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Analysis of Frass Excreted by *Tenebrio molitor* for Use as Fertilizer

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ABSTRACT

Insect production is a rapidly growing industry worldwide, as it presents a promising solution for the effective recycling of organic waste. The most abundant by-product of insect production is insect feces, scientifically known as 'frass'. Frass is produced in greater volumes than the actual insect products and therefore should be utilized in order to maintain a circular economy. Thus, this study focused on analyzing the potential of using frass from the yellow mealworm (*Tenebrio molitor*) as a fertilizer. Mineral analysis of frass was investigated using spectroscopic methods and in terms of nitrogen, phosphorus, and potassium, mineral content was found to be 3.3 %, 2.8 %, and 2.3 % respectively. These values were in cognition with values that were found in literature, meaning that the frass can be used as a fertilizer replacement or complement. The fertilizing ability of frass was also investigated and results showed the best harvest from soil treated with complement (frass and mineral fertilizer), followed by frass having quality seeds, then mineral fertilizer which had a relatively good yield, and lastly control which had no treatment of the soil. However, more research should be conducted, especially on the immune-stimulating aspect of frass, to collaborate on the promising results obtained during this project.

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

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INTRODUCTION

Soil degradation and poor waste management are severe threats to Sub-Saharan Africa's environmental health and food and nutrition security (SSA). Approximately 40 % of soils in SSA are lacking in the majority of nutrients essential for crop growth, with 25 % influenced by aluminum toxicity, 18 % susceptible to leaching, and 8.5 % defined by phosphorus fixation [1]. However, most small-scale farmers frequently use mineral fertilizers though their effectiveness is still limited by poor soil organic matter, lack of micronutrients, and high soil acidity [2]. Although farmers are willing to utilize organic fertilizer and can afford it, there has not been much uptake because of poor quality, a lengthy production process, and a scarcity of sources of organic matter on farms [3]. Thus, the purpose of this study was to analyze the potential of using frass as a fertilizer. Frass is characterized as insect excrement which has been reported to improve plant growth and increase plant tolerance to abiotic stresses, such as drought and flooding [4]. It contains uneaten remnants of feed substrate as well as chitin-rich exoskeleton fragments from the insect which are believed to be able to trigger plant immunological responses, which may boost the plant's resistance to pests and diseases [5]. Depending on the insect species and its diet, frass can often account for 80–95 % of overall production output, which is 4–20 times greater than insect biomass output [6]. Therefore, the anticipated expansion of the insect industry, particularly *Tenebrio molitor* for food production will inevitably result in a corresponding rise in the production of frass, causing major waste problems for producers. Thus, there is a need to characterize frass

excreted by *Tenebrio molitor* for possible use as a fertilizer.

However, previous work by Poveda *et al*., showed that the diet of the yellow mealworm greatly influenced both the nutritional and the microbial content of its frass, yielding fertilizers with significantly different growth-promoting abilities [6]. It was found that a diet containing 66 % carbohydrates, 6 % fat, and 28 % protein resulted in the best-performing frass fertilizer, with nitrogen, phosphorus, and potassium (NPK) values of approximately 3 %, 2 %, and 2 % respectively. Houben *et al.,* found frass to be as effective as mineral NPK fertilizer in growth trials with barley (*Hordeum vulgare*), and the nutrient content of leaves was similar between the two fertilizer treatments [7]. The authors propose that frass can substitute mineral NPK fertilizer partially or completely, without compromising biomass output. They also found that the presence of frass increases the diversity as well as metabolic activity of soil microbiota, supporting the research on microorganisms in frass by Poveda *et al*. [6].

MATERIALS AND METHODS

Sample preparation

Frass was obtained from the larvae of *Tenebrio molitor* which were fed with wheat bran and carrots. Collected frass was sterilized by the method of heating frass for an hour at 100 °C as previously reported [8].

Microbiology of frass

A 5 g sample of frass was placed in a conical flask and 200 ml of distilled water was added and then incubated for 72 hrs at 27 °C and 120 rpm. This was done in triplicate with all the flasks tightly covered along with another flask that contained only distilled water as the control. Using the quadrant streak method, the incubated solutions were applied to the agar plates and left to grow overnight at 27 °C.

Gram staining

Using 70 % alcohol, object glass slides were disinfected. One of the bacterium isolates was aseptically removed from a loop, spread on a slide, and repeatedly fixed on the bunsen lamp fire. After one minute, two drops of crystal violet were used to etch the bacterium isolates. Additionally, isolates were dried and rinsed with distilled water. Primary staining was done where iodine was then dropped onto the slide and allowed to sit there for a minute. Using distilled water, isolates were cleaned and dried off. Rapid decolorization was performed with ethanol, and after progressively combining drops of 95 % alcohol with bacteria isolates for 30 seconds, the isolates were rinsed with distilled water and dried out. Counterstaining was performed with safranin as it slowly leaked isolates for 30 seconds. The isolates were subsequently rinsed with distilled water and air-dried once more.

Microscopy (examining the gram stain)

The prepared bacteria isolates were examined under a light microscope, and the slides were observed under magnification of X10, X40, and X100.

Mineral analysis

Phosphorus

A 2 g sample was taken for ashing for 4 hours at a temperature of 600 °C. The residue was cooled after ashing and 5 ml of 6N HCl along with several drops of Nitric acid were added before being heated to dissolve the ash completely. The solution was cooled and transferred to a 100 ml volumetric flask where distilled water was added to the mark. Aliquots of 2 ml and 5 ml were pipetted respectively into two separate 100 ml volumetric flasks and 20 ml of molybdovanadate reagent was added. Using distilled water the solution was diluted to the mark for both flasks. The color was allowed to develop for 10 minutes and the absorbance for each solution was read at 400 nm using a phosphorus standard curve with aid of a UV-VIS Spectrophotometer.

Potassium

A 2 g sample was weighed and ashed, then placed into a beaker and 5 ml of 6N HCl was added. In addition, 4 drops of concentrated nitric acid were added and the beaker was placed on a hot plate. The mixture was heated until almost dry and distilled water was added. The mixture was then transferred into a 100 ml volumetric flask and 1 ml aliquots were taken into 100 ml, 200 ml, and 250 ml volumetric flasks respectively. A volume of 20 ml of

phosphovanadate was added into each flask and then topped up to the mark with distilled water. Standards of 100 ppm of KH_2PO_4 were prepared to be used to calibrate the atomic absorption spectroscopy machine. In this respect, 1 ml, 2 ml, and 3 ml of the standard solution were taken and placed into 3 different 100 ml volumetric flasks respectively and 20 ml of phosphovanadate was added in each flask. A blank of 20 ml of phosphovanadate and the readings were taken at a wavelength of 400 nm with the use of atomic absorption spectrum spectroscopy.

Nitrogen

This was achieved by using the Kjeldahl method, whereby 1 g of frass was weighed into a 50 ml Kjeldahl flask and 40 ml of concentrated Sulphuric acid was added. Anti-bumping chips were added into the flask together with 10 g of selenium catalyst. The mixture was shaken and left for 10-15 minutes before being digested for 2 hours at 150 °C. After digestion, the solution was left to cool and thereafter diluted with 200 ml of distilled water and left to cool again. Then followed the distillation process, and 2 drops of methyl red indicator were added with 150 ml of 50 % sodium hydroxide into the receiving flask. A handful of zinc granules were added and a solution of 100 ml was collected after distillation. This volume was then taken to the third stage of the analysis which is titration, where the volume was titrated with N/4 sodium hydroxide. Calculations were done using the excess acid to obtain the percentage amount of nitrogen present in the frass.

pH analysis

A 5 g sample of the soil to be analyzed (there were 5 samples in total) was taken and placed into tightly closed containers. Calcium chloride (75 ml) was added into each container with a sample, tightly closed, and put on a shaker for an hour. After shaking, calibration of the pH meter was done using buffer 4 and buffer 7 with washing after the use of each. The pH was determined by dipping the meter into each container and taking the reading of each.

Investigation of the fertilizing ability of frass sowing

The impact of frass on the availability of nutrients to plants was investigated in a pot experiment. Twelve plastic pots were used to plant the wheat seeds (SC Smart) which were red in color. The pots were kept in a controlled, dark environment before planting. The pots were moved to the Natural Science and Technology Research (NSTR) laboratory and put in a randomly chosen order, where sunlight was easily accessed. Three wheat seeds were planted in each pot. The plastic potted plants were put behind a large transparent window to allow sunlight availability for the plants. The seeds were watered 3 times per week.

Different fertilizer treatments

The experiment was done in triplicate and there were four categories of treatments of the soil to be analyzed namely frass, mineral fertilizer, complement (both frass and fertilizer), and the control of the experiment. The conventional fertilizer that was used was bought from a fertilizer shop. The frass that was used was obtained from the *Tenebrio molitor* larvae and typically heated for one hour at 100 °C. The complement treatment comprised equal masses of frass and fertilizer of a gram each mixed to treat the soil. The fertilizer treatment was treated with 2 g of fertilizer and for frass 2 g was made use of as well. The control had soil that was not treated or added any minerals for growth. Frass top-ups were done after every 2 weeks as pointed out in the literature because of the reduced amount of mineral elements in frass. Petri dishes were put under the plastic pots to prevent loss of water along with nutrients upon watering of plants. Each treatment was labeled and the growth was observed over 12 weeks.

RESULTS AND DISCUSSION

Determination of microbial activity

The aim of performing the microbial activity in frass was to initially check if microbes were present even after sterilization. Sterilized frass samples showed the presence of microbes as shown in **(Figure 1).**

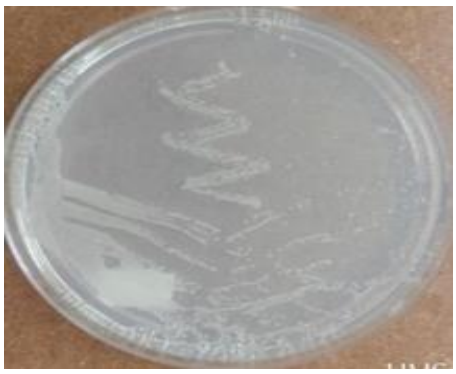

Figure 1. Bacterial colonies were observed after sub-culturing and incubation at 27 °C for 24 hours

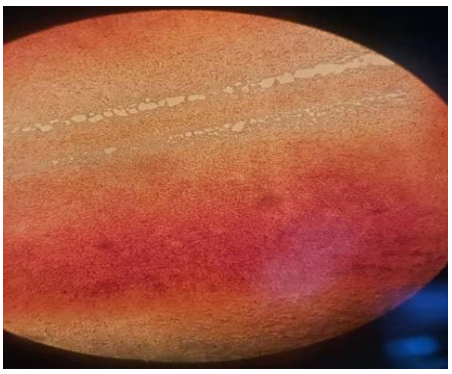

Figure 2. The microscopic examination of the pure colonies of bacteria grown on nutrient agar plates was viewed under a light microscope.

Microscopic examination

The microscopic examination of microbes obtained from frass excreted by *Tenebrio molitor* was done using a light microscope. It was deduced from the appearance of the bacteria that the microbes were gram-positive bacteria. The observable appearance of the obtained bacteria was its round or circular shape and the color depicted which was a light purplish **(Figure 2)**.

Mineral analysis

Phosphorus

Phosphorus content was determined by measuring the intensity of the yellow color in the presence of molybdovanadate using a spectrophotometer. Phosphorus % content was calculated using the equation:

Absorbance x Dilution factor

Phosphorus – absorbance

X200 = 1.10 x 2.29 (dilution factor) = 2.519 % (1)

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

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

Potassium

Potassium % content was calculated using the equation below and results were given in 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 % (2)

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

Nitrogen

The samples were taken for distillation and titration, where in titration the excess acid was found to be 9.5 ml. This was the crucial value used to calculate the % total nitrogen in formulae:

Excess acid x 0.35 = % total nitrogen (3)

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 profile of the mealworm frass obtained showed good levels of both macronutrients as well as several micronutrients, which suggests promising fertilizing qualities. According to the literature the NPK values that were found were 3 %, 2 %, and 2 % respectively [9]. This suggests that the mineral nutrients found in this study were of good value, as NPK values were found to be (3.3 % N), (2.3 % P), and (2.8 % K). Poveda *et al*. reported that mealworm frass can have a nitrogen content ranging from 2.7 % to 7.8 %, indicating that the frass from (3.3 % N) is somewhat low in nitrogen. Yet, during growth trials and abiotic stress experiments, the authors found that frass with 3.3 % nitrogen performed much better than the one containing 7.8 % nitrogen at a 2 % volume inclusion, a result attributed to the low carbon-to-nitrogen ratio in the latter frass version [6, 10]. Most research has focused on frass from the black soldier fly larvae, but those that looked at mealworm frass concluded that it is a very promising fertilizer [6, 11].

pH determination

Lab analyses alone were not sufficient proof so plant growth trials were also carried out in this study to demonstrate the fertilizing qualities of mealworm frass. The initial soil pH of soil that was used for all the potted plants was taken before planting and treatment of the soil. The pH of the soil after planting and after treatment was then taken for comparison to see if treating the soil affected the pH as shown 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

The pH that was read and observed before planting was 5.9 and this was for all the soil in every different pot. However, after subsequently adding treatments, the pH was altered in all four cases with complement exhibiting pH 5.6, fertilizer showing pH 5.5, frass exhibiting pH 6.0, and the control pH 6.2.

The aspect of pH in the soil is an important one in agriculture and gravely affects the availability or accessibility of nutrients by plants [12]. The accessibility of practically all vital plant nutrients is determined by pH, which is fundamental to plant growth [13]. Most nutrients are accessible for plant use with a soil pH of 55-6.5. If the pH of the soil is excessively acidic, certain nutrients, particularly phosphorus, become less available, whereas others, such as aluminum and manganese, can become hazardous [14]. Acidic pH levels are also unfavorable to beneficial microorganisms. Alkaline soils make nutrients like iron, manganese, copper, zinc, and phosphorus unavailable [15]. Plants typically require a lot of iron, particularly evergreens, and struggle on alkaline soils. The Department of Research and Specialist Services under the Ministry of Agriculture in Zimbabwe stipulate that the acceptable pH range is 5.5-6.3. In a more acidic or basic soil environment nutrients bind tightly to the soil, diminishing the availability of nutrients for the plant [16]. All pH values were in the acceptable range hence the deductions made were based on a good foundation, which was good soil pH that did not interfere with the treatments done to the soil in improving nutrient content.

Potted Plant Investigation

Observation of plant growth

The growth of the wheat plants over 2 weeks is shown in **(Figure 3)**, where the frass fertilized plants had growth greater than all the other plants.

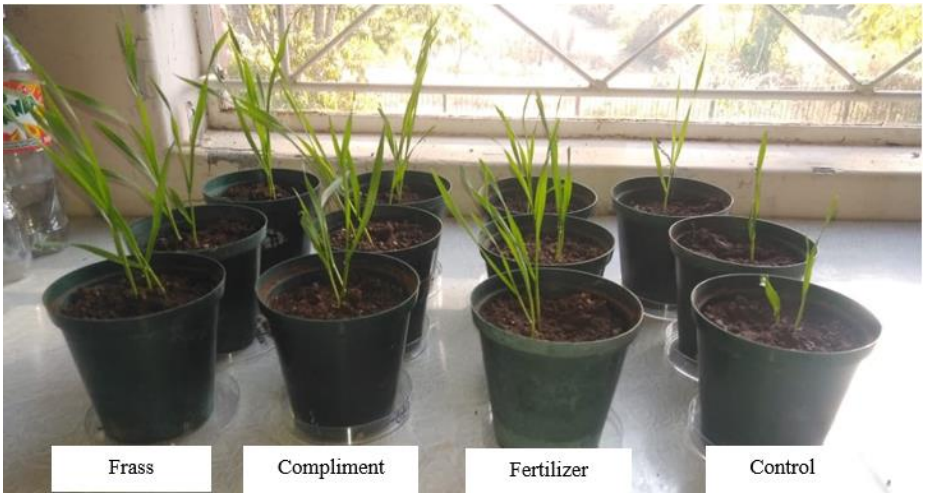

Figure 3. Growth trend of wheat plants over 2 weeks.

It is however possible that the bacteria that was found in frass in its predominant nature was beneficial to the plant growth. In addition to improving plant growth, treatment with frass may have also increased plant tolerance to abiotic stresses. Frass is also believed to be able to trigger plant immunological responses, which may boost the plant's resistance to pests and diseases [5, 17]. There are no adverse signs and symptoms of a microbial attack on the plants as shown in **(Figure 3)** hence microbes in frass have no harmful effects on plants, but rather beneficial aspects.

Figure 4 shows the growth trend of wheat over 12 weeks where the frass fertilized plants had an exponential growth greater than all the other plants from the first week to the fourth week. From the 4th week, another steep rise in growth is shown between week 5 and week 6. This trend of overpowering the other plants continued until week 8 when it was overtaken by complement and commercial fertilizer fertilized plants in week 8. The compliment had the most flourishing plants from the 8th week till the 12th week. The control as expected had the least growth of all the wheat plants, as clearly shown in **(Figure 4)**.

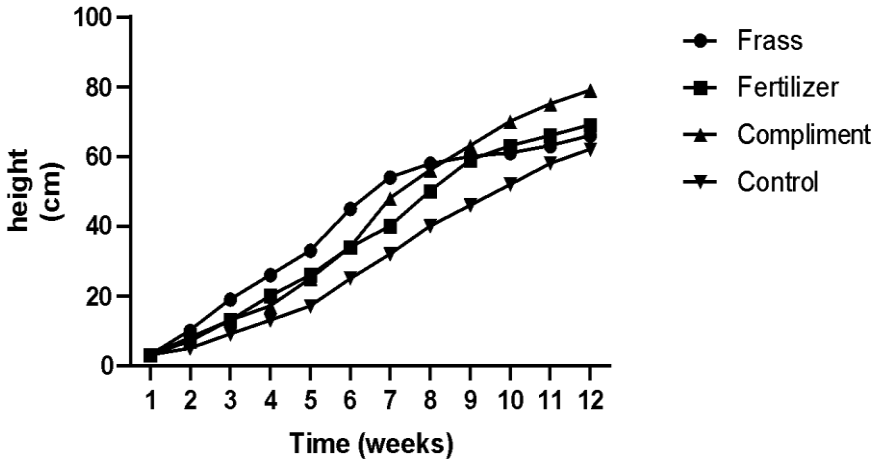

Figure 4. Growth trend of wheat plants over a period of 12 months

Stem and root development

As shown in **(Figure 5)**, the stems and roots of the complement, frass, and fertilizer were more distinct and developed more than the control. The stems for the compliment were thick and exhibited a healthy outlook more than the one for frass and fertilizer. The roots were more defined, with distinct fibrous roots. The number

of roots for the compliment was greater than all the others. The frass-fertilized plants also had thick stems and a healthy appearance of the plants. The roots were also healthy and more fibrous than the fertilized plants and the control. The number of roots was many as well as defined and thick. The plants that were treated with commercial fertilizer also exhibited a relatively healthy appearance with roots that were reduced in number and thickness but were distinct and defined better than the ones for control. The control had thin stems and few roots that were not as defined as the others.

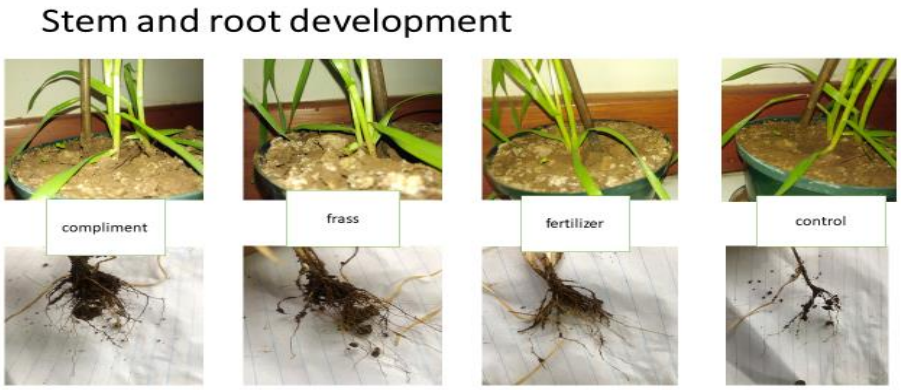

Figure 5. The stem and root development of the wheat plants are treated with different fertilizers

Quality of seed and harvest

The number of seeds harvested from the spikelet that had the most yields was chosen on the best plants of each group. As shown in **(Figure 6)**, the complement had the most yield and the seeds were desirable and of good quality, with a golden brown color for its seeds. The size of the seeds was greater than all the others and was more defined and strong as well. The seeds had a mature feel in the hands and had a healthy appearance.

Furthermore, the frass also exhibited a good yield that competed with the compliment. The seeds were of really good quality and the color of golden brown color was still exhibited in the seeds which added to their quality appearance. The seeds looked mature, defined, and healthy as well as strong upon squeezing. The size of the seeds was large and of good quality. The plants that were fertilized with commercial fertilizer had a relatively or moderately good yield with a brown color. The quality was relatively good and its seeds were defined but were not as strong as the frass' and complementary plants. The seeds had a good size as well. Lastly, the control showed the least quality in seeds and had the least yield which was not as desirable, most of the seeds were not mature or defined. Most of the seed coats were empty as well and the color was not as desirable or healthy looking.

Figure 6. The seed and harvest quality of the same species of wheat plant but treated with different fertilizers

CONCLUSION

The mineral content of frass in terms of NPK was found to be 3.3 %, 2.8 %, and 2.3 % respectively. These values were in cognition with values that were found in literature,

meaning that the frass was of high value and can be used as a fertilizer replacement or complement.

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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;142:110-22. 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