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Examination of *Tenebrio molitor* Frass for Potential Fertilizer Applications

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ABSTRACT

The insect production industry is expanding rapidly across the globe, offering an effective approach to recycling organic waste. One of the most important by-products of this process is insect feces, commonly referred to as 'frass.' Since frass is generated in larger quantities than the insects themselves, its use is essential to promote a sustainable circular economy. This research focused on assessing the feasibility of employing frass from yellow mealworms (*Tenebrio molitor*) as a fertilizer. Spectroscopic techniques were used to analyze its mineral composition, revealing nitrogen, phosphorus, and potassium levels of 3.3%, 2.8%, and 2.3%, respectively. These concentrations aligned with existing data, supporting the idea that frass could serve as either a replacement for or an enhancement to conventional fertilizers. The study also evaluated its fertilizing efficiency, and the results showed that the highest productive yield came from soil treated with both frass and mineral fertilizer. This was followed by frass applied alongside high-quality seeds, followed by mineral fertilizer alone, which still resulted in a fairly good yield, while untreated soil produced the lowest output. Despite these promising findings, further research is necessary, particularly on the immune-boosting properties of frass, to build upon the insights gained in this study.

Keywords: Fertilizer, *Tenebrio molitor*, Frass, Yellow mealworms, Mineral content

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Introduction

Soil degradation and ineffective waste disposal present significant risks to environmental health and food security in Sub-Saharan Africa (SSA). Around 40% of soils in this region are deficient in key nutrients required for crop development, with 25% affected by aluminum toxicity, 18% vulnerable to leaching, and 8.5% experiencing phosphorus fixation [1]. Although many small-scale farmers rely on mineral fertilizers, their efficiency is often hindered by low soil organic matter, a lack of micronutrients, and high acidity levels [2]. While organic fertilizers are both affordable and accepted by farmers, their use remains limited due to inconsistent quality, prolonged production processes, and insufficient sources of organic material on farms [3]. In light of these constraints, this study aimed to examine the potential of frass as a fertilizer.

Frass, which consists of insect excrement, has been found to support plant growth and enhance tolerance to abiotic stresses such as flooding and drought [4]. It contains uneaten feed particles along with chitin-rich exoskeleton fragments, which are believed to activate plant immune responses, potentially strengthening resistance to diseases and pests [5]. Depending on the insect species and its diet, frass can make up 80–95% of total output, producing 4–20 times more waste than insect biomass [6]. With the anticipated growth of the insect farming industry, particularly the use of *Tenebrio molitor* for food production, frass generation is expected to increase significantly,

posing a waste management challenge for producers. Therefore, characterizing the frass excreted by *Tenebrio molitor* is essential to evaluate its suitability as a fertilizer.

Prior research by Poveda *et al*. [6] demonstrated that the nutritional composition and microbial profile of yellow mealworm frass are heavily influenced by the insect's diet, leading to variations in its effectiveness as a fertilizer. Their study indicated that the most beneficial frass fertilizer was derived from a diet consisting of 66% carbohydrates, 6% fat, and 28% protein, with nitrogen, phosphorus, and potassium (NPK) levels measuring approximately 3%, 2%, and 2%, respectively. Similarly, Houben *et al*. [7] conducted growth trials with barley (Hordeum vulgare) and found that frass performed comparably to conventional mineral NPK fertilizers, with leaf nutrient content remaining similar across both treatments. Their findings suggested that frass could serve as either a partial or full replacement for mineral fertilizers without reducing crop yield. Additionally, their research revealed that frass contributed to greater microbial diversity and enhanced soil metabolic activity, reinforcing the conclusions drawn by Poveda *et al*. [6] regarding the role of microorganisms in frass. Given these findings, this study aimed to evaluate the potential of yellow mealworm (*Tenebrio molitor*) frass as a fertilizer.

Materials and Methods

Sample preparation

Frass was sourced from *Tenebrio molitor* larvae raised on a diet of wheat bran and carrots. The collected frass underwent sterilization through a heating process at 100 °C for one hour, following the procedure outlined in previous research [8].

Microbiology of frass

A 5 g portion of frass was transferred into a conical flask, followed by the addition of two hundred milliliters of distilled water, and incubated for 72 hours at 27 °C with continuous shaking at 120 rpm. This procedure was performed in triplicate, with all flasks securely sealed, alongside a control flask containing only distilled water. The incubated mixtures were then streaked onto agar plates using the quadrant streak technique and left to incubate overnight at 27 °C.

Gram staining

The glass slides were disinfected using 70% alcohol. A bacterial isolate was carefully transferred onto a slide with a sterile loop and heat-fixed by passing the slide through the flame of a Bunsen burner. After one minute, crystal violet was added to the slide in two drops to stain the bacterial isolate. The slide was then dried and rinsed with distilled water. For primary staining, iodine was applied to the slide and allowed to sit for one minute. Afterward, the isolates were washed with distilled water and dried. To decolorize, 95% ethanol was added drop by drop, followed by a 30-second incubation, after which the isolates were rinsed with distilled water and dried again. Finally, the slide was counterstained with safranin for 30 seconds, rinsed with distilled water, and air-dried.

Microscopy (examining the gram stain)

The bacterial isolates were analyzed using a light microscope, with the slides examined at magnifications of 10 x, 40 x, and 100 x.

Mineral analysis

Phosphorus

A 2-gram sample was subjected to ashing at 600 °C for 4 hours. After ashing, the residue was allowed to cool, and 5 milliliters of 6N HCl, along with a few drops of nitric acid, were added before heating to fully dissolve the ash. The solution was then cooled and transferred to a one hundred milliliters volumetric flask, where distilled water was added to reach the calibration mark. Aliquots of two milliliters and 5 milliliters were transferred into 2 separate 100 milliliters volumetric flasks, and twenty milliliters of molybdovanadate reagent was introduced into each. Distilled water was then added to both flasks to bring the solution to the mark. After allowing the color to develop for ten minutes, the absorbance of each solution was measured at 400 nanometers using a phosphorus standard curve, with readings taken on a UV-VIS spectrophotometer.

Potassium

A 2-gram sample was measured, ashed, and transferred into a beaker, where 5 milliliters of 6N HCl were added along with four drops of concentrated nitric acid. The beaker was heated on a hot plate until the mixture nearly dried, after which distilled water was added. The resulting mixture was then moved into a 100-milliliter volumetric flask. Aliquots of 1 milliliter were pipetted into separate 100, 200, and 250-milliliter volumetric flasks. To each flask, 20 milliliters of phosphovanadate was added, and the volume was adjusted with distilled water. To calibrate the atomic absorption spectrometer, standards of one hundred ppm KH2PO4 were prepared by transferring 1 milliliter, 2 milliliters, and 3 milliliters of the standard solution into three different 100-milliliter volumetric flasks. Each flask also received 20 milliliters of phosphovanadate. A blank solution consisting of 20 milliliters of phosphovanadate was prepared, and absorbance was measured at 400 nanometers using atomic absorption spectroscopy.

Nitrogen

The Kjeldahl method was employed to determine the nitrogen content, starting with the weighing of 1 gram of frass into a 50 milliliter Kjeldahl flask, to which 40 milliliters of concentrated sulfuric acid was added. Anti-bumping chips and 10 grams of selenium catalyst were introduced into the flask, and the mixture was shaken and allowed to stand for ten to fifteen minutes before being digested for two hours at 150 °C. Following digestion, the solution was allowed to cool, then diluted with 200 milliliters of distilled water and left to cool again. Distillation was then performed by adding two drops of methyl red indicator to the receiving flask containing 150 milliliters of 50% sodium hydroxide, followed by the addition of zinc granules. A 100-milliliter solution was collected after distillation. This solution was then titrated in the third stage of the analysis using N/4 sodium hydroxide. The nitrogen content in the frass was calculated by determining the excess acid used during the titration process.

pH analysis

Five 5-gram soil samples were collected and placed in securely sealed containers. To each container, 75 milliliters of calcium chloride were added, and the containers were tightly sealed before being placed in a shaker for one hour. After shaking, the pH meter was calibrated using buffer solutions 4 and 7, with thorough rinsing between each use. The pH was measured by inserting the meter into each container and recording the readings for each sample.

Investigation of the fertilizing ability of frass sowing

The influence of frass on nutrient availability to plants was examined through a pot experiment. 12 plastic pots were selected for planting red wheat seeds (SC Smart). Initially, the pots were stored in a controlled dark space before planting. Afterward, they were transferred to the Natural Science and Technology Research (NSTR) laboratory, arranged randomly in a location with abundant sunlight. 3 wheat seeds were placed in each pot. To ensure sufficient sunlight, the pots were positioned behind a large transparent window. The plants received water three times per week.

Different fertilizer treatments

The experiment was conducted in triplicate and included four treatment categories: frass, mineral fertilizer, a combination of both (fertilizer and frass), and a control. The mineral fertilizer was purchased from a commercial supplier, while the frass was collected from *Tenebrio molitor* larvae and heated for one hour at 100 °C. The combined treatment involved an equal mass of fertilizer and frass, each weighing one gram, to treat the soil. The fertilizer treatment used 2 grams of fertilizer, and the frass treatment used 2 grams of frass. The control group consisted of untreated soil with no added nutrients. Frass was replenished every two weeks to account for the depletion of mineral content, as indicated in previous studies. Petri dishes were placed beneath the plastic pots to prevent the loss of water and nutrients during plant watering. Each treatment was labeled, and plant growth was monitored over 12 weeks.

Results and Discussion

Determination of microbial activity

The purpose of assessing microbial activity in frass was to determine whether microbes remained present after the sterilization process. The results from the sterilized frass samples revealed the presence of microbes, as depicted in **(Figure 1)**.

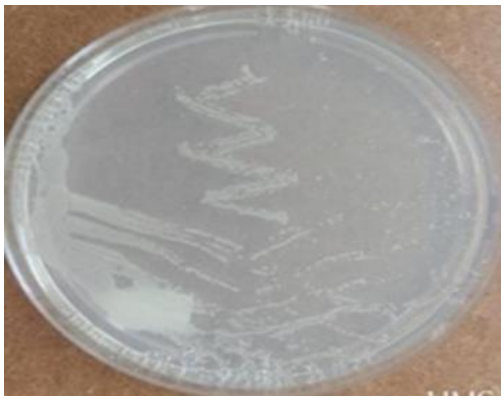

Figure 1. After sub-culturing and incubating at 27 °C for 24 hours, bacterial colonies were detected.

Microscopic examination

The microbial examination of frass from *Tenebrio molitor* was carried out using a light microscope. The bacteria observed were identified as gram-positive based on their appearance. They exhibited a round or circular shape and a light purplish color, as seen in **(Figure 2)**.

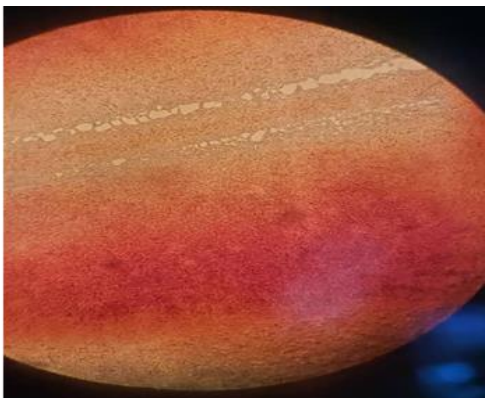

Figure 2. The pure bacterial colonies cultivated on nutrient agar plates were observed under a light microscope.

Mineral analysis

Phosphorus

Phosphorus levels were quantified by measuring the yellow color intensity produced in the presence of molybdovanadate with a spectrophotometer. The phosphorus percentage was calculated based on the equation

$$\text{Phosphorus} - \text{absorbance} = \frac{\text{Absorbance x Dilution factor}}{\text{X200} = 1.10 \times 2.29 \text{ (dilution factor)} = 2.519\%} \quad (1)$$

X100 = 1.89 x 1.15 (dilution factor) = 2.277%

Therefore after averaging: % Phosphorus = 2.3% to 2 s.f

Potassium

The percentage of potassium content was determined using the equation below, with the results expressed in parts per million (ppm).

Absorbance x Dilution factor/10000

50 x 20 x 5 x 5.70 = 28 500ppm
28 500/10 000 = 2.85%

50 x 50 x 5 x 2, 26 = 28 250pp
28 250/10 000 = 2.825%

The two values were averaged to give 2.8375 %
Hence % Potassium = 2.8% to 2 s.f

Nitrogen

The samples underwent distillation and titration, with the excess acid measured at 9.5 ml. This value was essential for determining the percentage of total nitrogen using the following formula:

Excess acid x 0.35 = % total nitrogen

0.35 came from the calculation of nitrogen's total mass in a gram which was 0.0035 g. If multiplied by 100% becomes 0, 35%

9.5ml x 0.35 = 3.325%

Nitrogen = 3.3% to 2 s.f

The nutrient composition of the frass obtained from mealworms revealed satisfactory levels of both macronutrients and several micronutrients, indicating its potential as an effective fertilizer. The NPK values found in this research were 3%, 2%, and 2%, which aligns with those reported in the literature [9]. Specifically, the mineral nutrient concentrations in this research were determined to be (3.3% N), (2.3% P), and (2.8% K). According to Poveda *et al*. [6], mealworm frass typically contains nitrogen levels between 2.7% and 7.8%, meaning the frass in this study, with its 3.3% nitrogen content, is on the lower end of this range. However, the authors observed that frass with 3.3% nitrogen outperformed that with 7.8% nitrogen in growth trials and under abiotic stress conditions, likely due to a lower carbon-to-nitrogen ratio in the frass with higher nitrogen [6, 10]. While much research has concentrated on frass from black soldier fly larvae, studies examining mealworm frass have also highlighted its promising potential as a fertilizer [6, 11].

pH determination

In addition to laboratory analyses, plant growth trials were conducted to validate the fertilizing potential of mealworm frass. Before planting and applying treatments, the pH of the soil used for all the potted plants was measured. After planting and soil treatment, the pH was re-measured for comparison to determine whether the soil's pH was influenced by the treatment, as presented in **(Table 1)**.

Table 1. pH values of the soil samples before and after planting wheat.

Type of treatment	Before planting	After planting and treatment
Control	5.9	6.2
Frass	5.9	6.0
Fertilizer	5.9	5.5
Complement	5.9	5.6

Before planting, the soil pH measured 5.9 across all pots. However, following the application of treatments, the pH values varied among the four treatments: complement had a pH of 5.6, fertilizer resulted in a pH of 5.5, frass had a pH of 6.0, and the control had a pH of 6.2.

Soil pH plays a crucial role in agriculture, significantly influencing the availability of nutrients to plants [12]. It is essential for plant growth since pH directly impacts the accessibility of key nutrients [13]. Most nutrients are available within a pH range of 5.5-6.5. If the soil becomes too acidic, certain nutrients, such as phosphorus, become less accessible, while others like aluminum and manganese may become toxic [14]. Acidic conditions also hinder beneficial microorganisms. Conversely, alkaline soils limit the availability of nutrients such as iron, copper, manganese, zinc, and phosphorus [15]. Many plants, particularly evergreens, require significant amounts of iron and struggle in alkaline soils. According to the Department of Research and Specialist Services in Zimbabwe's Ministry of Agriculture, an ideal pH range is 5.5-6.3. In environments where the soil is too acidic or basic, nutrients bind tightly to the soil, reducing their availability to plants [16]. Given that all pH values fell

within the acceptable range, the conclusions drawn were based on sound evidence, ensuring that the soil pH didn't interfere with the effectiveness of the treatments in improving nutrient content.

Potted plant investigation

Observation of plant growth

The wheat plant growth after two weeks is illustrated in **Figure 3**, where the plants treated with frass fertilizer exhibited the highest growth compared to the other treatments.

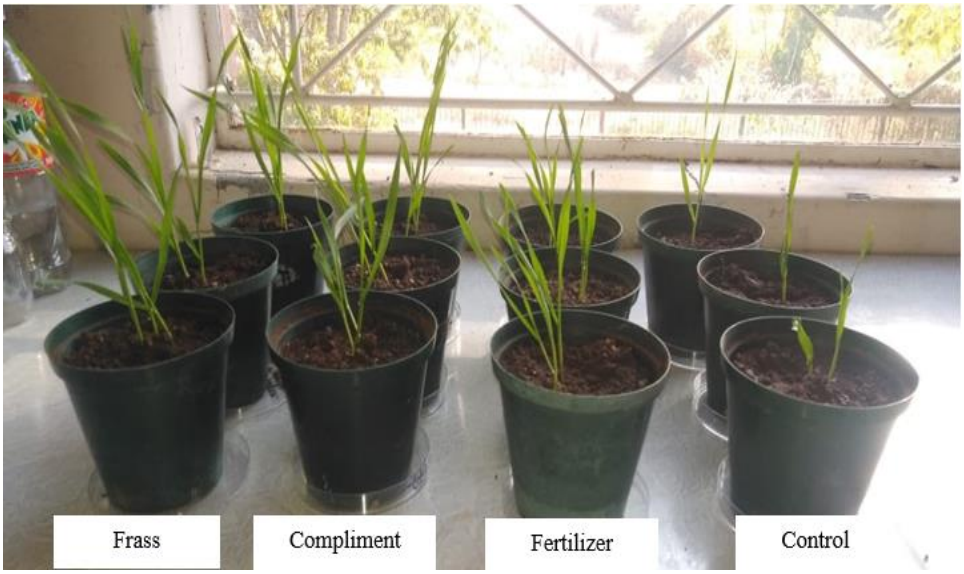

Figure 3. Wheat plant growth progression over two weeks.

The bacteria found in frass may have played a positive role in promoting plant growth. Besides enhancing growth, frass treatment may have also contributed to increased tolerance to environmental stresses. Additionally, frass is believed to stimulate plant immune responses, potentially strengthening the plants' defenses against diseases and pests [5, 17]. No signs of microbial damage were observed in the plants, as displayed in **(Figure 3)**, indicating that the microbes present in frass are beneficial rather than harmful.

Figure 4 illustrates the wheat plant growth trends over 12 weeks. Plants treated with frass experienced rapid growth, surpassing all other treatments from week 1 to week 4. A second surge in growth was observed between weeks 5 and 6. This trend continued until week 8, when plants treated with a combination of frass and fertilizer, along with commercial fertilizer, outpaced the frass-treated plants. From week 8 onward, the plants receiving the complement treatment showed the most robust growth, continuing through to week 12. As anticipated, the control group exhibited the least growth, as depicted in **Figure 4.**

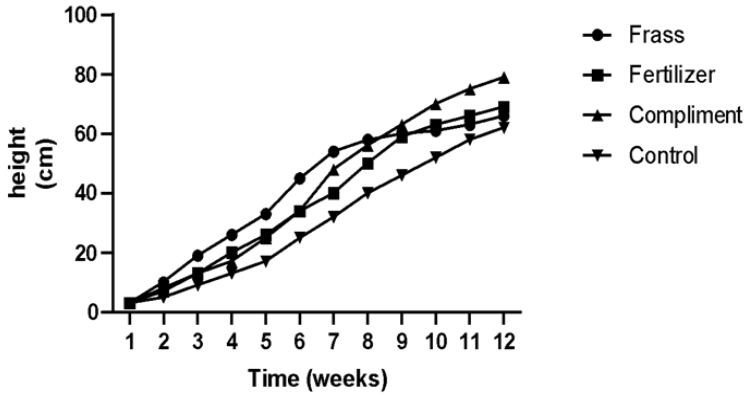

Figure 4. The growth progression of wheat plants over 12 months.

Stem and root development

As seen in **Figure 5**, the plants treated with complement, frass, and fertilizer showed more pronounced development in both stems and roots compared to the control. The complement-treated plants had thick, healthy stems, which were more robust than those of the frass and fertilizer plants. Their roots were well-defined, with a higher number of fibrous roots. The frass-treated plants also demonstrated strong stems and a healthy overall appearance. Their roots were fibrous and abundant, surpassing the commercial fertilizer and control groups in both definition and thickness. While the plants treated with commercial fertilizer appeared relatively healthy, their roots were fewer in number, and less thick, but still well-defined compared to the control. The control plants had thinner stems and fewer, less distinct roots.

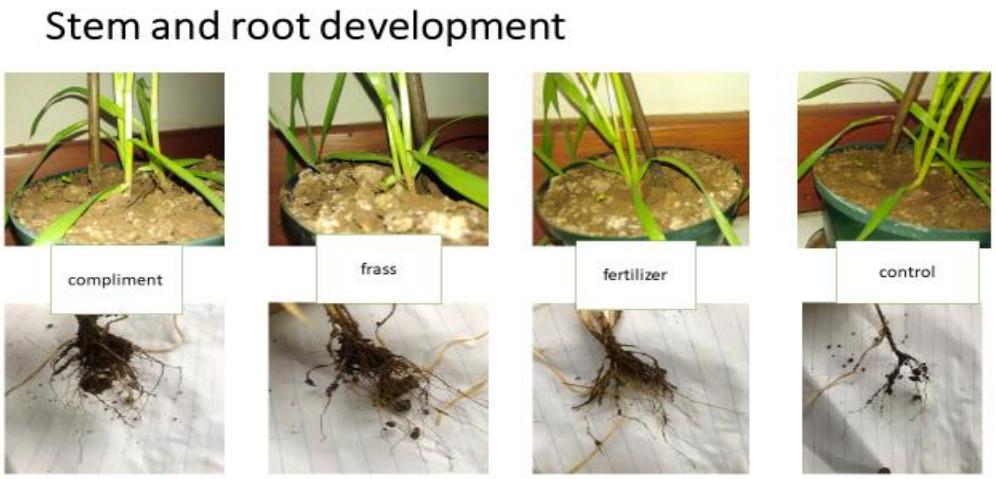

Figure 5. Development of stems and roots in wheat plants treated with various fertilizers.

Quality of seed and harvest

The yield from the spikelet with the highest production was selected from the best plants of each treatment group. As displayed in **Figure 6**, the complement treatment produced the highest yield, with seeds that were of excellent quality and a golden brown color. The size of the seeds was larger than those from other treatments, with a defined and robust appearance. The seeds felt mature and healthy to the touch.

Additionally, the frass-treated plants also showed a competitive yield. Their seeds were of high quality, with the same golden brown hue, adding to their visual appeal. The seeds appeared mature, well-defined, and healthy, with a firm texture when squeezed. The seed size was large and of good quality.

Commercial fertilizer-treated plants had a moderate yield, with brown-colored seeds. Although the quality was fair, the seeds were not as strong or defined as those from the frass and complement treatments. The seed size was still good.

The control plants, on the other hand, had the least desirable yield, with seeds of lower quality. Most of the seeds were underdeveloped and poorly defined, and the seed coats were often empty. The color was dull, and the overall appearance was not as healthy.

Figure 6. Seed yield and quality of wheat plants from the same species, subjected to various fertilizer treatments.

Conclusion

The mineral composition of the frass, specifically its NPK values, was found to be 3.3%, 2.8%, and 2.3%, respectively. These figures correspond closely with those found in the literature, indicating that the frass has high fertilizing potential and could serve as a viable alternative or supplement to traditional fertilizers.

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Conflict of Interest: None

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Ethics Statement: None

References

1. Nowakowski AC, Miller AC, Miller ME, Xiao H, Wu X. Potential health benefits of edible insects. Crit Rev Food Sci Nutr. 2022;62(13):1-10.
2. Dulaurent AM, Daoulas G, Faucon MP, Houben D. Earthworms (Lumbricus terrestris L.) mediate the fertilizing effect of frass. Agronomy. 2020;10(6):783. doi:10.3390/agronomy10060783
3. Choi S, Hassanzadeh N. BSFL Frass: a novel biofertilizer for improving plant health while minimizing environmental impact. CSFJ. 2019;2(2):41-6. doi:10.18192/csfj.v2i220194146
4. Specht K, Zoll F, Schümann H, Bela J, Kachel J, Robischon M. How will we eat and produce in the cities of the future? From edible insects to vertical farming—a study on the perception and acceptability of new approaches. Sustainability. 2019;11(16):4315. doi:10.3390/su11164315
5. Quilliam RS, Nuku-Adeku C, Maquart P, Little D, Newton R, Murray F. Integrating insect frass biofertilisers into sustainable peri-urban agro-food systems. J Insects Food Feed. 2020;6(3):315-22. doi:10.3920/jiff2019.0049
6. Poveda J, Jiménez-Gómez A, Saati-Santamaría Z, Usategui-Martín R, Rivas R, García-Fraile P. Mealworm frass as a potential biofertilizer and abiotic stress tolerance-inductor in plants. Appl Soil Ecol. 2019;142(1):110-22. doi:10. 1016/j.apsoil.2019.04.016
7. Houben D, Daoulas G, Faucon MP, Dulaurent AM. Potential use of mealworm frass as a fertilizer: Impact on crop growth and soil properties. Sci Rep. 2020;10(1):4659. doi:10.1038/s41598-020-61765-x
8. Joemat-Pettersson T. Fertilizers, farm feeds, agricultural remedies and stock remedies act, 1947. Staatskoerant, 2012.
9. Ahmad F, Singh A, Kamal A. Salicylic acid-mediated defense mechanisms to abiotic stress tolerance. InPlant Signaling Molecules 2019 Jan 1 (pp. 355-369). Woodhead Publishing.
10. Farooq M, Pisante M, editors. Innovations in sustainable agriculture. Cham, Switzerland: Springer International Publishing; 2019. doi:10.1007/978-3-030-23169-9
11. Gärttling D, Kirchner SM, Schulz H. Assessment of the N- and P-fertilization effect of black soldier fly (Diptera: Stratiomyidae) by-products on maize. J Insect Sci. 2020;20(5):1-11. doi:10.1093/jisesa/ieaa089
12. Beesigamukama D, Mochoge B, Korir N, Musyoka MW, Fiaboe KK, Nakimbugwe D, et al. Nitrogen fertilizer equivalence of black soldier fly frass fertilizer and synchrony of nitrogen mineralization for maize production. Agronomy. 2020;10(9):1395. doi:10.3390/agronomy10091395
13. Sánchez-Bayo F, Wyckhuys KA. Worldwide decline of the entomofauna: a review of its drivers. Biol Conserv. 2019;232:8-27.
14. Kathpalia R, Bhatla SC. Plant mineral nutrition. InPlant physiology, development and metabolism 2018 (pp. 37-81). Springer, Singapore. doi:10.1007/978-981-13-2023-1_2
15. Capdevila-Cortada M. Electrifying the Haber–Bosch. Nat Catal. 2019;2(12):1055. doi:10.1038/s41929-019-0414-4

16. FAO. The state of world fisheries and aquaculture. 2020. Retrieved April 12, 2021. Available from: http://www.fao.org/3/ca9229en/ca9229en.pdf
17. Cheval C, Samwald S, Johnston MG, de Keijzer J, Breakspear A, Liu X, et al. Chitin perception in plasmodesmata characterizes submembrane immune-signaling specificity in plants. Proc Natl Acad Sci U S A. 2020;117(17):9621-9. doi:10.1073/pnas.1907799117