

Application of Black Soldier Fly Frass, *Hermetia illucens* (Diptera: Stratiomyidae) as Sustainable Organic Fertilizer for Lettuce, *Lactuca sativa* Production

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Abstract

In recent years, black soldier fly (BSF) has been shown to efficiently convert organic waste into nutrient-rich larval biomass while generating frass as the main by-product. This work aims to investigate the potential of BSF frass (BSFF) as an organic fertilizer for agricultural production. BSFF was produced by recycling household waste using BSF larvae, and a portion was taken to the lab for physicochemical and microbial analyses before the field trial on lettuce growth and health. The field trial consisted of two doses of BSFF (15 t·ha⁻¹ and 30 t·ha⁻¹) and one dose of urea (0.214 t·ha⁻¹). An unfertilized plot was prepared and used as a control, and the effects on lettuce growth were assessed using agronomic parameters while the health effects were assessed using parameters such as: the number of leaves affected per plant, the incidence of affection, the severity of the affection and the microbial analysis. BSFF exhibited acceptable physicochemical properties as an organic fertilizer. Its application improved the growth parameters of lettuce plants compared to urea and control. The application rates of 15 t·ha⁻¹ and 30 t·ha⁻¹ did not reveal any significant difference ($p > 0.05$). Further studies are therefore needed to determine the minimum applicable dose. The health parameters of the lettuce plants were slightly altered regardless of the treatment and the microbial analysis of the affected leaves revealed pathogenic microorganisms. We therefore recommend that decontamination methods be considered when producing BSFF as an organic fertilizer.

Keywords

Biofertilizer, Biowaste, Insect Frass, Plant Growth, Sustainable Agriculture

1. Introduction

The depletion of raw material sources and soils leads to hunger and increased food insecurity. The situation is more noticeable in urban areas with high rates of urbanization and scarcity of agricultural land [1]. To cope with such a situation, there is a need for strategies that can match future food demands with increasing population growth while conserving the soil resources. Most African countries rely on agriculture for livelihood, which is a better way to increase food production. However, the use of agricultural land without a conservation method leads to a rapid decline in its fertility which is a major cause of the decline in production yields. Organic fertilizers offer a promising way to sustainably improve soils and crop productivity [2]. They are a good source of nitrogen, which is one of the most limiting soil nutrients for agricultural production [3]. Organic fertilizers improve soil microbial activities and provide macronutrients necessary for the growth of crops [4]. The use of organic fertilizers, such as compost and manure, constitutes a direct input of carbon (C) to the soil, which can be stabilized through physical, chemical, and biochemical mechanisms [5], contributing to long-term storage of C in soils [6] [7] [8]. In addition, organic fertilizers supply secondary nutrients (Calcium, Magnesium, and Sulfate) and micronutrients (zinc, boron, and manganese), which play a key role in the uptake and utilization of macronutrients such as nitrogen, phosphorous, and potassium [9] [10].

Despite its many advantages, the use of organic fertilizers in agriculture is still largely limited because of their low availability. Most organic manures are used to feed animals and leave little or no organic resources for use in agricultural production [11] [12]. Therefore, improving soil productivity using organic resources requires a venture into new organic fertilizer sources. In recent years, black soldier fly (BSF) has been shown to efficiently convert organic wastes into high quality essential nutrients [13] [14]. Globally, this has attracted interest in the face of rising prices of animal feedstuff and accumulating amounts of waste. The main by-product of this bioconversion technology is BSF Frass (BSFF), which can be used as an organic fertilizer in agriculture [15].

Frass refers to the excrement of insects and will often contain other components such as exoskeleton sheds, death insects and insect food residue. The frass of commercially produced insect larvae, including BSF have great potential as a sustainable fertilizer source with many applications, since commercially produced insects are a way of utilizing pre-consumer food by-products that may otherwise create waste streams [16]. Additionally, the use of insect frass can reduce the need for chemical fertilizers [17]. Insect frass is rich in chitin from the shed exoskeleton, which is known to have several beneficial effects on plant growth and health [18] [19]. It functions as a nematicide and fungicide, and promotes mycorrhization, but in some cases appears to be phytotoxic [20]. Also, BSFF is reported to have insecticidal and insect-detering properties [21].

In natural conditions, it is well known that frass deposition to soil has a great

impact on soil fertility due to its high nutrient and labile carbon content [22] [23]. Therefore, several insect companies have already decided to sell it as organic fertilizer [24], and even some farmers are already reporting its beneficial effects on crops [25]. However, there is still very limited information on its ability to improve soil fertility [26]. It still needs further research before a major scaling up in the industry. For example, its effects on soil characteristics and plant growth, its nutrient composition and influencing factors on variation, and the ecological aspects of its application. To contribute to these knowledge gaps, this work aimed at investigating some physicochemical and microbiological characteristics of BSFF and its fertilizing potential on lettuce growth and health.

2. Material and Methods

2.1. Black Soldier Fly Frass

The BSFF was produced at the BSF rearing unit of the Agri-Business Vocational Training Center in Dschang, Cameroon, following the bioconversion process of organic waste by BSF larvae (BSFL) as described by Dzepe *et al.* [27]. **Figure 1** summarizes the engineering of the production system, which integrates the BSF life cycle and the recovery process of bio-waste by the BSFL. The bio-waste used in this study was household waste from the students' guesthouse. It consisted mainly of food waste and was collected on a weekly basis and subjected to the BSFL from a pre-established BSF colony. The BSF colony was established using young BSFL from a wild-trapped BSF population reared since 2016 at the International Institute of Tropical Agriculture (IITA) in Yaounde, Cameroon.

Upon receipt, the young larvae were fed in a plastic container with chicken feed until mature, and then they were taken to a new container with pupation substrate (wood shavings) to undergo metamorphosis. The maturity of BSFL was marked by a change in skin color from white to dark-brown. At this stage, they are called pre-pupae and have taken a few days to become pupae, characterized by complete immobility and a hardening of the exoskeleton. The pupae were transferred to an untreated mosquito net cage where they completed their metamorphosis and emerged as adult flies. As the adult BSF do not feed on solid food but can take in liquid substances, they were provided with water twice a day to ensure their survival, and an egg-laying medium was also provided to them in the cage to collect the eggs which were incubated to obtain young larvae used to renew the cycle. Water was sprayed directly onto the cage, while egg-laying medium was adapted from Dortmans *et al.* [28].

Every two days, clusters of eggs from the established BSF colony were collected and incubated following the procedure described by Dzepe *et al.* [29]. After hatching, the neonate larvae were fed chicken feed for four days, before being transferred into the household waste from the students' guesthouse. The treatment took place in plastic containers (60 × 40 × 12 cm), at a rate of 100 mg of waste per larva per day as recommended by Dortmans *et al.* [28] and lasted 15 days, which is the normal time required by BSFL to effectively process organic



Figure 1. Engineering of the bio-waste recycling process using black soldier fly technology.

waste. At the end of the treatment, BSFLs were separated manually, and the remaining residue (frass) was collected and stored at room temperature for further use as organic fertilizer. A small fraction was sampled for physicochemical and microbiological analyses.

2.2. Soil Preparation and Experimental Design

The fertilizing potential of the BSFF was evaluated on a single piece of land at the Agri-Business Vocational Training Center in Dschang, Cameroon. Before setting up the experimental design, the land was cleaned, plowed, and sampled for physico-chemical analyzes. It was then divided into 16 random plots of 0.1 m² each, corresponding to four treatments with four repetitions. The first treatment was considered a control and received no fertilizer (no fertilizer). The second treatment received 0.0214 kg of urea, which is equivalent to 0.214 t·ha⁻¹ (Urea 0.214 t·ha⁻¹), and the third and fourth treatments received respectively quantities of 1.5 and 3.0 kg of BSFF, which are equivalents of 15 t·ha⁻¹ (BSFF 15 t·ha⁻¹) and 30 t·ha⁻¹ (BSFF 30 t·ha⁻¹). After applying the fertilizers, the different experimental plots were watered using a watering can and used for growing *Batavia lettuce (Lactuca Sativa)*, which is an interesting vegetable for human con-

sumption [30]. The experimental field had an area of 27.73 m² and was divided into 16 experimental plots of 0.1 m² each (1.25 × 0.8 m). The plots were separated from each other vertically by a space of 0.5 m and horizontally by a space of 0.3 m. The treatments were randomly distributed among the plots and each plot was considered a replicate for a given treatment.

2.3. Physicochemical and Microbiological Analyses

Soil and BSFF samples were transferred to the laboratory of soil sciences and environmental chemistry and the Laboratory of Plant Pathology and Agricultural Zoology of the University of Dschang for physicochemical and microbiological analyses, respectively.

The physicochemical analyzes were carried out according to the standard protocols applied in the laboratory. The pH was measured using a pH meter 24 hours after mixing 10 g of each sample with 25 ml of distilled water. Organic matter (OM) was determined by dry matter and weight loss on ignition at 550°C for 4 h in a muffle furnace. While the organic carbon (OC) content was determined using the classic dichromate oxidation method of Walkley and Black [31]. The total nitrogen (N) was determined using the Kjeldhal method which successively includes mineralization, distillation and titration. The available phosphorus (P) was determined by the Bray-2 method (1945). This method combines the extraction of phosphorus in an acidic medium (Hydrogen Chloride) with ammonium fluoride (NH₄F). The determination of the phosphorus extracted was then carried out using spectrophotometry with Molybdenum blue. Calcium (Ca), potassium (K), sodium (Na) and magnesium (Mg) were extracted with ammonium acetate (CH₃COONH₄) using NH₄⁺ ions which saturate the complex and release the cations (Ca²⁺, Mg²⁺, K⁺, Na⁺) which will be then determined. The concentrations of Ca and Mg were determined by complexometry with EDTA (Ethylene diamine tetraacetic acid), while those of K and Na were determined by flame spectrophotometry. The soil texture was also determined using the texture triangle method based on the USDA grain size classification.

Only BSFF was considered for microbiological analysis. A suspension of 0.1 g of sample in 10 ml of sterile water was prepared, and 0.1 ml of the suspension was then diluted serially (ten-fold) and used in the estimation of bacterial and fungal populations by the standard spread-plate dilution method described by Seeley and VanDemark [32]. Nutrient agar containing 0.015% (w/v) nystatin (to inhibit fungi growth) was used for the isolation of bacteria and incubation took place at 35°C for 05 days. While potato dextrose agar mixed with 0.05% (w/v) chloramphenicol (to inhibit bacteria growth) was used for fungal isolation, and the incubation took place at 28°C for 07 days. Pure isolates of representative communities were maintained on agar slant at 4°C, and identification was based on cultural, microscopic, and biochemical characteristics with reference to Bergey's manual of determinative bacteriology for bacteria, and fungal identification kit of Botton *et al.* [33] for fungi.

2.4. Black Soldier Frass and Lettuce Growth

The agronomic potential of BSFF was investigated using young lettuce plants from a pre-established lettuce nursery. The nursery was carried out on a 3.6 m² (3 × 1.2 m) plot of unfertilized soil using lettuce seeds of the Batavia variety purchased from a local market. Using a string and poles, seeding lines spaced 20 cm apart from each other were materialized on the previously plowed plot, and the lettuce seeds were sown at a rate of 2 g·m⁻². The nursery was watered twice a day, and germination was observed from day 4 to day 10 before transplanting into the experimental plot. At the time of transplantation, only healthy plants bearing 03 or 04 leaves were selected for the experiment. Transplantation was done very early in the morning and each experimental plot described above received 24 lettuce seedlings. BSFF (BSFF 15 t·ha⁻¹ and BSFF 30 t·ha⁻¹) was applied to the soil one day before transplanting, while urea (Urea 0.214 t·ha⁻¹) was applied around the lettuce plants on days 10 and 15 after transplanting. The experiment lasted six weeks and the influence of different treatments on lettuce growth was monitored weekly using parameters such as: a) Number of leaves; b) Plant height; c) Crown diameter; d) Largest leaf length; e) Largest leaf width; f) Leaf area.

a) The number of leaves per plant was counted manually in each plot and included all the leaves of the plant.

b) Plant height was measured from collar to apex using a graduated ruler.

c) Crown diameter was measured for each plant using a digital caliper.

d) The length of the largest leaf was measured from the leaf blade using a graduated ruler

e) The width of the largest leaf was also measured using a graduated ruler.

f) The leaf area was calculated using the length of the largest leaf and the width of the largest leaf according to the following formula: Leaf area = $(\pi L I)/4$; where $\pi = 3.14$, L = largest leaf length and I = largest leaf width.

At the end of the experiment, the lettuce plants were harvested by treatment, weighed using an electronic scale, and the production yield was determined using the following formula: $Y (\text{t}\cdot\text{h}^{-1}) = W (\text{t}) \times S (\text{ha})^{-1}$; Where $Y (\text{t}\cdot\text{h}^{-1})$ is the production yield in tonnes per hectare, $W (\text{t})$ the weight of lettuce harvested in tonnes, and $S (\text{ha})$ the area harvested in hectares.

2.5. Black Soldier Frass and Plant Health

The influence of BSFF on the health of lettuce plants was evaluated in the different treatments using parameters such as: a) the number of leaves affected per plant, b) the incidence of the affection and, c) the severity of the affection.

a) The number of leaves affected includes all leaves that show signs of disease and have been counted manually.

b) The affection incidence was determined using the number of affected plants according to the following formula: $A_i (\%) = N_a \times N_t^{-1} \times 100$; Where A_i is the affection incidence, N_a the number of affected plants, and N_t the total

number of plants.

c) The severity of affection was determined by field observation using the severity scale of Horsfall and Barrat [34].

For each treatment, all affected leaves were collected and transferred to the laboratory for microbiological analysis. The analysis consisted in identifying the possible micro-organisms responsible for the affections observed in the field and were carried out according to the same procedure as those of the BSFF previously described.

2.6. Statistical Analyses

The effects of BSFF on lettuce growth and health were statistically tested using one-way analysis of variance (ANOVA). In case of significant difference, the Tukey's HSD (honestly significant difference) post hoc test was performed. Analyses were performed using the SPSS v. 22.0 Software, and the tests were considered significant at $P < 0.05$.

3. Results

3.1. Experimental Soil and Black Soldier Fly Frass Characteristics

Table 1 presents the physicochemical characteristics of soil and BSFF and their evaluations according to Beernaert and Bitondo [35]. The experimental soil had low clay (16.5%) content and high sand (64.65%) content. According to the textural triangle of the USDA classification, it exhibited a texture of sandy loam (loamy soil with a moderately coarse texture). With a low C:N ratio ($18 < 25$), the experimental soil presented a poor quality of organic matter. The concentration of major elements N and K was high, while that of trace elements P and Ca was very low. According to the NFU44 051 standard for organic fertilizers, the BSFF produced in this study from household waste was rich in major elements N, P, K and trace elements Ca and Mg (**Table 1**). It had an organic matter content of 90% and a high C/N ratio ($85 > 25$). It also had a relatively alkaline pH (7.8).

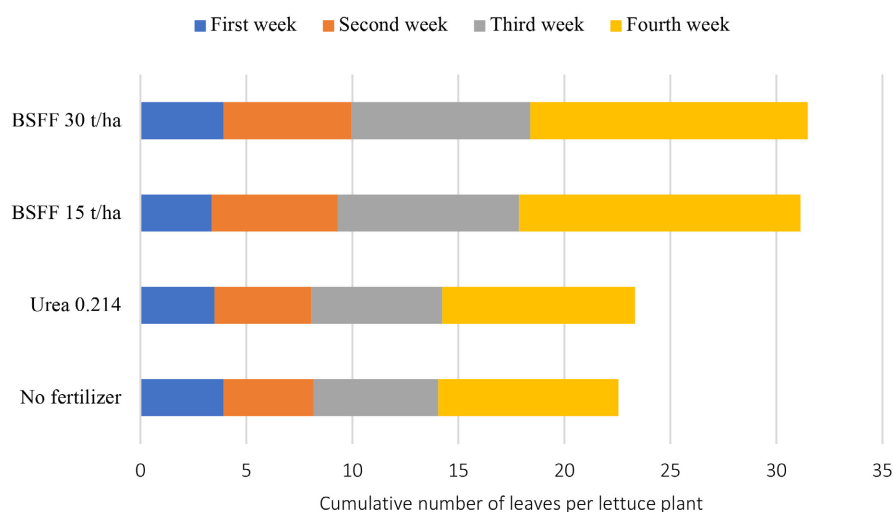
3.2. Influence of Black Soldier Fly Frass on Lettuce Growth

As shown in **Figure 2**, the average number of leaves per lettuce plant was significantly influenced from the 2nd to the 4th week ($p < 0.05$). At the end of the 1st week, the number of leaves recorded per plant varied from 3.37 ± 0.87 to 3.93 ± 0.76 and no significant difference was observed ($p > 0.05$). From the 2nd week, the BSFF 15 t·ha⁻¹ and BSFF 30 t·ha⁻¹ treatments recorded significantly ($p < 0.05$) higher values and averages of 13.28 ± 4.61 and 13.09 ± 4.17 leaves per plant were observed respectively at the 4th week, against 8.5 ± 2.27 recorded with the Control, no fertilizer and 9.09 ± 2.87 recorded with the Urea 0.214 t·ha⁻¹.

The leaf area of lettuce plants was also significantly affected by BSFF application ($p < 0.05$). At the end of the first week, leaf area varied between 10.34 ± 3.54 and 21.44 ± 3.85 cm² and the plants under the BSFF 15 t·ha⁻¹ treatment showed a significant demarcation (**Figure 3**). During weeks 2 and 3, the leaf areas of the

Table 1. Physicochemical characteristics of experimental soil and black soldier fly frass.

Parameters	Experimental soil	Appreciation of soil elements [35]	BSFF	Standard NFU44 051 for organic fertilizers
Texture (%)				
Sand	64.65	-	-	-
Silt	18.85	-	-	-
Clay	16.5	-	-	-
Organic elements (%)				
Organic matter (OM)	8.62	>6, Very high	90	≥20
Organic carbon (OC)	5	-	45	-
Nitrate (N)	0.28	0.225 - 0.3, High	0.52	<2
C:N ratio	18	14 - 20, Poor quality of organic matters	85	>8
Mineral elements (meq/100 g)				
Calcium (Ca)	2.65	<7, Very low	0.39	-
Magnesium (Mg)	1.13	0.5 - 1.5, Low	0.15	-
Potassium (K)	1.06	0.6 - 1.2, High	1.8	<30
Sodium (Na)	0.015	<0.1, Very low	0.055	-
Phosphorus (P)	2.29	<7, Very low	1.08	<30
Cation exchange capacity (meq/100 g)	18.2	10 - 25, Medium	-	-
pH	6.3	6 - 7, Slightly acidic	7.84	= 7

**Figure 2.** Influence of black soldier fly frass on leaf reach of lettuce plants.

plants under the Urea 0.214 t·ha⁻¹, BSFF 15 t·ha⁻¹ and BSFF 30 t·ha⁻¹ treatments evolved considerably and the BSFF 15 t·ha⁻¹ treatment still recorded the highest value (39.99 ± 5.67 cm²). On the other hand, at week 4, the highest value was recorded with the plants under the BSFF 30 t·ha⁻¹ (65.45 ± 6.98 cm²) treatment, followed by the plants under the Urea 0.214 t·ha⁻¹ (48.79 ± 4.26 cm²) treatment.

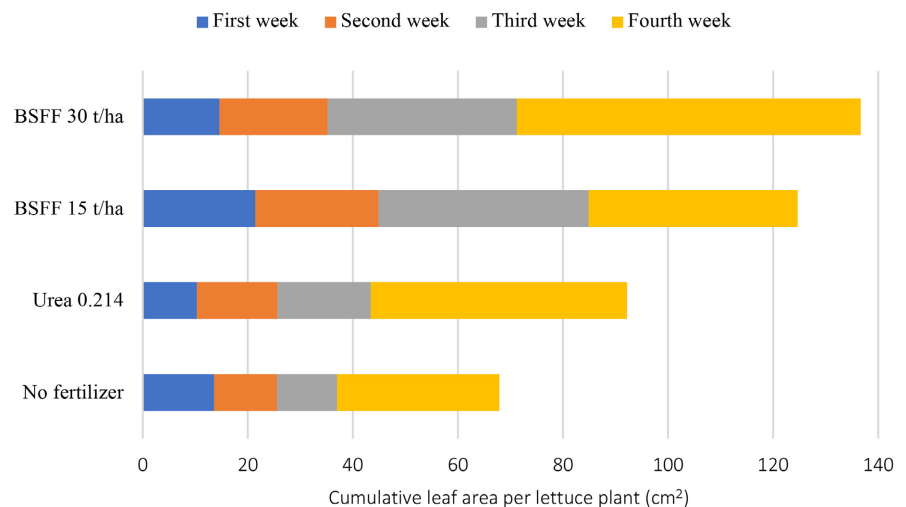


Figure 3. Influence of black soldier fly frass on leaf area of lettuce plants.

Figure 4 illustrates the influence of BSFF on the crown diameter of lettuce plants during the experiment. During the first week, the diameters recorded independently of the different treatments varied from 0.52 ± 0.07 to 1.05 ± 0.26 cm and plants subjected to Urea $0.214 \text{ t}\cdot\text{ha}^{-1}$ recorded a significantly high value ($p < 0.05$). From week 2 to week 4, the highest diameters were recorded in the BSFF $15 \text{ t}\cdot\text{ha}^{-1}$ and BSFF $30 \text{ t}\cdot\text{ha}^{-1}$ treatments compared to the controls. These two treatments showed no significant difference throughout the experimental period ($p > 0.05$).

Figure 5 shows the effect of BSFF on lettuce plant height. Regardless of the different treatments, plant height ranged from 6.37 ± 1.75 to 7.43 ± 2.08 cm during week 1 and from 6.31 ± 2.63 to 8.57 ± 3.46 cm during week 2 and no significant differences were observed ($p > 0.05$), although the BSFF $15 \text{ t}\cdot\text{ha}^{-1}$ and BSFF $30 \text{ t}\cdot\text{ha}^{-1}$ treatments showed relatively higher values. During week 3 and week 4, on the other hand, the latter recorded significantly higher heights compared to the control ($p < 0.05$). In week 4, an average height of 17.05 ± 4.65 cm was recorded in the BSFF $15 \text{ t}\cdot\text{ha}^{-1}$ treatment and 16.94 ± 4.31 cm in the BSFF $30 \text{ t}\cdot\text{ha}^{-1}$ treatment, compared to 10.98 ± 3.41 cm and 12.20 ± 3.69 cm recorded in the Control, no fertilizer and the Urea $0.214 \text{ t}\cdot\text{ha}^{-1}$, respectively.

Lettuce yield was significantly influenced by the different treatments applied during the study ($p < 0.05$). It ranged from 31.17 ± 5.77 to $63.28 \pm 7.34 \text{ t}\cdot\text{ha}^{-1}$ and the BSFF $30 \text{ t}\cdot\text{ha}^{-1}$ treatment had the highest influence, followed by the BSFF $15 \text{ t}\cdot\text{ha}^{-1}$ treatment ($55.57 \pm 6.89 \text{ t}\cdot\text{ha}^{-1}$). The lowest yield was recorded in the Control, no fertilizer, while the Urea $0.214 \text{ t}\cdot\text{ha}^{-1}$ had an intermediate value (**Figure 6**).

3.3. Influence of Black Soldier Fly Frass on Lettuce Health

The application of BSFF influenced the health parameters of lettuce plants during the experiment. **Figure 7** presents the average number of leaves per plant that showed signs of pathology during the study. It appears that until day 21

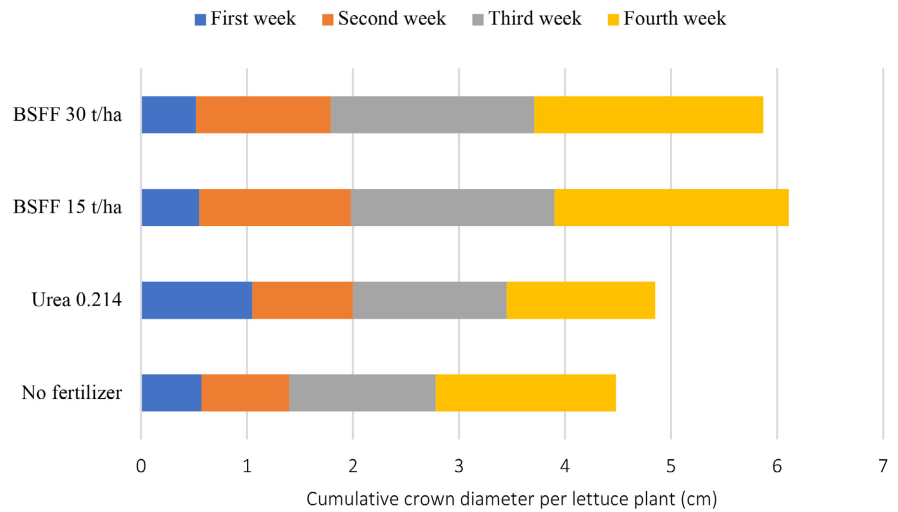


Figure 4. Influence of black soldier fly frass on crown diameter of lettuce plants.

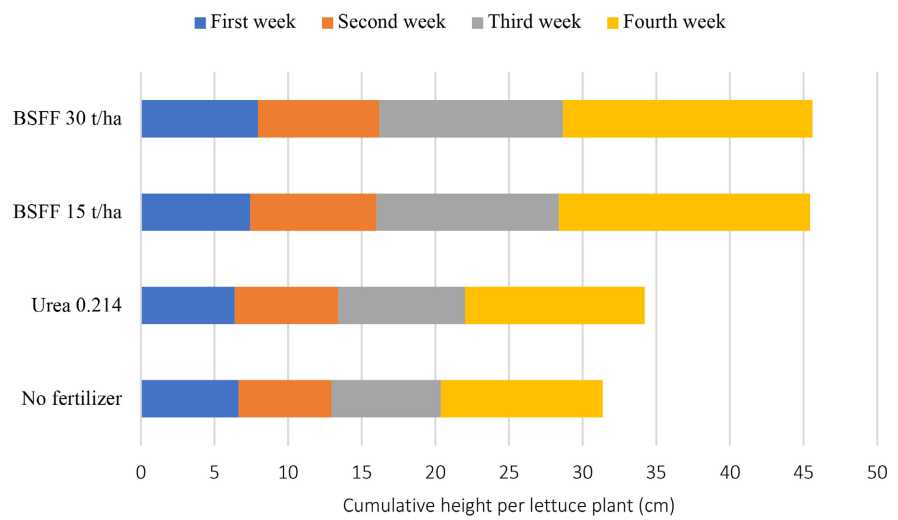


Figure 5. Influence of black soldier fly frass on the height of lettuce plants.

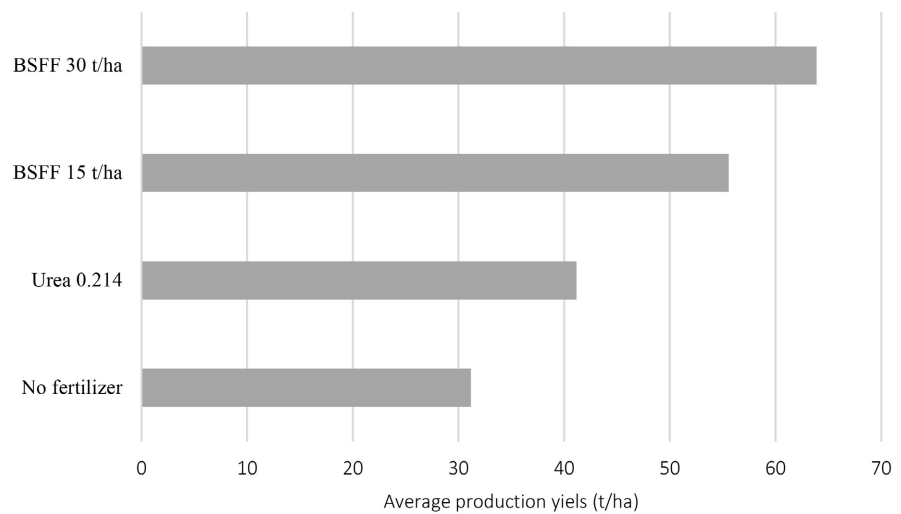


Figure 6. Influence of black soldier fly frass on lettuce production yield.

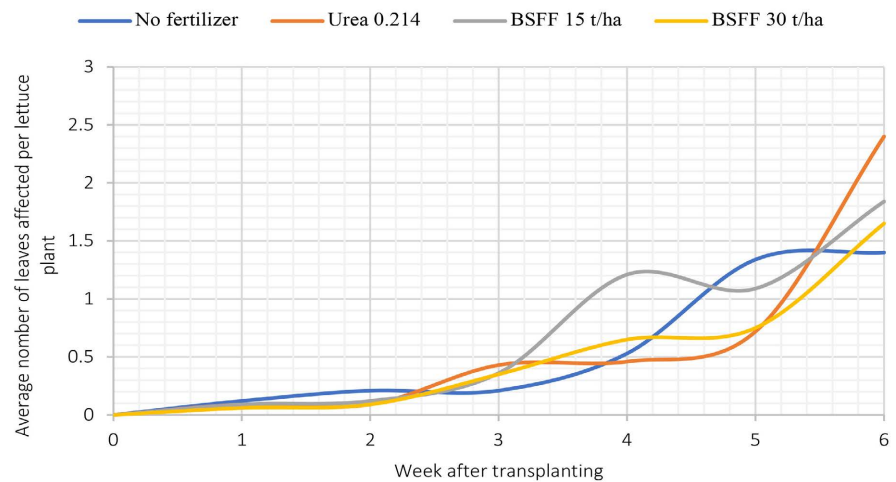


Figure 7. Influence of black soldier fly frass on lettuce leaf health.

after transplanting, less than one leaf per plant was affected whatever the treatment and no significant difference was observed ($p > 0.05$). On the other hand, from week 4, the plants of the BSFF 15 t·ha⁻¹ treatment were significantly more affected (1.21 ± 0.14 leaves per plant), followed by the plants of the Control, no fertilizer (1.34 ± 0.85 leaves per plant) at week 5. At week 6, plants from the Urea 0.214 t·ha⁻¹ were more affected (2.40 ± 1.03 leaves per plant), followed by plants from the BSFF 15 t·ha⁻¹ treatment (1.84 ± 0.67 leaves per plant).

Figure 8 presents the affection dynamics of lettuce plants according to the different treatments during the study. The incidence of the affection increased with time whatever the treatment and reached more than 50% of the plants before the harvest period (day 42). At the end of week 5, the affection incidence recorded with the 1st Control, no fertilizer was significantly higher compared to the other treatments. Similarly, at the end of week 6, the value recorded with the BSFF 30 t·ha⁻¹ treatment was significantly lower ($p < 0.05$).

A similar trend was recorded with the severity of the affection. Disease incidence was generally low (below 20%) on day 7 after transplanting and had increased to about 60% on day 42 for most of the treatments (**Figure 9**). Lettuce plants in the control, no fertilizer and the Urea 0.214 t·ha⁻¹ were more susceptible to the disease compared to plants in the BSFF 15 t·ha⁻¹ and BSFF 30 t·ha⁻¹ treatments. Throughout the study period, the plants treated with BSFF 30 t·ha⁻¹ showed less signs of disease than the rest of the treatments.

The microbiological analysis of the affected leaves collected in the fields revealed the presence of two genera of fungi (*Cercospora* and *Aspergillus*) and three genera of bacteria (*Actinomyces*, *Bacillus* and *Clostridium*), whatever the treatment. The genus *Cercospora* is a pathogenic fungus and has also been found in the BSFF, along with all the other genera previously listed.

4. Discussion

As previously depicted in **Figure 1**, the application of BSF technology generates two main products: a nutrient-rich larval biomass and a waste residue commonly

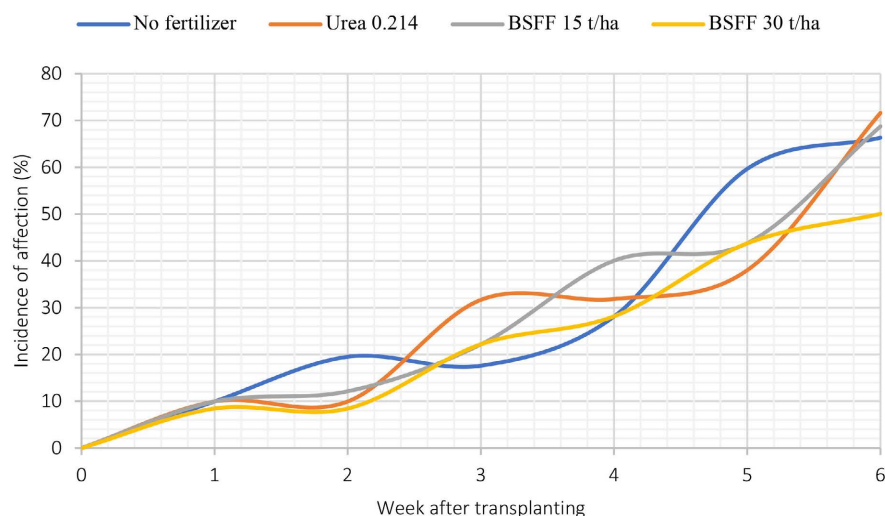


Figure 8. Influence of black soldier fly frass on the occurrence of pathologies in lettuce plants.

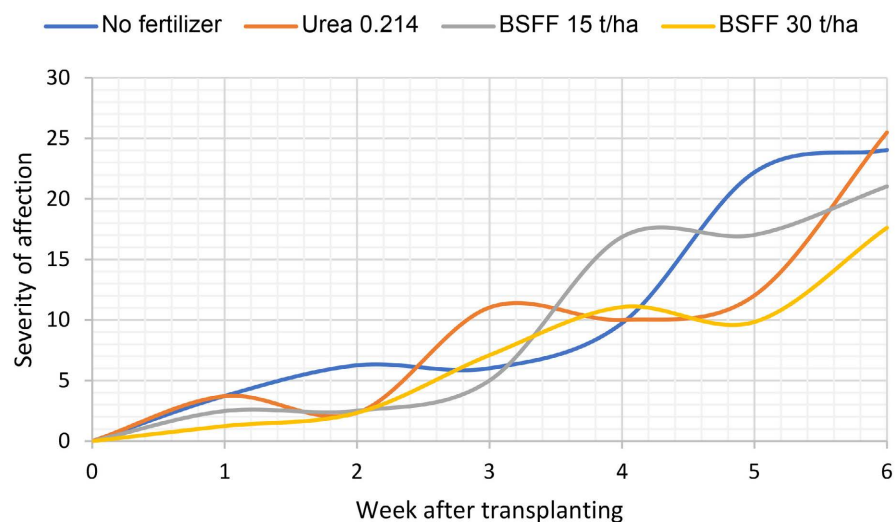


Figure 9. Influence of black soldier fly frass on the severity of lettuce pathologies.

referred to as frass, known for its valuable fertilizing potential. A physicochemical analysis of frass revealed desirable values for N, P_2O_5 and K_2O , which respect the quality standards recommended by the WHO for organic composts (0.1% - 1.8% for N, 0.1% - 1.7% for P_2O_5 and 0.1% - 2.3% for K_2O). N'dienor *et al.* [36] report that the N content of a good organic fertilizer is between 0.5% and 6% depending on its basic elements, and that its application in sufficient quantity makes it possible to improve the properties of the soil. According to the NFU44 051 standard for organic fertilizers, the following parameters must be taken into consideration when producing organic fertilizers: $MO \geq 20\%$, total N < 3%, total P_2O_5 < 3%, total K_2O < 3%, C:N ratio > 8, and the sum of nutrients (total N + total P_2O_5 + total K_2O) < 7%. For the present study, all these parameters were respected, which testifies to the quality of BSFF. The C:N ratio was higher compared to that obtained by Gajalakshmi [37] with compost from solid urban

waste. Indeed, Ajaweed [38] characterized compostable urban waste and reported that the nature of the latter could influence the C:N ratio, as well as other nutrient elements in the compost. The BSFF mineral element concentrations obtained in this study were different from those obtained by Anyega *et al.* [38] with larvae fed on brewers' spent grain, and Gärttling *et al.* [15] with larvae fed with a mixture of urban waste. However, all values were within the suitable range for organic fertilizers. These observations confirm the hypothesis that the physico-chemical characteristics of BSFF vary widely depending on the type of substrate used to rear the BSF larvae. Poveda *et al.* [26] also found that this feeding substrate can affect the microbial composition of frass as well as its nutrient content. In the present study, the pH of BSFF was slightly alkaline (7.84) like that of most organic fertilizers [39]. It therefore provides favorable conditions for improving soil biological properties and cation availability. It could also be used to increase the pH of acidic soils.

The growth parameters of lettuce plants had been significantly improved by the application of BSFF. In general, the mean number of leaves, plant height, crown diameter and leaf area of plants grown under the BSFF 15 t·ha⁻¹ and BSFF 30 t·ha⁻¹ treatments were significantly higher compared to those of the plants in the control treatments. BSFF had made available to plants the nutrients necessary for their growth. These results are in line with the work of Steven [40] who reports that organic compost provides the plant with the nitrogen necessary for its aerial growth and improves its stem/leaf ratio. Rosmiati *et al.* [41] also report good growth performance in lettuce plants with BSFF produced from Coffee Husk. The same observation was made with the production yield where the values obtained with the BSFF treatments were significantly higher compared to those of the control treatments. Varying the BSFF application rate had no significant effect on lettuce growth and production, although the yield recorded with the BSFF 30 t·ha⁻¹ treatment was relatively higher. Although urea provides enough nitrogen to the plant compared to BSFF, the latter also provides other nutrients such as phosphorus, potassium, calcium, and magnesium essential for plant growth. Also, with a C:N ratio > 25, the application of BSFF can lead to nitrogen mineralization in the soil and release accessible forms for plants. These preliminary results confirm the fertilizing potential of BSFF which can be used as a sustainable alternative to chemical fertilizers. With the increasing importance of the insect production sector, BSFF will soon be a highly available organic fertilizer. Current insect producers are mostly small or medium-sized enterprises, of which about 80% raise BSF [13]. Present production sums up to a few thousand tonnes per year but will expand substantially in the coming years and frass will emerge toward a considerable by-product. Frass represents about 70% to 90% of the total output in a BSF production system [42] and must be valued for the system to be profitable.

Microbial analyzes revealed the presence of a pathogenic fungus (*Cercospora sp*) both in the lettuce plants and in the BSFF. This fungus has been shown to be the main cause of affection in lettuce leaves, and we hypothesize that *Cercospora*

sp is transmitted from BSFF to plants, and plants in treatments without BSFF would have been accidentally contaminated by wind or during handling. Another fungus of the genus *Aspergillus* has also been identified. Although, it is not pathogenic to lettuce plants, this fungus (*Aspergillus*) can cause allergies or respiratory illnesses in humans. Fuchs and Larbi [43], report that not all organic fertilizers have the potential to protect plants against infections. Some can constitute sources of contaminants if they are improperly treated. Bacteria found in BSFF (*Actinomyces sp*, *Bacillus sp* and *Clostridium sp*), were also found on the affected lettuce leaves. This could be explained by the anatomy of lettuce plants, where leaves are found very close to the ground, thus facilitating the migration of bacteria from the soil to the plants.

The results found in this study suggest that BSFF can be used as an organic fertilizer and improve crop growth and production yield. The variation of two doses of BSFF (BSFF 15 t·ha⁻¹ and BSFF 30 t·ha⁻¹) did not reveal any significant difference in the parameters studied. Further studies are therefore needed to determine the minimum applicable dose, in order to better advise farmers for sustainable use. The microbial analysis revealed the presence of pathogenic microorganisms capable of affecting plants, as well as humans. It is therefore necessary that decontamination methods be considered when producing BSFF for soil fertilization. This can be done by composting the BSFF for a few more weeks after production.

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Authors' Contributions

D.D., Y.A. and R.D. conceived and designed the study. T.K.M. performed the experiments. D.D. and T.K.M. analysed and interpreted the data. D.D. did the original draft preparation. G.N., H.M. and S.Y.C. reviewed and edited the paper. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declared that there are no conflicts of interest.

References

- [1] Food and Agricultural Organization of the United Nations (2014) Food Waste Footprint: Full-Cost Accounting.
- [2] Vanlauwe, B., Descheemaeker, K., Giller, K.E., Huising, J., Merckx, R., Nziguheba, G. and Wendt, J. (2015) Integrated Soil Fertility Management in Sub-Saharan Africa: Unravelling Local Adaptation. *Soil*, **1**, 491-508.
<https://doi.org/10.5194/soil-1-491-2015>
- [3] Tully, K., Sullivan, C., Weil, R. and Sanchez, P. (2015) The State of Soil Segregation in Sub-Saharan Africa: Baselines, Trajectories and Solutions. *Sustainability*, **7**, 6523-

6552. <https://doi.org/10.3390/su7066523>
- [4] Islam, M., Islam, S., Akter, A., Rahman, M. and Nandwani, D. (2017) Effect of Organic and Inorganic Fertilizers on Soil Properties and the Growth, Yield and Quality of Tomato in Mymensingh, Bangladesh. *Agriculture*, **7**, Article No. 18. <https://doi.org/10.3390/agriculture7030018>
- [5] Six, J., Conant, R.T., Paul, E.A. and Paustian, K. (2002) Stabilization Mechanisms of Soil Organic Matter: Implications for C-Saturation of Soils. *Plant Soil*, **241**, 155-176. <https://doi.org/10.1023/A:1016125726789>
- [6] Yan, X. and Gong, W. (2010) The Role of Chemical and Organic Fertilizers on Yield, Yield Variability and Carbon Sequestration-Results of a 19-Year Experiment. *Plant Soil*, **331**, 471-480. <https://doi.org/10.1007/s11104-009-0268-7>
- [7] Brar, B.S., Singh, K. and Dheri, G.S. (2013) Balwinder-Kumar Carbon Sequestration and Soil Carbon Pools in a Rice-Wheat Cropping System: Effect of Long-Term Use of Inorganic Fertilizers and Organic Manure. *Soil and Tillage Research*, **128**, 30-36. <https://doi.org/10.1016/j.still.2012.10.001>
- [8] Tautges, N.E., Chiartas, J.L., Gaudin, A.C.M., O'Geen, A.T., Herrera, I. and Scow, K.M. (2019) Deep Soil Inventories Reveal That Impacts of Cover Crops and Compost on Soil Carbon Sequestration Differ in Surface and Subsurface Soils. *Global Change Biology*, **25**, 3753-3766. <https://doi.org/10.1111/gcb.14762>
- [9] Tittonell, P., Corbeels, M., Van Wijk, M.T., Vanlauwe, B. and Giller, K.E. (2008) Combining Organic and Mineral Fertilizers for Integrated Soil Fertility Management in Smallholder Farming Systems of Kenya: Explorations Using the Crop-Soil Model FIELD. *Agronomy Journal*, **100**, 1511-1526. <https://doi.org/10.2134/agronj2007.0355>
- [10] Wortmann, C.S., Kaizzi, K.C., Maman, N., Cyamweshi, A., Dicko, M., Garba, M., et al. (2019) Diagnosis of Crop Secondary and Micro-Nutrient Deficiencies in Sub-Saharan Africa. *Nutrient Cycling in Agroecosystems*, **113**, 127-140. <https://doi.org/10.1007/s10705-018-09968-7>
- [11] Rufino, M.C., Dury, J., Tittonell, P., van Wijk, M.T., Herrero M., Zingore, S., Mapfumo, P. and Giller, K.E. (2011) Competing Use of Organic Resources, Village-Level Interactions between Farm Types and Climate Variability in a Communal Area of NE Zimbabwe. *Agricultural Systems*, **104**, 175-190. <https://doi.org/10.1016/j.agsy.2010.06.001>
- [12] Rusinamhodzi, L., Corbeels, M. and Giller, K.E. (2016) Diversity in Crop Residue Management across an Intensification Gradient in Southern Africa: System Dynamics and Crop Productivity. *Field Crops Research*, **185**, 79-88. <https://doi.org/10.1016/j.fcr.2015.10.007>
- [13] Derrien, C. and Boccuni, A. (2018) Current Status of the Insect Producing Industry in Europe. In: Halloran, A., Flore, R., Vantomme, P. and Roos, N., Eds., *Edible Insects in Sustainable Food Systems*, Springer Publishing, New York, 471-479. https://doi.org/10.1007/978-3-319-74011-9_30
- [14] Dzepe, D., Nana, P., Mube, K.H., Kimpara, J.M., Magatsing, O., Tchuinkam, T. and Djouaka, R. (2021) Feeding Strategies for Small-Scale Rearing Black Soldier Fly Larvae (*Hermetia illucens*) as Organic Waste Recycler. *SN Applied Sciences*, **3**, Article No. 252. <https://doi.org/10.1007/s42452-020-04039-5>
- [15] Gärtling, D., Kirchner, S.M. and Schulz, H. (2020) Assessment of the N- and P-Fertilization Effect of Black Soldier Fly (Diptera: Stratiomyidae) By-Products on Maize. *Journal of Insect Science*, **8**, 1-11. <https://doi.org/10.1093/jisesa/ieaa089>
- [16] Schmitt, E. and de Vries, W. (2020) Potential Benefits of Using *Hermetia illucens*

- Frass as a Soil Amendment on Food Production and for Environmental Impact Reduction. *Current Opinion in Green and Sustainable Chemistry*, **25**, Article ID: 100335. <https://doi.org/10.1016/j.cogsc.2020.03.005>
- [17] Berggren, A., Jansson, A. and Low, M. (2019) Approaching Ecological Sustainability in the Emerging Insects-as-Food Industry. *Trends in Ecology & Evolution*, **34**, 132-138. <https://doi.org/10.1016/j.tree.2018.11.005>
- [18] Debode, J., De Tender, C., Soltaninejad, S., Van Malderghem, C., Haegeman, A., Van der Linden, I., Cottyn, B., Heyndrickx, M. and Maes, M. (2016) Chitin Mixed in Potting Soil Alters Lettuce Growth, the Survival of Zoonotic Bacteria on the Leaves and Associated Rhizosphere Microbiology. *Frontiers in Microbiology*, **7**, Article No. 565. <https://doi.org/10.3389/fmicb.2016.00565>
- [19] Quilliam, R.S., Nuku-Adeku, C., Maquart, P., Little, D., Newton, R. and Murray, F. (2020) Integrating Insect Frass Biofertilisers into Sustainable Peri-Urban Agro-Food Systems. *Journal of Insects as Food and Feed*, **6**, 315-322. <https://doi.org/10.3920/JIFF2019.0049>
- [20] Sharp, R. (2013) A Review of the Applications of Chitin and Its Derivatives in Agriculture to Modify Plant-Microbial Interactions and Improve Crop Yields. *Agronomy*, **3**, 757-793. <https://doi.org/10.3390/agronomy3040757>
- [21] Vickerson, A., Radley, R., Marchant, B., Kaulfuss, O. and Kabaluk, T. (2013) *Hermetia illucens* Frass Production and Use in Plant Nutrition and Pest Management. U.S. Patent No. 9844223 B2.
- [22] Frost, C.J. and Hunter, M.D. (2004) Insect Canopy Herbivory and Frass Deposition Affect Soil Nutrient Dynamics and Export in Oak Mesocosms. *Ecology*, **85**, 3335-3347. <https://doi.org/10.1890/04-0003>
- [23] Kagata, H. and Ohgushi, T. (2012) Positive and Negative Impacts of Insect Frass Quality on Soil Nitrogen Availability and Plant Growth. *Population Ecology*, **54**, 75-82. <https://doi.org/10.1007/s10144-011-0281-6>
- [24] Dicke, M. (2018) Insects as Feed and the Sustainable Development Goals. *Journal of Insects as Food and Feed*, **4**, 147-156. <https://doi.org/10.3920/JIFF2018.0003>
- [25] Halloran, A., Roos, N., Eilenberg, J., Cerutti, A. and Bruun, S. (2016) Life Cycle Assessment of Edible Insects for Food Protein: A Review. *Agronomy for Sustainable Development*, **36**, Article No. 57. <https://doi.org/10.1007/s13593-016-0392-8>
- [26] Poveda, J., Jiménez-Gómez, A., Saati-Santamaría, Z., Usategui-Martín, R., Rivas, R. and García-Fraile, P. (2019) Mealworm Frass as a Potential Biofertilizer and Abiotic Stress Tolerance Inductor in Plants. *Applied Soil Ecology*, **142**, 110-122. <https://doi.org/10.1016/j.apsoil.2019.04.016>
- [27] Dzepe, D., Magatsing, O., Kuitche, H.M., Meutchieye, F., Nana, P., Tchuinkam, T. and Djouaka, R. (2021) Recycling Organic Wastes Using Black Soldier Fly and House Fly Larvae as Broiler Feed. *Circular Economy and Sustainability*, **3**, 895-906. <https://doi.org/10.1007/s43615-021-00038-9>
- [28] Dortmans, B.M.A., Egger, J., Diener, S. and Zurbrugg, C. (2021) Black Soldier Fly Biowaste Processing—A Step-by-Step Guide. 2nd Edition. Eawag: Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland.
- [29] Dzepe, D., Nana, P., Fotso, A., Tchuinkam, T. and Djouaka, R. (2020) Influence of Larval Density, Substrate Moisture Content and Feedstock Ratio on Life History Traits of Black Soldier Fly Larvae. *Journal of Insects as Food and Feed*, **6**, 133-140. <https://doi.org/10.3920/JIFF2019.0034>
- [30] Ekoungoulou, R. And Mikouendanandi, E.B.R.M. (2020) Lettuce (*Lactuca sativa* L.) Production in Republic of Congo Using Hydroponic System. *Open Access Library Journal*, **7**, e6339. <https://doi.org/10.4236/oalib.1106339>

- [31] Walkley, A. and Black, I.A. (1934) An Examination of Degtjareff Method for Determining Soil Organic Matter and a Proposed Modification of the Chromic Acid Titration Method. *Soil Science*, **37**, 29-37.
<https://doi.org/10.1097/00010694-193401000-00003>
- [32] Seeley, H.W. and Van Demark, P.J. (1981) *Microbes in Action. A Laboratory Manual of Microbiology*. 3rd Edition, W.H Freeman and Company, New York, 350 p.
- [33] Botton, B., Breton, A., Fevre, M., Gauthier, S., Guy, P.H., *et al.* (1990) Moisissures utiles et nuisibles: Importance industrielle. Masson, Paris, 34-428.
- [34] Horsfall, J.G. and Barratt, R.W. (1945) An Improved Grading System for Measuring Plant Diseases. *Phytopathology*, **35**, Article No. 655.
- [35] Beernaert, F. and Bitondo, D. (1992) Simple and Practical Methods to Evaluate Analytical Data of Soil Profiles. CUDs Dschang Soil Sciences Department, Belgium Cooperation, Dschang.
- [36] N'Dienor, M., Dabat, M.H., Ramanarivo, R., Randriamiharisoa, F., Rajoelison, J. and Aubry, C. (2006) A Trend towards Urban Integration and Organization of the Tomato Subsector in Antananarivo, Madagascar. *Acta Horticulturae*, **699**, 317-326.
<https://doi.org/10.17660/ActaHortic.2006.699.37>
- [37] Gajalakshmi, S. and Abbasi, S.A. (2008) Solid Waste Management by Composting: State of the Art. *Critical Reviews in Environmental Science and Technology*, **38**, 311-400. <https://doi.org/10.1080/10643380701413633>
- [38] Ajaweed, A.N., Hassan, F.M. and Hyder, N.H. (2022) Evaluation of Physio-Chemical Characteristics of Bio Fertilizer Produced from Organic Solid Waste Using Composting Bins. *Sustainability*, **14**, Article No. 4738.
<https://doi.org/10.3390/su14084738>
- [39] Anyega, A.O., Korir, N.K., Beesigamukama, D., Changeh, G.J., Nkoba, K., Subramanian, S., van Loon, J.J.A., Dicke, M. and Tanga, C.M. (2021) Black Soldier Fly-Composted Organic Fertilizer Enhances Growth, Yield, and Nutrient Quality of Three Key Vegetable Crops in Sub-Saharan Africa. *Frontiers in Plant Science*, **12**, Article ID: 680312. <https://doi.org/10.3389/fpls.2021.680312>
- [40] McGeehan, S.L. (2012) Impact of Waste Materials and Organic Amendments on Soil Properties and Vegetative Performance. *Applied and Environmental Soil Science*, **2012**, Article ID: 907831. <https://doi.org/10.1155/2012/907831>
- [41] Rosmiati, M., Nurjanah, K.A., Suantika, G. and Putra, R.E. (2017) Application of Compost Produced by Bioconversion of Coffee Husk by Black Soldier Fly Larvae (*Hermetia illucens*) as Solid Fertilizer to Lettuce (*Lactuca sativa* Var. Crispa): Impact to Growth. *Proceedings of the International Conference on Green Technology*, **8**, 38-44.
- [42] Devic, E. (2016) *Assessing Insect-Based Products as Feed Ingredients for Aquaculture*. University of Stirling, Stirling, United Kingdom.
- [43] Fuchs, J. and Larbi, M. (2004) Disease Control with Quality Compost in Pot and Field Trails. *I International Conference on Soil and Composts Eco-Biology*, León, Spain, 15-17 September 2004, 157-166.