Article
Transformation Capability Optimization and Product Application Potential of *Proteatia brevitarsis* (Coleoptera: Cetoniidae) Larvae on Cotton Stalks

Guangjie Zhang 1, Yeshan Xu 1, Shuai Zhang 1, Andong Xu 1, Zhuo Meng 1, Hao Ge 1, Jing Li 1, Yusheng Liu 2,* and Deying Ma 1,*

1 Engineering Research Centre of Cotton, Ministry of Education, Key Laboratory of the Pest Monitoring and Safety Control on Crop and Forest, College of Agronomy, Xinjiang Agricultural University, 311 Nongda East Road, Urumqi 830052, China
2 College of Plant Protection, Shandong Agricultural University, Tai’an 271018, China
* Correspondence: ysl8877@163.com (Y.L.); mdyxnd@163.com (D.M.);
Tel.: +86-131-0538-6599 (Y.L.); +86-189-9984-6225 & +86-131-7683-1480 (D.M.)

Abstract: Cotton stalks (CS) are a potential agricultural biomass resource. We investigated the use of CS as a feed for *Proteatia brevitarsis* Lewis larvae and the resulting frass (larvae dung-sand) as a fertilizer. Based on a three-factor experiment (decomposition inoculant, fermentation duration, and cattle manure ratio), the optimal parameters for the transformation of CS using *P. brevitarsis* larvae were determined as 40–50% of cattle manure, the use of VT inoculant and a fermentation duration of 25–30 days. Regarding the products of the transformation, the protein content of the larval body was as high as 52.49%, and the fat content was 11.7%, which is twice more than that of the organic fertilizer standard (NY525-2021, Beijing, China, TNPK ≥ 4.0%). The application of CS as feed (larval body) and fertilizer (larvae dung-sand) is feasible, promoting the utilization of both CS and cattle manure.

Keywords: cotton stalks; manure; decomposition inoculant; *Proteatia brevitarsis* Lewis; biotransformation; feed; fertilizer

1. Introduction

The Xinjiang Uyghur autonomous region is the most important area for cotton (*Gossypium hirsutum* L.) production in China. The cotton planting area is about 2.5 million hectares, and the cotton yield exceeds 5.0 million tons [1]. This area also produces cotton stalks (CS) equivalent to five times the cotton yield. Excluding the cotton leaves and root stubble, the...
CS yield that can be mechanically harvested is approximately 12 million tons [2]. With the characteristics of high calorific value, prominent cellulose and lignin content, and abundant nutrients, CS is used as a renewable agricultural biomass resource for energy [3,4], industrial raw materials [5], fertilizer [6], and feed [7,8]. However, more than 80% of CS is currently crushed and returned to the field directly as fertilizer [9,10]. The fertilizer effect of CS has been diminishing due to the direct return to the field in successive years. Meanwhile, the disadvantageous effects (e.g., aggravation of cotton Verticillium wilt (Verticillium dahliae kieb), deterioration of the soil structure) on cotton growth, yield, and quality have become more apparent [11–15]. For this reason, the indirect return of CS to the field has been attracting increased attention. In recent years, technologies and the utilization of micro-livestock (e.g., environmental insects, earthworms) to transform organic waste (e.g., crop residues, livestock manure) into feed and fertilizer have been attracting greater attention [16–26]. Micro-livestock has notable advantages in reducing greenhouse gas emissions (e.g., CO2, CH4) and promoting carbon peaking and carbon neutrality strategies [27–29]. In particular, the application potential of *Proteatia brevitarsis* Lewis larvae to transform crop stalks and animal manure is outstanding [30,31].

*P. brevitarsis* is an insect belonging to the genus *Protaetia*, the family Cetoniidae, and the order Coleoptera, which is widely distributed in China, Russia, North Korea, Mongolia, and other countries [32,33]. Adults are phytophagous or saprophagous, which are harmful in nature [34]. The larvae are saprophagous, which have strong transformation capability and can transform crop stalks [35–37], animal manure [38–40], edible fungus chaff [41–44] and other organic wastes efficiently. Dry larvae are a relatively high-quality protein feed ingredient with a protein content of about 50% [45–48]. Frass (larvae dung-sand) is rich in humic acids (HAs), beneficial microorganisms and nutrient elements, and it has suitable granularity and stable properties [49,50]. Dung-sand is an excellent raw material for bio-fertilizer and has shown promising effects in the cultivation of horticultural crops [51–54]. The larvae, together with the larvae of other Scarabaeoidae (i.e., *Holotrichia parallela* Motschulsky), are known as grubs. As the traditional medicine and feed insects in China and Korea, grubs have functions in anticancer [55,56], antibacterial [57], antioxidant [58], and anti-inflammation [59,60]; therefore, *P. brevitarsis* has suitable development prospects in food and feed industries [61]. On the other hand, the genome and transcriptome sequencing of *P. brevitarsis* has been completed, which lays the foundation for in-depth research and development of its resource value of *P. brevitarsis* [62,63]. In conclusion, *P. brevitarsis* has potential resources in the fields of transforming organic wastes, pharmaceutical applications, feed ingredients and organic fertilizers.

Decomposition microorganisms promote pre-decomposition and humification of materials and provide assistance to carrion feeders (e.g., earthworms, dung beetles, wood-eating beetles, the black soldier fly (*Hermetia illucens* L.), etc.) in feeding and digesting food [64–69]. Studies have shown that fermentation of lignin- and cellulose-rich organic materials with specific microbial inoculants followed by vermicomposting or insect composting can not only improve the yield of production and nutritional value of frass but also shorten the time for organic materials to become standard organic fertilizer [70–75]. Based on the previous work, this study initially screened five decomposition inoculants suitable for the pre-treatment of organic waste from the transformation of *P. brevitarsis* larvae [31,40]. On the other hand, the C/N ratio is essential for material decomposition [76–78]. This study chose cattle manure, which is plentiful in the Xinjiang region and is a better feed for *P. brevitarsis* larvae, as the auxiliary material to adjust the C/N ratio of the raw materials [79]. Previous studies have shown that fermentation duration is another key factor affecting the transformation capability of *P. brevitarsis* larvae [37,46]. We carried out a three-factor (decomposition inoculant, fermentation duration, and cattle manure ratio) five-level orthogonal experiment to explore the best technical parameters of the transformation capability for CS using *P. brevitarsis* larvae and to evaluate the application potential of the larval body as a feed ingredient and larvae dung-sand as organic fertilizer. The significance of
this study is to provide a method reference for improving the transformation capability of organic waste and promoting the utilization of cotton stalks and cattle manure.

2. Materials and Methods

2.1. Experimental Site

The experimental site was located in the Industrialization Research Base of Environmental Insect Transforming Organic Waste, Xinjiang Agricultural University, in Manas County (44°13′49″ N, 86°23′3″ E), Changji Prefecture, China.

2.2. Experimental Materials

Cotton stalks (CS) and cattle manure were taken from farmers or herders around the base. The larvae of *P. brevitarsis* were self-reproduced in the base. Materials such as decomposition inoculants (Table 1), cucumber (*Cucumis sativus* L.) seeds (Changchun Mithorn, Xinjiang Lianchuang Seed Co., Ltd., Urumqi, China; for the determination of the seed germination index), electronic balance (LT3002, Changshu Tianliang Instrument Co., Ltd., Changshu, China) and experimental tools were purchased or previously owned.

### Table 1. Introduction and instructions for decomposition inoculants.

| Decomposition Inoculants | Brand and Production Company | Main Functional Bacteria | Effective Number of Viable Bacteria (100 million/g) | Recommended Dosage (kg/t) |
|--------------------------|-----------------------------|--------------------------|---------------------------------------------------|--------------------------|
| LK                       | Organic material decomposing inoculant, stalks type, Zhongnong Lvkang Biotechnology Co., Ltd., Beijing, China | *Bacillus*, *Trichoderma*, and yeast | 8 | 0.5 |
| LL                       | Organic fertilizer decomposing inoculant, Shandong Lvlong Biotechnology Co., Ltd., Zhucheng, China | *Bacillus subtilis*, *Bacillus licheniformis*, yeast, and *Trichoderma viride* | 200 | 10 |
| NFK *                    | Organic material decomposing inoculant, Henan NongFukang Biotechnology Co., Ltd., Zhengzhou, China | Mainly *Bacillus licheniformis*, *Candida utilis*, *Bacillus subtilis*, *Lactobacillus*, and *Enterococcus-like bacteria* Bacteria (*Bacillus subtilis*, *Bacillus licheniformis*, and *Bacillus jelly*), filamentous fungi, and yeast | 0.1 | 30 |
| RW                       | RW decomposing inoculant, stalks type, Hebi Renyuan Biological Co., Ltd., Hebi, China | *Bacillus*, actinomycetes, lactic acid bacteria, and molds | 100 | 10 |
| VT                       | VT-1000, stalks type, Beijing VOTO Biotechnology Co., Ltd., Beijing, China | *Bacillus* | 200 | 1 |

* Decomposition inoculants need to be activated in advance.

2.3. Experimental Methods

2.3.1. Preliminary Selection of the Optimal Combination of Decomposition Inoculant, Fermentation Duration, and Cattle Manure Ratio

CS and cattle manure were dried and crushed for use. The three-factor five-level orthogonal experiment (Table 2) of decomposition inoculant, cattle manure ratio and fermentation duration were conducted in September 2020. A total of 25 treatments were designed by IBM SPSS Statistics 23.0 (SPSS 23.0) (L25 (5^2) orthogonal table) and recorded as A_{1,5} B_{1,5} C_{1,5}. The CK groups were the CS fermented for 0, 10, 15, 20, 25, and 30 days. The initial materials for every treatment were 90 kg (dry weight, the same as below). The decomposition inoculants were added at the recommended amount. The water content...
(WC) of the materials was adjusted to 65 (±5)%. Then, the materials were mixed and piled into a cone shape. The ambient temperature and fermentation temperature of material pile (20 cm depth) were recorded daily. Samples were taken from 20 to 30 cm below the surface of material pile (five-point sampling method) according to the days of fermentation duration for each treatment. Each sample weighed 3 kg (fresh weight) and was frozen and stored in the refrigerator. In strict accordance with the process of turning the material pile every 5 days and sampling first and then turning the pile, and the material fermentation and sampling experiments were finished after 30 days.

Table 2. Orthogonal experimental factors and levels.

| Level | Decomposing Inoculants (A) | Cattle Manure Ratio (B/%) | Fermentation Duration (C/d) |
|-------|---------------------------|--------------------------|---------------------------|
| 1     | LK                        | 10                       | 10                        |
| 2     | LL                        | 20                       | 15                        |
| 3     | NFK                       | 30                       | 20                        |
| 4     | RW                        | 40                       | 25                        |
| 5     | VT                        | 50                       | 30                        |

The samples were thawed naturally, and each culture box (1 L) was filled with 280 g of fresh material (about 80 g dry weight), 10 larvae (the 3rd instar and 15th day) of *P. brevitarsis* were put into the box. Thereafter, the transformation experiment was carried out for 15 days. Each treatment was repeated four times. On the 16th day, weighing larvae weight gain, feed intake and dung-sand weight, the feed utilization rate, dung-sand conversion rate and mortality were calculated by Liu (2012) [80]. The optimum technical parameters were selected by making a comprehensive comparison of the transformation capability of larvae.

Calculation formula (Mass unit/mg):

Feed utilization rate = \((\text{total feed weight} - \text{remaining feed weight}) / \text{total feed weight} \times 100\%\) \hspace{1cm} (1)

Dung-sand conversion rate = \(\text{Dung-sand weight} / (\text{feeding weight} - \text{dry larvae weight gain}) \times 100\%\) \hspace{1cm} (2)

Mortality = \(\text{number of dead larvae} / \text{number of tested larvae} \times 100\%\) \hspace{1cm} (3)

2.3.2. Validation of the Optimal Technical Parameters for CS as Feed and Fertilizer

The validation experiment was carried out in May 2021. The optimal combination based on the experimental results of Section 2.3.1 was \(A_5B_4C_4\): VT inoculant, the ratio of cattle manure was 40%, and the fermentation duration was 25 days. The control feed (CK) was cotton stalks fermented for 25 days, and the specific operation is referred to in Section 2.3.1. Thereafter, we determined the transformation capability data of the *P. brevitarsis* larvae to CS and verified the feasibility of the optimal technical parameters.

2.3.3. Determination of Related Nutritional Indicators for CS Transformation Products as Feed and Fertilizer

The feed or fertilizer nutrition indicators of the raw materials (CS and cattle manure), fermented materials (fermented CS and \(A_5B_4C_4\) feed), and products (dry larvae and larvae dung-sand) of the optimal treatment and control were determined (refer to GB 13078-2017 and NY525-2021 standards, Beijing, China, and tested by Sichuan Weil Testing Technology Co., Ltd., Chengdu, China. The seed germination index was determined by referring to the appendix of NY525-2021, Beijing, China). To explore the application potential of CS transformation by *P. brevitarsis*.

2.4. Data Processing

SPSS 23.0 was used to conduct a three-factor five-level analysis of variance with repeated observations and no interaction. One-Way ANOVA was performed for the CK
groups and the three factors, and Tukey’s multiple comparison analysis was performed for the differences between different treatments ($p < 0.05$). Microsoft Excel 2013 was used to record and organize data and draw tables. Sigma Plot 14 was used to draw graphs.

3. Results

3.1. Preliminary Selection of the Optimal Combination of Decomposition Inoculant, Fermentation Duration, and Cattle Manure Ratio

3.1.1. Effect of Fermentation Duration on Transformation Capability to CS Using *P. brevitarsis* Larvae

As shown in Table 3, the transformation capability of the *P. brevitarsis* larvae on CS was significantly different under different fermentation duration. The optimal indexes of feed intake, larvae weight gain, and feed utilization rate were 25 days after fermentation. The dung-sand weight was the best after 20 days of fermentation, but the difference was insignificant compared with 25 days of fermentation. The dung-sand conversion rate was optimal after 15 days of fermentation, which was not significantly different from that after 20 days of fermentation. The mortality of larvae was the lowest at the 15 and 25 days of fermentation duration, and there was no significant difference among all treatments. Comprehensive analysis showed that the transformation capability of the *P. brevitarsis* larvae on CS was the best for 25 days after fermentation.

| Fermentation Duration (d) | Feed Intake (g) | Larvae Weight Gain (g) | Dung-Sand Weight (g) | Feed Utilization Rate (%) | Dung-Sand Conversion Rate (%) | Mortality (%) |
|---------------------------|-----------------|------------------------|----------------------|---------------------------|------------------------------|---------------|
| 0                         | 48.50 ± 1.18a   | 1.89 ± 0.09a           | 19.16 ± 0.28d        | 54.78 ± 1.33b             | 41.17 ± 1.27d               | 5.00 ± 2.89a  |
| 10                        | 37.68 ± 1.13c   | 1.81 ± 0.10a           | 28.32 ± 0.30c        | 44.11 ± 1.32c             | 79.17 ± 2.65ab              | 2.50 ± 2.50a  |
| 15                        | 36.33 ± 0.44c   | 1.82 ± 0.10a           | 30.91 ± 0.31b        | 45.14 ± 0.55c             | 89.57 ± 0.63a               | 2.50 ± 2.50a  |
| 20                        | 49.11 ± 0.64a   | 2.04 ± 0.13a           | 36.98 ± 0.60a        | 62.83 ± 0.81a             | 78.54 ± 0.48ab              | 2.50 ± 2.50a  |
| 25                        | 49.24 ± 0.46a   | 2.18 ± 0.10a           | 35.24 ± 0.61a        | 64.66 ± 0.60a             | 74.86 ± 0.85c               | 0.00 ± 0.00a  |
| 30                        | 41.58 ± 0.50b   | 1.92 ± 0.04a           | 32.30 ± 0.75b        | 55.03 ± 0.67b             | 81.45 ± 1.41b               | 2.50 ± 2.50a  |

Data in the table are mean ± standard error (SE). Different letters in the same column indicate a significant difference ($p < 0.05$). The same is below.

3.1.2. Influence of Three Factors on the Fermentation Temperature of Materials

As shown in Table 4, under the fermentation cycle of every 5 days, the influence of the decomposition inoculant on the fermentation temperature of the material pile did not reach a significant difference level, and the overall situation was relatively stable. The influence of the ratio of cattle manure on the fermentation temperature of the material pile reached a significant difference level on the 10th, 20th, and 30th days. In the first 20 days, the fermentation temperature of the material pile at the 10% cattle manure group was the highest, and that of the 50% cattle manure group was lower. After 25 days, the temperature showed an opposite trend. In terms of fermentation duration, only 25 days of fermentation showed a significant difference level, which should be the inflection point of material fermentation temperature. After 30 days of fermentation, except for the CK group, the fermentation temperature of 25 treatments was above 30 °C, which was much higher than the ambient temperature on the same day. In the early stage, the temperature of the CK group was high, but the temperature dropped sharply after 20 days. The temperature of the 25 treatments only dropped significantly after 25 days of fermentation, which was related to the degree of material fermentation entering the later stage and also related to the low ambient temperature (the average temperature after 20 days was lower than 10 °C). The trend of temperature variation among different treatments showed that adding decomposition inoculant and cattle manure could maintain the temperature of the material pile in a high and stable range and then promote the fermentation of CS.
Table 4. Effect of the decomposition inoculant, cattle manure ratio, and fermentation duration on the fermentation temperature of the material pile.

| Factor and Level | Temperature (°C) | 1 d  | 5 d  | 10 d | 15 d | 20 d | 25 d | 30 d |
|------------------|-----------------|------|------|------|------|------|------|------|
| Decomposing inoculants (A) |                  |      |      |      |      |      |      |      |
| LK               | 41.80 ± 2.51a   | 55.58 ± 3.78a | 48.50 ± 1.74a | 48.88 ± 1.31a | 47.72 ± 0.52a | 47.88 ± 1.35a | 43.30 ± 2.04a |
| LL               | 41.04 ± 2.07a   | 53.18 ± 2.75a | 48.20 ± 1.69a | 46.08 ± 1.64a | 47.86 ± 1.39a | 49.40 ± 1.79a | 43.60 ± 0.46a |
| NFK              | 42.98 ± 2.74a   | 52.68 ± 3.94a | 48.16 ± 1.92a | 48.94 ± 2.44a | 48.94 ± 1.85a | 49.24 ± 1.81a | 44.66 ± 1.32a |
| RW               | 41.06 ± 1.89a   | 50.24 ± 3.63a | 49.86 ± 2.32a | 46.46 ± 0.92a | 46.64 ± 2.11a | 47.26 ± 1.75a | 42.58 ± 1.40a |
| VT               | 40.76 ± 1.22a   | 54.82 ± 1.35a | 49.78 ± 2.43a | 48.78 ± 1.03a | 46.86 ± 1.34a | 48.52 ± 1.67a | 42.14 ± 2.86a |
| Cattle manure ratio (B/%) |                  |      |      |      |      |      |      |      |
| 10               | 43.80 ± 2.13a   | 58.34 ± 1.74a | 53.22 ± 1.70a | 50.36 ± 1.06a | 51.10 ± 1.35a | 50.30 ± 1.96a | 38.78 ± 2.00b |
| 20               | 40.86 ± 2.98a   | 55.64 ± 2.89a | 49.50 ± 1.18ab | 47.76 ± 1.24a | 48.20 ± 0.22ab | 50.96 ± 1.48a | 45.20 ± 0.98a |
| 30               | 42.88 ± 1.18a   | 50.70 ± 2.77a | 46.42 ± 1.71ab | 47.66 ± 1.13a | 47.24 ± 1.39ab | 46.76 ± 1.70a | 43.48 ± 1.71ab |
| 40               | 41.42 ± 1.80a   | 53.98 ± 3.87a | 48.36 ± 1.95ab | 44.92 ± 2.30a | 45.08 ± 1.41b | 46.20 ± 1.07a | 42.98 ± 1.05ab |
| 50               | 36.68 ± 1.43a   | 47.84 ± 2.38a | 46.10 ± 1.43b | 48.44 ± 1.14a | 46.40 ± 1.35ab | 48.08 ± 0.68ab | 45.84 ± 0.78ab |
| Fermentation duration (C/d) |                |      |      |      |      |      |      |      |
| 10               | 39.68 ± 1.69a   | 51.14 ± 2.01a | 49.40 ± 2.21a | 46.30 ± 1.53a | 47.78 ± 0.35a | 48.22 ± 1.61ab | 43.74 ± 0.97a |
| 15               | 42.36 ± 2.25a   | 52.26 ± 2.65a | 47.00 ± 1.60a | 47.98 ± 1.00a | 47.62 ± 0.70a | 45.14 ± 0.42b | 40.72 ± 1.47a |
| 20               | 41.74 ± 2.06a   | 59.20 ± 1.40a | 48.92 ± 2.27a | 48.10 ± 2.36a | 48.64 ± 2.51a | 48.28 ± 2.01ab | 43.60 ± 1.77a |
| 25               | 40.48 ± 2.14a   | 50.60 ± 3.50a | 48.88 ± 2.25a | 47.70 ± 1.61a | 46.88 ± 1.79a | 49.12 ± 0.75ab | 42.60 ± 2.58a |
| 30               | 43.38 ± 2.27a   | 53.30 ± 4.41a | 49.40 ± 1.63a | 49.06 ± 1.35a | 47.10 ± 1.46a | 51.54 ± 1.53a | 45.62 ± 1.08a |
| CK               | 48.50           | 57.60           | 52.90           | 45.60           | 40.10           | 21.90           | 17.30           |
| Ambient temperature | 16.50         | 15.50         | 20.50         | 18.50         | 12.00         | 9.50         | 6.50         |

3.1.3. Differences in the Transformation Capability of the *P. brevitarsis* Larvae on CS Considering Three Factors

Table 5 has shown that the transformation capability of the *P. brevitarsis* larvae with different decomposition inoculants was significantly different in the indexes of feed intake and weight gain but not significantly different in the other four indexes, and VT inoculant was the best. As for the factor of cattle manure ratio, 40% and 50% groups showed the best performance, and the indexes of feed intake, dung-sand weight, feed utilization rate, and dung-sand conversion rate were significantly different from the 10% and 20% groups. The transformation capability of the *P. brevitarsis* larvae was the best at 25 days and 30 days after fermentation, and the feed intake, dung-sand weight, and feed utilization rate of the third instar larvae were significantly higher than those at 10 days after fermentation. The difference in transformation capability of the larvae under the three factors provided suitable support for optimizing the technical parameters of the transformation of CS using the *P. brevitarsis*.

3.1.4. Test of Inter-Subjects Effects under Three Factors

It can be seen from Table 6 that the effects of the three factors on feed intake, dung-sand weight, feed utilization rate, and dung-sand conversion rate were significantly different, while the differences in larvae weight gain and mortality were not significant. This experiment mainly analyzed four indexes with significant differences. According to the comparison of the type III sum of squares, the order of influencing factors for the feed intake was from largest to smallest: B > C > A. For the three assessment indicators of dung-sand weight, feed utilization, and dung-sand conversion rate, the order of the three effect factors was C > B > A.
Table 5. Effect of the decomposition inoculant, cattle manure ratio, and fermentation duration on the transformation capability of the 3rd instar larvae of *P. brevitarsis*.

| Factor and Level | Feed Intake (g) | Larvae Weight Gain (g) | Dung-Sand Weight (g) | Feed Utilization Rate (%) | Dung-Sand Conversion Rate (%) | Mortality (%) |
|------------------|-----------------|------------------------|----------------------|---------------------------|-------------------------------|---------------|
| Decomposing inoculants (A) |                 |                        |                      |                           |                               |               |
| LK               | 52.48 ± 2.16ab  | 1.833 ± 0.043ab       | 38.35 ± 1.95a       | 72.99 ± 3.02a             | 75.46 ± 1.37a                 | 0.50 ± 0.50a  |
| LL               | 54.32 ± 1.33ab  | 1.928 ± 0.051ab       | 40.34 ± 1.56a       | 75.14 ± 2.57a             | 76.78 ± 1.89a                 | 1.00 ± 0.69a  |
| NFK              | 54.33 ± 1.22ab  | 1.886 ± 0.048ab       | 40.99 ± 0.99a       | 76.81 ± 1.78a             | 78.19 ± 0.81a                 | 1.00 ± 0.69a  |
| RW               | 48.66 ± 1.69b   | 1.733 ± 0.054b        | 36.88 ± 1.57a       | 69.85 ± 3.08a             | 78.32 ± 1.07a                 | 1.50 ± 1.09a  |
| VT               | 55.53 ± 1.18a   | 1.949 ± 0.051a        | 40.81 ± 1.20a       | 78.10 ± 1.81a             | 76.06 ± 1.37a                 | 1.50 ± 0.82a  |
| Cattle manure ratio (B/%) |            |                        |                      |                           |                               |               |
| 10               | 49.76 ± 1.50bc  | 1.799 ± 0.060a        | 33.58 ± 1.03c       | 64.66 ± 2.39c             | 70.33 ± 1.37c                 | 2.50 ± 1.23a  |
| 20               | 47.43 ± 1.96c   | 1.846 ± 0.058a        | 34.43 ± 1.49c       | 65.60 ± 2.43c             | 75.53 ± 0.87b                 | 1.00 ± 0.69a  |
| 30               | 53.89 ± 1.52ab  | 1.895 ± 0.042a        | 39.68 ± 0.95b       | 77.80 ± 1.86b             | 76.64 ± 1.15b                 | 1.00 ± 0.69a  |
| 40               | 55.25 ± 0.61a   | 1.905 ± 0.047a        | 43.22 ± 0.79ab      | 79.19 ± 0.63ab            | 80.97 ± 0.90a                 | 0.50 ± 0.50a  |
| 50               | 58.99 ± 0.71a   | 1.904 ± 0.046a        | 46.45 ± 0.71a       | 85.64 ± 0.94a             | 81.35 ± 0.63a                 | 0.50 ± 0.50a  |
| Fermentation duration (C/d) |        |                        |                      |                           |                               |               |
| 10               | 46.34 ± 2.15c   | 1.863 ± 0.068a        | 34.60 ± 1.71b       | 65.45 ± 3.73b             | 73.62 ± 1.62a                 | 1.00 ± 1.00a  |
| 15               | 51.54 ± 1.15b   | 1.906 ± 0.040a        | 36.77 ± 1.50b       | 72.68 ± 2.13ab            | 73.68 ± 1.68a                 | 1.00 ± 0.69a  |
| 20               | 51.69 ± 1.20b   | 1.821 ± 0.040a        | 39.00 ± 1.23ab      | 74.47 ± 2.18a             | 78.16 ± 1.48a                 | 2.00 ± 0.92a  |
| 25               | 57.05 ± 0.81a   | 1.843 ± 0.047a        | 43.46 ± 0.98a       | 80.28 ± 1.28a             | 78.58 ± 0.74a                 | 0.50 ± 0.50a  |
| 30               | 58.70 ± 0.75a   | 1.917 ± 0.056a        | 43.53 ± 0.91a       | 80.01 ± 1.12a             | 76.78 ± 1.63a                 | 1.00 ± 0.69a  |

Table 6. Tests of inter-subjects effects.

| Source                      | Dependent Variable | Type III Sum of Squares | df  | Mean Square | F    | Sig. |
|-----------------------------|--------------------|-------------------------|-----|-------------|------|------|
| Corrected Model             | Feeding intake     | 418.966 ^a             | 12  | 348.916     | 29.758 | 0.000 |
|                            | Larval weight gain | 0.806 ^b               | 12  | 0.067       | 1.353  | 0.204 |
| Decomposition inoculant (A) | Feed utilization rate | 3987.502 ^c          | 12  | 332.292     | 57.961 | 0.000 |
|                            | Dung-sand weight   | 1.049 ^d               | 12  | 0.087       | 31.856 | 0.000 |
|                            | Mortality          | 0.206 ^e               | 12  | 0.017       | 9.815  | 0.000 |
| Cattle manure ratio (B)     | Feed intake        | 581.020                 | 4   | 145.255     | 12.388 | 0.000 |
|                            | Dung-sand weight   | 256.548                 | 4   | 64.137      | 11.187 | 0.000 |
|                            | Feed utilization rate | 0.085 ^f               | 4   | 0.021       | 7.760  | 0.000 |
|                            | Dung-sand conversion rate | 0.013 ^g             | 4   | 0.003       | 1.848  | 0.127 |
| Fermentation duration(C)    | Feed intake        | 1940.292                | 4   | 485.073     | 41.371 | 0.000 |
|                            | Dung-sand weight   | 1272.551                | 4   | 318.138     | 55.492 | 0.000 |
|                            | Feed utilization rate | 0.298 ^h               | 4   | 0.074       | 27.140 | 0.000 |
| Error                      | Feed intake        | 1665.684                | 4   | 416.421     | 35.516 | 0.000 |
|                            | Dung-sand weight   | 2458.403                | 4   | 614.601     | 107.204 | 0.000 |
|                            | Feed utilization rate | 0.666 ^i               | 4   | 0.166       | 60.666 | 0.000 |
| Corrected total            | Feed intake        | 1020.076                | 87  | 11.725      |       |      |
|                            | Larval dry weight  | 4.318                   | 87  | 0.050       |       |      |
|                            | Dung-sand weight   | 498.772                 | 87  | 5.733       |       |      |
|                            | Feed utilization rate | 0.239 ^j             | 87  | 0.003       |       |      |
|                            | Dung-sand conversion rate | 0.153 ^k             | 87  | 0.002       |       |      |
|                            | Mortality          | 0.109                   | 87  | 0.001       |       |      |

^a. R squared = 0.804 (adjusted R squared = 0.777), ^b. R squared = 0.157 (adjusted R squared = 0.041), ^c. R squared = 0.889 (adjusted R squared = 0.873), ^d. R squared = 0.815 (adjusted R squared = 0.789), ^e. R squared = 0.575 (adjusted R squared = 0.517), ^f. R squared = 0.078 (adjusted R squared = −0.049).
3.1.5. Intuitive Analysis and Tukey Test under Three Factors

As can be seen from Figure 1, when the feed intake (a) and dung-sand weight (b) were used as the screening indicators, the optimal combination of the decomposition inoculant (A), cattle manure ratio (B), and fermentation duration (C) was: VT inoculant, 40% (50%) of cattle manure ratio, and 30 days of fermentation duration.

When the dung-sand utilization rate (c) was used as the screening indicator, the optimal combination of the decomposition inoculant, cattle manure ratio, and fermentation duration was: VT inoculant, 40% (50%) of cattle manure ratio, and 30 days of fermentation duration.

When the dung-sand conversion rate (d) was used as the screening indicator, the RW and NFK inoculant, 40% of cattle manure ratio, and 30 days (25 days) of fermentation duration were optimum.

According to the results of intuitive analysis and Tukey’s test (Figure 1), and referring to the results that the transformation capability of the P. brevitarsis larvae was the best when CS was fermented for a duration of 25 days (Table 3), the principles of minimizing cattle manure ratio, shortening fermentation duration, and reducing treatment cost were also considered. The optimal combination was A5B4-5C4 (0.1% VT inoculant, 40–50% of cattle manure ratio, and 25–30 days of fermentation duration), and A5B4C4 was given preference.

Figure 1. Effect of the decomposition inoculant, cattle manure ratio, and fermentation duration on the feed intake (a), dung-sand weight (b), feed utilization rate (c), and dung-sand conversion rate (d) of the 3rd instar larvae of P. brevitarsis. Tukey’s multiple-range tests were used for the analysis. The same factor with a different letter indicated a significant difference ($p < 0.05$, $n = 20$).
3.2. Validation of the Optimal Technical Parameters for the Transformation of CS Using *P. brevitarsis* Larvae

CS fermentation and transformation experiments were performed under the optimal combination (A$_5$B$_4$C$_4$). The results are shown in Table 7.

**Table 7.** Transformation capability of the 3rd instar larvae of *P. brevitarsis* under the optimal combination.

| Treatments | Feed Intake (g) | Larvae Weight Gain (g) | Dung-Sand Weight (g) | Feed Utilization Rate (%) | Dung-Sand Conversion Rate (%) | Mortality (%) |
|------------|-----------------|------------------------|----------------------|---------------------------|-----------------------------|---------------|
| CK         | 51.92 ± 0.37    | 2.030 ± 0.102          | 40.48 ± 0.39         | 64.90 ± 0.46              | 81.13 ± 0.38                | 2.50 ± 2.50 * |
| A$_5$B$_4$C$_4$ | 64.06 ± 0.52 *  | 2.338 ± 0.049          | 52.19 ± 0.60 *       | 80.07 ± 0.65 *            | 84.55 ± 0.53 *              | 0.00 ± 0.00   |

Using independent sample T-test, * means significantly different (Tukey test, $p < 0.05$, n = 4).

It can be seen from Table 7 that under the optimal technology combination, the transformation capability of the *P. brevitarsis* larvae on the A$_5$B$_4$C$_4$ feed was significantly different in feed intake, dung-sand weight, feed utilization rate, dung-sand conversion rate, and mortality with that of CK, and the feed utilization rate and dung-sand conversion rate were over 80%. Therefore, the optimal technical parameters for CS resource utilization were determined as A$_5$B$_4$C$_4$: 0.1% VT inoculant, 40% of cattle manure ratio, and 25 days of fermentation duration. The fresh weight of fermentation material (A$_5$B$_4$C$_4$ feed) was weighed, the water content was measured, and the yield of the material was calculated to be 62.85%. It can be concluded that 104.75 g of A$_5$B$_4$C$_4$ feed can be obtained by adding 66.67 g of cattle manure for every 100 g of CS raw material. A total of 70.92 g of larvae dung-sand can be obtained by the third instar larvae of *P. brevitarsis*, and the weight gain of the dry larvae is 3.06 g, and 20.88 g of residue is left.

3.3. Determination of Relevant Nutritional Indicators of Raw Materials, Fermentation Materials, and Products

3.3.1. Determination of Nutritional Indicators of Raw Materials, Fermented Materials, and Insect Bodies as Feed

It can be seen from Table 8 that the protein content of fermented CS increased by 41.9%, the crude fiber content decreased slightly, the content of gross energy (GE) was slightly increased, and the contents of crude ash and water-soluble chlorides increased greatly. The crude protein (CP) content of A$_5$B$_4$C$_4$ feed reached 13.18%, which was slightly lower than 14.16% of cow manure and was 1.29 and 1.84 times that of the fermented and unfermented CS. Compared to the fermented CS, the A$_5$B$_4$C$_4$ feed significantly reduced crude fiber content, increased the crude ash and water-soluble chloride content, and decreased GE. The content of free gossypol (FG) in fermented materials was about 50% lower than that in raw materials. The FG in the A$_5$B$_4$C$_4$ feed was not detected in the larvae of *P. brevitarsis* (detection limit is 20 mg/kg). The protein (52.49%) and fat (11.7%) content of the *P. brevitarsis* dry larvae were much higher than those of the A$_5$B$_4$C$_4$ feed, while the content of crude fiber was only 6.1%, and the content of water-soluble chloride was lower than that of the A$_5$B$_4$C$_4$ feed. The GE (19.20 KJ/g) was intermediate between carbohydrate (17.5 KJ/g) and protein (23.64 KJ/g). The insect-microorganism composite systems can improve the nutrition indicators of CS as a feed, and the larval body was 7.31, 19.50, and 1.16 times higher than that of CS in protein, fat, and total energy and more than 50% lower in FG, and the content of crude fiber is only 1/6 of CS.
Table 8. Key nutritional indicators for raw materials, fermented materials, and dry larvae as feed ingredients.

| Material Types       | WC (%) | CP (%) | Crude Fat (%) | Crude Fiber (%) | Crude Ash (%) | Water-Soluble Chloride (%) | FG (mg/kg) | GE (KJ/g) |
|----------------------|--------|--------|---------------|-----------------|---------------|---------------------------|------------|-----------|
| CS                   | 8.6    | 7.18   | 0.6           | 43.3            | 5.1           | 0.40                      | 96         | 16.57     |
| Cattle manure        | 79.2   | 14.16  | 0.6           | 27.4            | 17.6          | 1.20                      | 114        | 14.74     |
| Fermented CS         | 69.7   | 10.19  | 0.3           | 43.2            | 9.6           | 0.75                      | 47         | 17.1      |
| A₅B₄C₄ feed         | 71.2   | 13.18  | 0.3           | 34.7            | 15.9          | 1.60                      | 59         | 15.32     |
| Dry larvae           | 72.0   | 52.49  | 11.7          | 6.1             | 15.6          | 1.00                      | -          | 19.2      |

3.3.2. Determination of Nutritional Indicators for Raw Materials, Fermentation Materials, and Larvae Dung-Sand as Organic Fertilizer

As shown in Table 9, the organic matter (OM) content of the six materials was above 54%, and the CS was the highest (67%). Their total nutrient (TNPK) content was more than 4.0%. The total nutrient (TNPK) and potassium (TK) content of the A₅B₄C₄ feed were 9.04% and 4.44%. For the germination index (GI), the unfermented CS (47.09%) and manure (66.87%) had certain toxicity to seed germination, the GI of the remaining four materials was more than 70%, indicating that it was non-toxic to seed germination, and the GI of fermented CS was 102.88, which could promote the seed germination. The pH value of the six materials ranged from 6.6 to 9.5, and it was neutral to alkaline overall. OM decreased, HAs and GI increased first and then decreased, and TNPK, water-soluble chloride, and pH values increased in the insect-microorganism composite process from raw materials to fermentation materials and then to larvae dung-sand. In addition to pH value, two kinds of fermentation materials and two kinds of larvae dung-sand were in line with the latest standards of organic fertilizers in China in terms of OM, NPK, and GI (NY525-2021, NPK ≥ 4%, DOM ≥ 30%, GI ≥ 70%, pH 5.5–8.5).

Table 9. Main nutritional indicators for raw materials, fermentation materials, and larvae dung-sand as organic fertilizer.

| Material Types                  | WC (%) | OM (%) | HAs (%) | TN (%) | TP (%) | TK (%) | TNPK (%) | pH   | Water-Soluble Chloride (%) | GI (%) |
|---------------------------------|--------|--------|---------|--------|--------|--------|----------|------|----------------------------|-------|
| CS                              | 8.6    | 67.0   | 1.06    | 1.29   | 0.99   | 2.35   | 4.63     | 6.6  | 0.40                       | 47.09 |
| Manure                          | 79.2   | 58.9   | 1.59    | 2.3    | 1.29   | 2.18   | 5.77     | 8.9  | 1.20                       | 66.87 |
| Fermented CS                    | 69.7   | 65.9   | 2.31    | 2.23   | 0.42   | 3.84   | 6.49     | 9.3  | 0.75                       | 102.88|
| A₅B₄C₄ feed                    | 71.2   | 59.5   | 1.82    | 2.54   | 1.16   | 4.13   | 7.83     | 9.5  | 1.60                       | 98.73 |
| CS-based larvae dung-sand       | 65.6   | 61.3   | 1.38    | 2.68   | 0.87   | 4.55   | 8.1      | 9.4  | 0.95                       | 77.35 |
| A₅B₄C₄d feed-based larvae dung-sand | 68.7   | 54.8   | 0.81    | 2.93   | 1.67   | 4.44   | 9.04     | 9.2  | 1.60                       | 75.90 |

4. Discussion

This study showed that for every 100 g of cotton stalks supplemented with 66.67 g of manure, 104.75 g of A₅B₄C₄ feed was obtained, and 70.92 g of dung-sand was obtained after transformation by the third instar larvae of P. brevitarsis. The weight gain of the dry larvae was 3.06 g, and 20.88 g of residue remained. The larvae of the P. brevitarsis had a 27.41-fold ability to transform fermented materials (FCR = weight of feed intake/weight gained), which was nearly six times higher than that of the black soldier fly (FCR = 4.5), and had a higher feed utilization rate (80.07% ± 0.65%) and dung-sand conversion rate (84.55% ± 0.53%) [73]. Compared with other dung beetles, P. brevitarsis are more suitable to perform the ecological function of converting organic waste in concentrated agricultural and livestock areas because of their high reproductive ability and their tendency to gather to lay eggs and feed [34,46,65,81,82]. A previous study showed that the ratio of material
surface/volume was positively correlated with the fermentation effect, and future work could improve the transformation capability of *P. brevitarsis* larvae on cotton stalks by reducing the crushing particle size and other measures [75,83]. Previous studies have only focused on the transformation efficiency of the larvae of *P. brevitarsis* for fermented material; this study also paid specific attention to the productivity from raw materials to fermented materials. According to the calculation results, the productivity of the A₅B₄C₄ feed was 62.85%, which was theoretically higher than the rate of traditional organic fertilizer production methods, as judged by the 25 days required for fermentation duration [70–72,84,85]. The productivity of fermentation materials can provide data support for the productivity from raw materials to dry larvae and dung-sand.

Some researchers have shown that long-term feeding of excessive amounts of non-detoxified cotton by-products (e.g., cotton leaves, cottonseed meal, and cotton stalks) to vertebrates can lead to the accumulation of free gossypol (FG) in the fed animals, causing poisoning and acute respiratory distress, anorexia, fatigue, and even death [86–88]. This has hindered the application of cotton stalks as fodder. In this study, the contents of FG in cotton stalks, cattle manure, fermented cotton stalks, and A₅B₄C₄ feed were 96, 114, 47, and 59 mg/kg. The decomposition of inoculant fermentation can significantly reduce the content of FG, which is consistent with the reduction of FG content in feed through fermentation in previous studies [89–91]. Interestingly, no FG was detected in the *P. brevitarsis* larvae after feeding on the A₅B₄C₄ feed, indicating that the FG did not accumulate in the larvae, which may be related to the larvae-degrading FG through feeding and metabolism or the short feeding time. The specific reason is the direction of future research. The insect-microorganism composite systems can undoubtedly reduce the content of FG, and the study of its degradation mechanism may provide a reference for reducing the toxicity of FG in livestock feeding on cotton by-products. The protein and fat content of the larval body were 52.49% and 11.7%. It was a suitable-quality, high-protein, insect-derived feed ingredient [92,93], and the nutrient composition of the larvae of *P. brevitarsis* was consistent with previous studies [46,48,94]. In conclusion, it is feasible to transform cotton stalks to dry larvae feed.

Organic matter (OM) and total nutrients (TNPK) are the most commonly used indicators for evaluating organic fertilizer. This study showed that the OM and TNPK indicators of cotton stalks and manure met the Chinese organic fertilizer standards (NY525-2021, China), but they cannot be applied directly as organic fertilizers [95,96]. Therefore, the evaluation of whether the materials can be used as organic fertilizers should refer to other indicators, such as the germination index (GI), humic acids (HAs), the number of beneficial microorganisms, and so on [44,49,50,69]. Furthermore, the application effect on crops is the core criterion for evaluating the quality of an organic fertilizer [97–99]. The larvae dung-sand obtained in this study was much better than the Chinese organic fertilizer standard in terms of OM, TNPK, and other nutrition indicators. However, the high pH value and water-soluble chloride content may be the reason for the low GI of seeds. The quality of larvae dung-sand as organic fertilizer can be improved by adjusting pH and other measures. On the other hand, larvae dung-sand has the characteristics of regular particles and uniform texture, which is easy to process and use and can be processed into prototype flower fertilizer [31]. In cash crops, it can be applied by sowing while fertilizing or using leaching solution drip irrigation, which has the potential to be used as dung-sand-based organic fertilizer [44,54,69].

5. Conclusions

The optimum technical parameters for transforming cotton stalks using *P. brevitarsis* larvae were supplementation with 40–50% of cattle manure, the addition of 0.1% VT inoculant, and a fermentation duration of 25–30 days. The dry larvae are a high-protein feed ingredient from an insect-derived, which can be fed and recycled into the ecological breeding industry. The larvae dung-sand is rich in nutrition and has the potential for fertilizer application. This study preliminarily proves the feasibility of cotton stalk feeding
and fertilizer dual-use technology based on the transformation of *P. brevitarsis* larvae. It possesses substantial significance for both theoretical and practical investigations related to boosting the recycling utilization of cotton stalks and cattle manure.

**Author Contributions:** Conceptualization, G.Z. and D.M.; methodology, Y.L.; validation, Y.X., S.Z. and A.X.; data curation, Z.M., H.G. and J.L.; writing—original draft preparation, G.Z.; writing—review and editing, D.M.; supervision, Y.L.; project administration, D.M. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the Autonomous Region “Tianshan Innovation Team Plan” Project, grant number 2020D14036 and the Autonomous Region Agricultural Technology Extension and Service Project, grant number 2021-41.

**Institutional Review Board Statement:** Ethical review and approval were waived for this study due to environmentally friendly use.

**Data Availability Statement:** Raw data used in this study are available on request from the authors.

**Acknowledgments:** The authors are grateful to teachers Song Qiang and Ye-ling Wang from the University of Xinjiang Agricultural University for their care in life. Additionally, we thank the reviewers for helping us to improve our original manuscript.

**Conflicts of Interest:** The authors declare no conflict of interest.

**References**

1. China National Bureau of Statistics. Announcement of the National Bureau of Statistics on Cotton Production in 2020. 2020. Available online: http://www.stats.gov.cn/tjsj/zxfb/202012/t20201218_1810113.html (accessed on 18 December 2020).
2. Yuyun, B. Study on Straw Resources Evaluation and Utilization in China. Ph.D. Thesis, Chinese Academy of Agricultural Sciences, Beijing, China, 2010.
3. Yingquan, C.; Haiping, Y.; Xianhua, W.; Shihong, Z.; Hanping, C. Biomass-based pyrolytic polygeneration system on cotton stalk pyrolysis: Influence of temperature. Bioresour. Technol. 2012, 107, 411–418.
4. Zhipu, W.; Like, X.; Kai, L.; Jian, W.; Henan, Z.; Qiang, S.; Xinqian, S. Co-pyrolysis of sewage sludge and cotton stalks. Waste Manag. 2019, 89, 430–438. [CrossRef]
5. Qingyue, W.; Nuerjiamali, T. Polyurethane foams and bio-polyols from liquefied cotton stalk agricultural waste. Sustainability 2020, 12, 4214. [CrossRef]
6. Qi, W.; Sheng, X.; Moyong, Z.; Maonan, Y.; Huijun, L.; Zixing, L. Operating procedures for high-yield cultivation of *Ganoderma lucidum* using cotton stalks. Cotton Sci. 2017, 39, 27–28.
7. Guoqing, Z.; Qiujiang, L.; Changjiang, Z.; Fengming, L.; Jirong, Z. Study on the nutritional value of cotton stalks and their effects on the digestion and metabolism of nutrients, growth and mutton safety of sheep. J. Anim. Nutr. 2018, 30, 3247–3257.
8. Xiaofang, Z.; Rui, G.; Junyu, Z. Research progress on feed utilization of cotton straws in ruminants. China Grass-Feed. Livest. 2020, 203, 24–27.
9. Pengpeng, Z.; Shou-zen, X.; Guoquan, Z.; Xiaozhen, P.; Jin, W.; Wangfeng, Z. Carbon cycle in response to residue management and fertilizer application in a cotton field in arid Northwest China. J. Integr. Agric. 2019, 18, 1103–1119. [CrossRef]
10. Jing, W.; Bing, C.; Jiliang, W.; Yongtao, L.; Min, W.; Yong, S.; Huangyong, H.; Fangyong, W. Effects of different mechanized methods of straw returning to the field on growth, yield and quality of cotton. Agric. Res. Arid Areas 2021, 39, 18–24, 56.
11. Wright, A.L.; Hons, F.M.; Lemon, R.G.; Mark, L.; McFarland, M.L.; Nichols, R.L. Stratification of nutrients in soil for different tillage regimes and cotton rotations. Soil Tillage Res. 2007, 96, 19–27. [CrossRef]
12. Chunli, H. Temporal and Spatial Variation of Soil Nutrients of Long-Term Monocultural Cotton Field and Sustainable Utilization in Xinjiang. Ph.D. Thesis, Shihezi University, Shihezi, China, 2010.
13. Tesio, F.; Vidotto, F.; Ferrero, A. Allelopathic persistence of *Helianthus tuberosus* L. residues in the soil. Sci. Hortic. 2012, 135, 98–105. [CrossRef]
14. Endeshaw, S.T.; Lodolini, E.M.; Neri, D. Effects of olive shoot residues on shoot and root growth of potted olive plant letts. Sci. Hortic. 2015, 182, 31–40. [CrossRef]
15. Yanbin, L.; Qin, Z. Effects of naturally and microbially decomposed cotton stalks on cotton seedling growth. Arch. Agron. Soil Sci. 2016, 62, 1264–1270. [CrossRef]
16. Subramanian, S.; Sivarajan, M.; Saravananpriya, S. Chemical changes during vermicomposting of sago industry solid wastes. J. Hazard. Mater. 2010, 179, 318–322. [CrossRef]
17. Arnold, V.H.; Joost, V.I.; Harmke, K.; Esther, M.; Afton, H.; Giulia, M.; Paul, V. Edible Insects: Future Prospects for Food and Feed Security, Food and Agriculture Organization of the United Nations: Rome, Italy, 2013.
18. Cickova, H.; Newton, G.L.; Lacy, R.C.; Kozanek, M. The use of fly larvae for organic waste treatment. Waste Manag. 2015, 35, 68–80. [CrossRef] [PubMed]
19. YuSheng, L. Scientific basis and technology system of macro agriculture circle economy. *Renew. Resour. Circ. Econ.* 2015, 8, 7–12.

20. Lim, S.L.; Lee, L.H.; Wu, T.Y. Sustainability of using composting and vermicomposting technologies for organic solid waste biotransformation: Recent overview, greenhouse gases emissions and economic analysis. *J. Clean. Prod.* 2016, 111, 262–278. [CrossRef]

21. Kiliç, E. Environmental friendly insects is *Tenebrio molitor* (Coleoptera Tenebrionidae). *Adv. Ecol. Res.* 2018, 1–13. Available online: https://www.researchgate.net/publication/323635871 (accessed on 25 March 2018).

22. Naseer, H.; Shahid, A. Efficacy of the vermicomposts of different organic wastes as “Clean” fertilizers: State-of-the-art. *Sustainability* 2018, 10, 1205. [CrossRef]

23. Soobhany, N. Insight into the recovery of nutrients from organic solid waste through biochemical conversion processes for fertilizer production: A review. *J. Clean. Prod.* 2019, 241, 118413. [CrossRef]

24. Kawasaki, K.; Kawasaki, T.; Hirayasu, H.; Matsumoto, Y.; Fujitani, Y. Evaluation of fertilizer value of residues obtained after processing household organic waste with black soldier fly larvae (*Hermetia illucens*). *Sustainability* 2020, 12, 4920. [CrossRef]

25. Rumbos, C.I.; Mente, E.; Karapanagiotidis, I.T.; Vlontzos, G.; Athanassiou, C.G. Insect-based feed ingredients for aquaculture: A case study for their acceptance in Greece. *Insects* 2021, 12, 586. [CrossRef]

26. Mertenat, A.; Diener, S.; Zurbrugg, C. Black soldier fly biowaste treatment-assessment of global warming potential. *Waste Manag.* 2019, 84, 173–181. [CrossRef] [PubMed]

27. Arnold, V.H.; Dennis, G.A.; Oonincx, B. The environmental sustainability of insects as food and feed. A review. *Agron. Sustain. Dev.* 2017, 37, 43. [CrossRef]

28. Borkent, S.; Hodge, S. Glasshouse evaluation of the black soldier fly waste product HexaFrasSTM as an organic fertilizer. *Insects* 2021, 12, 977. [CrossRef]

29. Xiaoan, T.; Fuping, S.; Jie, Z.; Rongmei, L.; Xingpeng, Z.; Jiangyan, D.; Changlong, S. Diversity of gut bacteria in larval *Protaetia brevitarsis* (Coleoptera: Scarabaeida) fed on corn stalk. *Acta Entomol. Sin.* 2017, 60, 632–641.

30. Guangjie, Z. Studies on the Transformation Techniques of Organic Waste Using *Protaetia brevitarsis* (Coleoptera: Cetoniidae). Master’s Thesis, Shandong Agricultural University, Tai’an, China, 2019.

31. Wenzhen, M. *Chinese Economic Entomology (Coleoptera, Cetoniidae)*; China Science and Technology Press: Beijing, China, 1995; Volume 46, pp. 94–95.

32. Baohong, J.; Shuwen, L.; Kai, Z. *Entomological Basis and Common Species Identification*; Science Press: Beijing, China, 2011; pp. 251–252.

33. Xuanye, W.; Yanhong, C.; Shaoliang, H.; Kun, Z.; Shangshu, H.; Qianqiang, W.; Qianru, H. Study on earthworm transformation evaluation of spent mushroom substrates as food for white spotted flower chafer, *Protaetia brevitarsis* (Coleoptera: Cetoniidae) larvae. *J. Agric. Resour. Environ.* 2021, 39, 201–208.

34. Arnold, V.H.; Dennis, G.A.; Oonincx, B. The environmental sustainability of insects as food and feed. A review. *Agron. Sustain. Dev.* 2017, 37, 43. [CrossRef]

35. Xiaoan, T.; Fuping, S.; Jie, Z.; Rongmei, L.; Xingpeng, Z.; Jiangyan, D.; Changlong, S. Diversity of gut bacteria in larval *Protaetia brevitarsis* (Coleoptera: Cetoniidae) fed on corn stalk. *Acta Entomol. Sin.* 2017, 60, 632–641.

36. Guangjie, Z. Studies on the Transformation Techniques of Organic Waste Using *Protaetia brevitarsis* (Coleoptera: Cetoniidae). Master’s Thesis, Shandong Agricultural University, Tai’an, China, 2019.

37. Wenzhen, M. *Chinese Economic Entomology (Coleoptera, Cetoniidae)*; China Science and Technology Press: Beijing, China, 1995; Volume 46, pp. 94–95.

38. Baohong, J.; Shuwen, L.; Kai, Z. *Entomological Basis and Common Species Identification*; Science Press: Beijing, China, 2011; pp. 251–252.

39. YuSheng, L.; Xiaoyan, X.; Li, Z. The study on the effect of *Protaetia brevitarsis* Lewis larvae transformation the corn straw. *J. Environ. Entomol.* 2015, 37, 122–127.

40. YuSheng, L.; Xiaoyan, X.; Li, Z. The study on the effect of *Protaetia brevitarsis* Lewis larvae transformation the corn straw. *J. Environ. Entomol.* 2015, 37, 122–127.

41. YuSheng, L.; Xiaoyan, X.; Li, Z. The study on the effect of *Protaetia brevitarsis* Lewis larvae transformation the corn straw. *J. Environ. Entomol.* 2015, 37, 122–127.

42. YuSheng, L.; Xiaoyan, X.; Li, Z. The study on the effect of *Protaetia brevitarsis* Lewis larvae transformation the corn straw. *J. Environ. Entomol.* 2015, 37, 122–127.

43. YuSheng, L.; Xiaoyan, X.; Li, Z. The study on the effect of *Protaetia brevitarsis* Lewis larvae transformation the corn straw. *J. Environ. Entomol.* 2015, 37, 122–127.

44. YuSheng, L.; Xiaoyan, X.; Li, Z. The study on the effect of *Protaetia brevitarsis* Lewis larvae transformation the corn straw. *J. Environ. Entomol.* 2015, 37, 122–127.

45. YuSheng, L.; Xiaoyan, X.; Li, Z. The study on the effect of *Protaetia brevitarsis* Lewis larvae transformation the corn straw. *J. Environ. Entomol.* 2015, 37, 122–127.
47. Seonmin, L.; Yun-Sang, C.; Kyung, J.; Tae-Kyung, K.; Hae-In, Y.; Samooel, J. Quality characteristics and protein digestibility of *Protaetia brevitarsis* larvae. *J. Anim. Sci.* 2020, 62, 741–752. [CrossRef]
48. Youn-Kyung, H.; Sam-Woong, K.; Dong-Heon, S.; Hyun-Wook, K.; Il-Suk, K. Nutritional Composition of White-Spotted Flower Chafer (*Protaetia brevitarsis*) Larvae Produced from Commercial Insect Farms in Korea. *Food Sci. Anim. Resour.* 2021, 41, 416–427. [CrossRef]
49. Yimei, L.; Tong, F.; Lili, G.; Yu, S.; Haiyan, C.; Fushun, L.; Chunqin, L.; Fuping, S.; Jie, Z.; Changlong, S. *Protaetia brevitarsis* larvae can efficiently convert herbaceous and ligneous plant residues to humic acids. *Waste Manag.* 2019, 83, 79–82. [CrossRef]
50. Huina, X.; Peiweng, G.; Baohai, D.; Lili, G.; Kui, W.; Kun, H.; Jie, Z.; Tianpei, H.; Changlong, S. Characterization of microorganisms from *Protaetia brevitarsis* larva frass. *Microorganisms* 2022, 10, 311. [CrossRef]
51. Fushun, L.; Xiaojie, F.; Guocheng, X.; Yu, W.; Qinglei, W. The effects of *Protaetia brevitarsis* larva manure application on the growth of cherry radish. *Hubei Agric. Sci.* 2018, 57, 44–46, 50.
52. Deqiang, L.; Qinglei, W.; Yu, W.; Changlong, S.; Yue, Z.; Chunqin, L. Effect of *Protaetia brevitarsis* Lewis larvae dung on development of pepper seedling stage under low temperature. *North. Hortic.* 2019, 8, 63–66.
53. Xiang, W.; Congyong, H.; Ruijie, C.; Xiaoyan, X.; Jinlong, W.; Xiaobo, W. Influence of frass organic manure on tomato growth and quality. *North. Hortic.* 2019, 426, 66–70.
54. Kyong-Hee, J.; Jong-Won, K.; Seul-Bi, L.; Da-Hyun, J.; Byung-Man, Y.; Sung-Mun, B.; Young-Ho, C.; Young Han, L.; Dong-Cheol, S. Effects of *Protaetia brevitarsis* larvae manure application on lettuce growth and soil chemical properties. *Korean J. Soil. Sci. Fert.* 2022, 55, 80–85. [CrossRef]
55. Hua, J.; Shu, S.; Baiyan, Y.; Wanshan, Y.; Tiefeng, J. Effects of the grub extract on apoptosis of MCF-7 human breast cancer cell line. *Chin. J. Pathophysiol.* 2008, 24, 93–96.
56. Ahn, E.M.; Myung, N.Y.; Jung, H.A.; Kim, S.J. The ameliorative effect of *Protaetia brevitarsis* larvae in HFD-induced obese mice. *Food Sci. Biotechnol.* 2019, 28, 1177–1186. [CrossRef]
57. Mingxu, X.; Guofu, G.; Shouyun, Y.; Jie, S.; Chongxing, Z.; Chunhua, X.; Yi, L.; Keyun, Z. Isolation and purification of antibacterial materials from *Protaetia brevitarsis* (Coleoptera) Larva. *Life Sci. Res.* 2008, 12, 53–56.
58. Hwa-Jin, S.; Chul, K. Antioxidant activity of aqueous methanol extracts of *Protaetia brevitarsis* Lewis (Coleoptera: Scarabaeidae) at different growth stages. *Nat. Prod. Res.* 2012, 26, 510–517. [CrossRef]
59. Eunjung, L.; Jin-Kyoung, K.; Soyoung, S.; Ki-Woong, J.; Juneyoung, L.; Dong-Gun, L.; Jae-Sam, H.; Yangmee, K. Enantiomeric 9-mer peptide analogs of proteaetiamycin with bacterial cell selectivities and anti-inflammatory activities. *J. Pept. Sci.* 2011, 17, 675–682. [CrossRef]
60. Minglu, Q. Study on Extraction, Separation, Purification and Anti-Inflammatory Property of *Protaetia brevitarsis* Lewis Larvae Protein. Master’s Thesis, Shandong Agricultural University, Tai'an, China, 2020.
61. Nihkkah, A.; Van Haute, S.; Jovanovic, V.; Jung, H.; Dewulf, J.; Cirkovic Velickovic, T.; Ghnimi, S. Life cycle assessment of edible insects (*Protaetia brevitarsis* Seulensis larvae) as a future protein and fat source. *Sci. Rep.* 2021, 11, 14030. [CrossRef]
62. Zhongjie, L.; Miaomiao, M.; Shasha, L.; Deng, B. The transcriptome analysis of *Protaetia brevitarsis* Lewis larvae. *PLoS ONE* 2019, 14, e0214001. [CrossRef]
63. Kui, W.; Pengpeng, L.; Yonggang, G.; Chunqin, L.; Qinglei, W.; Jiao, Y.; Jie, Z.; Lili, G.; Changlong, S. De novo genome assembly of the white-spotted flower chafer (*GigaScience* 2019, 8, giz019. [CrossRef]
64. Xiangzhen, L.; Brune, A. Digestion of microbial biomass, structural polysaccharides, and protein by the humivorous larva of *Pachnoda ephippiata* (Coleoptera: Scarabaeidae). *Soil Biol. Biochem.* 2005, 37, 107–116. [CrossRef]
65. Manning, P.; Slade, E.M.; Beynon, S.A.; Lewis, O.T. Functionally rich dung beetle assemblages are required to provide multiple ecosystem services. *Agric. Ecosyst. Environ.* 2016, 218, 87–94. [CrossRef]
66. HARDERSSEN, S.; ZAPPONI, L. Wood degradation and the role of saproxylic insects for lignoforms. *Appl. Soil Biol.* 2018, 123, 334–338. [CrossRef]
67. Wu, L.; Qing, L.; Yuanyuan, W.; Longyu, Z.; Yanlin, Z.; Ziniu, Y.; Huanchun, C.; Jibin, Z. Efficient bioconversion of organic wastes to value-added chemicals by soaking, black soldier fly (*Hermetia illucens* L.) and anaerobic fermentation. *J. Environ. Manag.* 2018, 227, 267–276. [CrossRef]
68. Lijie, Y.; Xiangfang, Z.; Shiyan, Q. Advances in research on solid-state fermented feed and its utilization: The pioneer of private customization for intestinal microorganisms. *Anim. Nutr.* 2021, 7, 905–916. [CrossRef]
69. Xiang, Z.; Ju-Pei, S.; Chang-Long, S.; Sheng-Sheng, J.; Hong, J.D.; Li-Mei, Z.; Ji-Zheng, H. Attenuation of antibiotic resistance genes in livestock manure through vermicomposting via *Protaetia brevitarsis* and its fate in a soil-vegetable system. *Sci. Total Environ.* 2022, 807, 150781. [CrossRef]
70. Anshu, S.; Satyawati, S. Composting of a crop residue through treatment with microorganisms and subsequent vermicomposting. *Bioresour. Technol.* 2002, 85, 107–111. [CrossRef]
71. Shweta, K.R.; Singh, B.L.; Deeppshikha, V. Integrating microbial composting and vermicomposting for effective utilization of by-products of sugar cane–processing industries. *Bioresid. J.* 2010, 14, 158–167. [CrossRef]
72. Moran-Salazar, R.G.; Marino-Marmolejo, E.N.; Rodriguez-Campos, J.; Davila-Vazquez, G.; Contreras-Ramos, S.M. Use of agave bagasse for production of an organic fertilizer by pretreatment with *Bjerkandera adusta* and vermicomposting with *Eisenia fetida*. *Environ. Technol.* 2016, 37, 1220–1231. [CrossRef]
73. Kashif-ur, R.; Rashid, U.R.; Abdul, A.S.; Minmin, C.; Longyu, Z.; Xiaopeng, X.; Asif, U.R.; Abdul, R.; Jeffery, K.T.; Ziniu, Y.; et al. Enhanced biodegradation of chicken and manure by the interaction of exogenous bacteria and black soldier fly larvae. *J. Environ. Manag.* 2019, 237, 75–83. [CrossRef]

74. Kui, W.; Peiwen, G.; Lili, G.; Chunqin, L.; Jie, Z.; Changlong. S. Lignocellulose degradation in *Protaetia brevitarsis* larvae digestive tract: Refining on a tightly designed microbial fermentation production line. *Microbiome* 2022, 10, 90. [CrossRef]

75. Baohai, D.; Huina, X.; Lili, G.; Weihang, L.; Jie, Z.; Wensheng, X.; Rongmei, L.; Changlong, S. Microflora for improving the *Auricularia auricula* spent mushroom substrate for *Protaetia brevitarsis* production. *iScience* 2022, 25, 105307. [CrossRef]

76. Fuzing, G. Research on the Effect of Different C/N Ratios on Fermentation of Organic Residue. Master’s Thesis, Hunan Agricultural University, Changsha, China, 2014.

77. Takahashi, N.; Mochizuki, S.; Masuda, K.; Shimada, I.; Osada, M.; Fukunaga, H. Influence of temperature, water content and C/N ratio on the aerobic fermentation rate of woody biomass. *Kagaku Kagaku Ronbunshu* 2017, 43, 231–237. [CrossRef]

78. Carotenuto, C.; Guarino, G.D.; Amelia, L.I.; Morrone, B.; Minale, M. The peculiar role of C/N and initial pH in anaerobic digestion of lactating and non-lactating water buffalo manure. *Waste Manag.* 2020, 103, 12–21. [CrossRef]

79. Tao, X. Technical Research on *Protaetia brevitarsis* Lewis Bioconversion of Cattle Farm Waste in Indoor and Outdoor. Master’s Thesis, Xinjiang Agricultural University, Urumqi, China, 2020.

80. Yusheng, L. *Insect Production Science*; Higher Education Press: Beijing, China, 2012; pp. 178–180.

81. Xiaofang, Z.; Liuyang, W.; Chunqin, L.; Yongqiang, L.; Xiangdong, M.; Zhongyue, W.; Tao, Z. Identification and field verification of an aggregation pheromone from the white-spotted flower chafer, *Protaetia brevitarsis* Lewis (Coleoptera: Scarabaeidae). *Sci. Rep.* 2021, 11, 22362. [CrossRef]

82. Doube, B.M. Ecosystem services provided by dung beetles in Australia. *Basic Appl. Ecol.* 2018, 26, 35–49. [CrossRef]

83. Gossner, M.M.; Lachat, T.; Brunet, J.; Isacsson, G.; Bouget, C.; Brustel, H.; Brandl, R.; Weisser, W.W.; Muller, J. Current near-to-nature forest management effects on functional trait composition of saproxylic beetles in beech forests. *Conserv. Biol.* 2013, 27, 605–614. [CrossRef]

84. Pardillo, N. Production efficiency of organic fertilizer from different composting methods. *Asia Pac. J. Multidiscip. Res.* 2018, 6, 45–51.

85. Yingkai, L.; Jiali, L.; Xiyue, S.; Yali, W.; Xiaolei, Y.; Wen, G.; Yinheng, L. Effect of adding cow dung and garden waste on sewage sludge vermicomposting process. *Chin. J. Environ. Eng.* 2020, 14, 197–208.

86. Lordelo, M.M.; Calhoun, M.C.; Dale, N.M.; Dowd, M.K.; Davis, A.J. Relative toxicity of gossypol enantiomers in laying and broiler breeder hens. *Poult. Sci.* 2007, 86, 582–590. [CrossRef] [PubMed]

87. Yunfeng, L.; Xiuqi, W.; Qingyu, Z.; Zhang Junmin, Z. Research situation on gossypol safety limit in feed and gossypol residues in livestock product. *Chin. Agric. Sci. Bull.* 2010, 26, 1–5.

88. Rehemujiang, H.; Yimamu, A.; Wang, Y.L. Effect of dietary cotton stalk on nitrogen and free gossypol metabolism in sheep. *Asian-Australas J. Anim. Sci.* 2019, 32, 233–240. [CrossRef] [PubMed]

89. Wenju, Z.; Zirong, X.; Shunhong, Z.; Jianyi, S.; Xia, Y. Development of a microbial fermentation process for detoxification of gossypol in cottonseed meal. *Anim. Feed Sci. Technol.* 2007, 135, 176–186. [CrossRef]

90. Vellaichamy, M.; Sharmila, B.M.; Kuppusamy, P. Isolation and identification of potential Gossypol degrading fungal strains from cotton growing soil. *Int. J. Microbiol.* 2017, 2017, 1–6. [CrossRef]

91. Xiuye, Q.; Quanxi, X.; Jiamin, Y.; Qian, Z.; Zhiyan, Z.; Haiyan, X.; Wei, G. Screening of free gossypol strain in high efficient cotton growing soil. *Chin. Agric. Sci. Bull.* 2016, 45–51. [CrossRef] [PubMed]

92. Van Huis, A. Potential of insects as food and feed in assuring food security. *Annu. Rev. Entomol.* 2013, 58, 563–583. [CrossRef]

93. Choi, S.U.; Choi, I.H.; Chung, T.H. Investigation of breast meat traits of broilers fed different amounts of *Hermetia illucens* and *Protaetia brevitarsis seleensis* powder. *Entomol. Res.* 2021, 51, 343–348. [CrossRef]

94. Deokyeol, J.; Namgyong, M.; Yeongbu, K.; Soo-Rin, K.; Ohseek, K. The effects of feed materials on the nutrient composition of *Protaetia brevitarsis* larvae. *Entomol. Res.* 2019, 50, 23–27. [CrossRef]

95. Quan, W.; Zhen, W.; Mukes, K.A.; Yahui, J.; Ronghua, L.; Xiuna, R.; Junchao, Z.; Feng, S.; Meiying, W.; Zengqiang, Z. Evaluation of medical stone amendment for the reduction of nitrogen loss and bioavailability of heavy metals during pig manure composting. *Bioresour. Technol.* 2016, 220, 297e304. [CrossRef]

96. Malinska, K.; Golanska, M.; Caceres, R.; Borat, A.; Weisser, P.; Slezak, E. Biochar amendment for integrated composting and vermicomposting of sewage sludge the effect of biochar on the activity of *Eisenia fetida* and the obtained vermicompost. *Bioresour. Technol.* 2017, 225, 206–214. [CrossRef] [PubMed]

97. Yan, H.; Rong, L.; Honjung, L.; Beibei, W.; Chenmin, Z.; Qirong, S. Novel resource utilization of refloated algal sludge to improve the quality of organic fertilizer. *Environ. Manag.* 2014, 55, 1658–1667. [CrossRef]

98. Yajuan, C.; Ji, L.; Yaofeng, Y. Dynamic change of key indicators and denitrifying bacteria in chicken manure sawdust aerobic composting process. *J. Chin. Agric. Univ.* 2016, 21, 67–75.

99. Xiuhong, W.; Xiangyuan, S.; Jitao, Z.; Yuxia, W.; Xinxin, L.; Jing, Z.; Hongye, Z. Analysis of maturity, heavy metal residues and microbial flora of chicken manure aerobic compost. *Shanxi Agric. Sci.* 2021, 49, 1094–1099.