |
| Infested with <i>S. litura</i> larvae |                |                   |                |                 |                   |                     |
| CK                                    | 1.80 ± 0.13 de | 0.500 ± 0.047 de  | 5.37 ± 0.91 b  | 3.24 ± 0.05 ab  | 0.398 ± 0.010 e   | 0.154 ± 0.130 abcde |
| CF                                    | 5.31 ± 0.17 a  | 0.682 ± 0.220 cde | 5.59 ± 0.00 b  | 3.33 ± 0.26 ab  | 0.478 ± 0.005 bcd | 0.168 ± 0.001 abcd  |
| VM                                    | 3.22 ± 0.71 bc | 0.850 ± 0.06 1abc | 6.67 ± 0.29 ab | 3.16 ± 0.12 abc | 0.474 ± 0.006 bcd | 0.184 ± 0.033 abcd  |
| VRM                                   | 4.16 ± 0.67 b  | 1.08 ± 0.053 a    | 7.04 ± 0.12 a  | 2.80 ± 0.11 bcd | 0.621 ± 0.019 a   | 0.197 ± 0.007 ab    |
| VPM                                   | 3.81 ± 0.15 b  | 0.961 ± 0.003 ab  | 6.35 ± 0.61 ab | 2.67 ± 0.11 cd  | 0.487 ± 0.006 bc  | 0.195 ± 0.005 abc   |
| VCM                                   | 1.52 ± 0.15 de | 0.776 ± 0.045 bc  | 5.69 ± 0.44 ab | 2.53 ± 0.25 d   | 0.389 ± 0.047 ef  | 0.233 ± 0.007 a     |

Note: Mean ± standard deviation. Means within a column followed by the same letters are not significantly different,  $p < 0.05$ , according to Fisher's protected least significant difference (LSD) test. The meaning of each code is the same as Table 2.

Relative to VCs, treatment with CK and CF had the highest TPC and TFC, respectively (Table 5). The DPPH free radical scavenging ability was higher under CK treatment than under other treatments. The above result was identical to that obtained by Sousa et al. [55], who reported that plants grown in soil with no applied amendment had higher TPC than those grown in soil treated with organic and chemical fertilizers. Higher TPC in plants was also observed in the N-deficient condition [56,57]. Similar to the experimental results for the growth exhibitions and the N content of the pak choi, the VRM and VPM had higher TFC than the VM and VCM among the four VCs used. Although many studies have reported that the TPC and TFC decreased under the application of N fertilizer [58–60], a contrary result was observed in this study. Besides environmental factors, the secondary metabolite content and antioxidant capacity of crops are determined by the application of N, and this decreases with unsuitable N application rates [56,61]. Although the application of VC was shown to increase the DPPH free radical scavenging ability of crops [22,62], it significantly decreased under the four VC treatments compared with CK. High Mg, K, and Ca content helps to enhance the antioxidant capacity of plants [26], but these effects were not observed in this study because the difference between treatments was not statistically



significant overall. A higher S content in crops was observed to increase the glucosinolate content of a crop, which had negative effects on the growth of insects [28]. The effect of increasing the S content of pak choi on the growth of *S. litura* larvae needs to be clarified in the future.

**Table 5.** The TPC, TFC, and DPPH free radical scavenging ability of *B. chinensis* of different treatments.

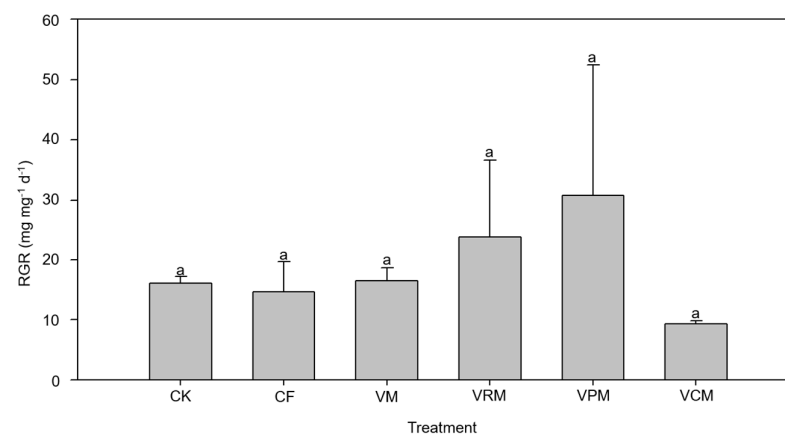
| Treatment | Total Phenolic (TPC)<br>(mg-GAE g-DW <sup>-1</sup> ) | Total Flavonoid (TFC)<br>(mg-QE g-DW <sup>-1</sup> ) | DPPH Free Radical Scavenging Ability<br>(%) |
|-----------|--|--|---|
|           |  | Without <i>S. litura</i> larvae                      |   |
| CK        | 6.96 ± 0.16 a  | 6.90 ± 1.07 cde                                      | 57.8 ± 1.0 abc                              |
| CF        | 5.65 ± 0.26 bc                                       | 14.4 ± 2.0 a   | 43.1 ± 3.2 cd                               |
| VM        | 4.69 ± 0.05 c  | 7.60 ± 1.14 cde                                      | 52.2 ± 3.7 abcd                             |
| VRM       | 5.39 ± 0.03 bc                                       | 11.9 ± 0.6 ab  | 56.2 ± 0.3 abc                              |
| VPM       | 5.76 ± 0.26 bc                                       | 9.73 ± 0.85 bc                                       | 58.0 ± 0.7 abc                              |
| VCM       | 6.37 ± 0.06 ab                                       | 6.67 ± 0.27 de                                       | 58.7 ± 8.5 ab                               |
|           |  | Infested with <i>S. litura</i> larvae                |   |
| CK        | 6.46 ± 0.29 ab                                       | 6.04 ± 0.28 e  | 66.1 ± 1.2 a                                |
| CF        | 4.64 ± 0.36 c  | 9.52 ± 0.50 bcd                                      | 40.1 ± 0.4 d                                |
| VM        | 5.30 ± 0.35 bc                                       | 6.68 ± 0.36 de                                       | 52.9 ± 5.8 abcd                             |
| VRM       | 4.81 ± 0.92 c  | 8.31 ± 1.70 cde                                      | 47.3 ± 10.0 bcd                             |
| VPM       | 5.26 ± 0.31 bc                                       | 7.46 ± 0.00 cde                                      | 56.7 ± 2.2 abc                              |
| VCM       | 4.97 ± 0.58 c  | 5.82 ± 0.21 e  | 53.6 ± 7.3 abcd                             |

Note: Mean ± standard deviation. Means within a column followed by the same letters are not significantly different,  $p < 0.05$ , according to Fisher's protected least significant difference (LSD) test. The meaning of each code is the same as Table 2. GAE: gallic acid equivalent; QE: quercetin equivalent.

### 3.2.3. VC's Effect on *S. litura* Larvae

The pak choi grown for six weeks under the various treatments were infested with third-instar *S. litura* larvae to assess the amendments' effects on the growth of larvae. Two replicates of each treatment were selected and infested with eight *S. litura* larvae. As recommended by Schoonhoven et al. [24], changes in the fresh weights of larvae were determined after infestation for one week, and the RGRs were calculated using Equation (1).

Although there was no statistical difference between the CF and the four VC treatments in the RGRs of the *S. litura* larvae compared with the CK treatment, the lowest RGR was found under the VCM treatment (Figure 2). The application of S-containing fertilizer clearly increased the synthesis of glucosinolate in Brassicaceae, which can inhibit the consumption of plants by insects and have a negative effect on insects' growth [28,29]. Since treatments with the CF and the four VCs did not significantly affect the secondary metabolite content nor the antioxidant capacity compared with CK (Table 5), the decrease in RGR possibly resulted from the higher S content under the VCM treatment (Table 4).



**Figure 2.** The relative growth rate (RGR) of *S. litura* larva in the pot experiment. The meaning of each code is the same as for Table 2. The same letters were not significantly different ( $p < 0.05$ ) according to Fisher's protected least significant difference (LSD) test.

#### 4. Conclusions

The properties of VCs were determined by the related food supplement, and the application of VCs increased not only OM content and concentrations of available P, available S, exchangeable K, exchangeable Ca, and exchangeable Mg in the soil, but also the growth of pak choi; however, the enhancing effect was still lower than with chemical fertilizer, possibly due to the lower mineralization rate of VC. Among the four VCs used in this study, VCM reduced infestation of pak choi with *S. litura* larvae the most, possibly due to the higher S content in the VCM and the leaves of pak choi.

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