Is biodigester effluent a suitable replacement for commercial fertilizers?

Assessing the efficacy of liquid biogas digestate for cultivation of tomato (*Solanum lycopersicum*) crops in Barbados

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1. Abstract

Apart from biogas, an increasingly desirable product of anaerobic digestion (AD) is biogas digestate or effluent, a byproduct that comes in solid and liquid fractions and can be applied as fertilizer. The core of this research examines the effects of a range of concentrations of post-AD liquid digestate on the physical characteristics and qualitative growth of tomato plants as well as potential effects on soil nutrient composition. Research shows various levels of efficacy of digestate used as plant fertilizer either on its own or in conjunction with a commercial inorganic fertilizer. Experimental data shows that effluent application of low (5-10%) concentration is associated with second highest tomato plant productivity in terms of number of fruits, height, and chlorophyll content, only after the inorganic fertilizer control group. This study provides a foundation for future investigation of the biogeochemical processes resulting from applying the liquid digestate as a soil amendment. More studies are needed to improve current knowledge on the exact concentrations of effluent (dose)–and thus soil composition–under which tomato plants thrive and achieve their optimal growth and fruiting (response). Subsequently, recommendations of proper digestate dosage can be made to farmers looking to improve the economic and environmental sustainability and sustainability of their agricultural systems.

Abbreviations:

Anaerobic digester (AD)

Dry matter (DM)

University of The West Indies - Cave Hill campus (UWI)

2. Introduction

Agriculture is known to extensively impact the world's natural resources, and with the impending effects of climate change, food production is now faced with limited key resources such as energy, land, water, and nutrients. Global issues related to agriculture include greenhouse gas emissions from the manufacturing and use of inorganic fertilizers, air and water pollution from emissions and excess nutrient leaching, and high fossil fuel-based energy consumption. (Lukehurst et al., 2010). Sustainable agricultural practices are designed to conserve the environment and are necessary for successful long-term agricultural production in Barbados. Fertilization is done in most crop systems to significantly increase the availability of certain nutrients in soil—the most essential being nitrogen (N), phosphorous (P), and potassium (K). However, there are different fertilization plans used–with organic fertilizers adding organic matter and releasing nutrients into the soil over a longer period of time compared to inorganic ones–and thus yield varied plant growth levels depending on method.)—with different types and nutrient ratios of fertilizers yielding varying plant growth effects. For instance, organic fertilizers supply carbon to the soil, whereas inorganic ones target specific nutrient needs more precisely but do not contribute to bulk soil.

In recent years, there has been demand for less costly, renewable alternatives to replace petroleum-based fertilizers. Anaerobic digestion of organic residues and wastes from agriculture and other industries can be used to produce both renewable energy and organic fertilizer as biogas (methane) and digestate (effluent), respectively. The processing of organic materials for biogas takes place in digester, in which acetogenic and methanogenic bacteria which break down organic material into various digestates in the absence of oxygen. Biogas is composed primarily of methane and CO_2 as well as small amounts of water vapor and other gases, and digestate. AD yields an alternative to fossil-fuel based energy source that can be employed while simultaneously retaining much of the nutrient content of the material. Because the technology is adaptable to digest a wide range of material, it also provides a sustainable strategy for managing organic wastes. At The University of The West Indies – Cave Hill Campus, the anaerobic digester biochemically converts organic plant material such as bagasse, sargassum, coconut husks, and grasses into biogas. Hence, research on the feasibility of replacing traditional fertilizers with biogas effluent produced from locally-sourced materials has potential to significantly impact sustainability within several areas of agriculture and natural ecosystems in Barbados.

3. Background and Literature Review

Barbados is considered as the “humid tropics” with a climate profiling typically high temperatures of 27.0ºC and uneven rainfall averaging 105.86 mm of rain per month (Barbados Official Meteorological Services, 2019). Soil in most parts of the island exceed 50% clay, formed over a parent material of calcified coral limestone, with these factors contributing partially to the generally poorly drainage, leached, and highly alkaline soils that limit crop production in Barbados (Vernon and Carroll, 1965). The primary way low fertility agricultural land has been ameliorated is through heavy inputs of chemical fertilizers, which bear large economic and ecological costs to the detriment of the social wellbeing of farmers and others. Therefore, in order to decrease the costs of dependence on inorganic fertilizers, more sustainable alternatives must be developed and employed–an area where AD technology could play a large role.

Further, in recent years, Barbados has been working towards enhancing its agriculture sector, which currently only contributes 1.6% of the GDP per capita mainly from sugarcane (CARDI, 2019). In 2013, the country's import rate was significantly higher than its domestic supply of produce (FAO, 2016). In order to boost agricultural production on the island and lessen farmers' costs, priority must be placed on addressing the barriers to agricultural development. For example, a lack of strict land use policies have led to deforestation and intensive cultivation of short-term monocrops on hilly land, further resulting in soil erosion leading to infertility and reduced land arability (Madramooto, 2000). Soil quality and nutrient composition are critical to the growth and development of crops, especially in Barbados where they are simultaneously impacted by challenges such as pest and disease persistence. Adding organic matter either in raw or digested form helps improve soil structure, nutrient retention, microbial activity, and resilience to climatic factors. Currently, horticulture on the island is predominantly done by small farmers with limited resources producing for the domestic market, and they are directly impacted by poor soil fertility, so developing sustainable, less costly alternatives to replace traditional agrochemicals is imperative (Madramooto, 2000).

3.1 Biogas digestion

Several environmental and agricultural reasons make anaerobic digestion appealing, as outlined in Figure 1. The central goal as of now is the production of biogas, a renewable energy source that cuts down on CO_2 emissions and fossil fuel energy costs while offering an efficient and ecological way to recycle nutrients and increase economic advantages for farmers (Comparetti et al., 2013).

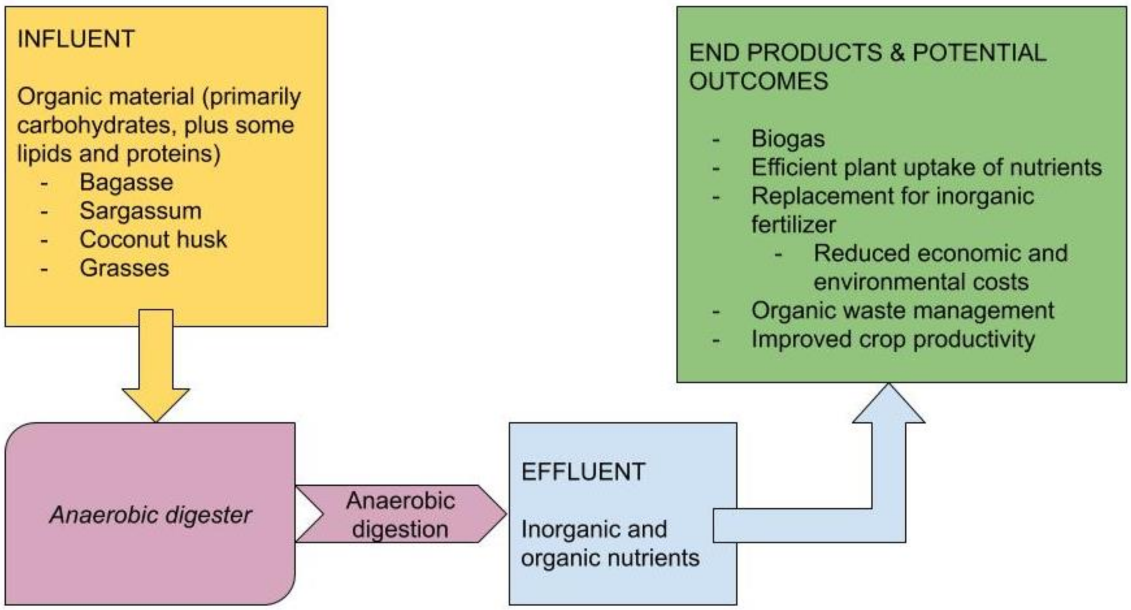


Figure 1: Overview of AD and potential outcomes. [Adapted from Harrison et al. (2019)].

In addition, anaerobic digestion for biogas yields two main byproducts: 1) a solid fraction containing fibers that the microorganisms were unable to utilize and 2) methanogenic digestate (i.e., effluent composed of bacterial cells), the liquid component that is rich in nutrients and is currently being investigated for its potential to substitute mineral fertilizer. Mineral elements contained in most AD effluents are nitrogen (ammonia and ammonium), phosphorous (phosphate), and potassium, which are all naturally occurring essential nutrients that promote plant growth just and are found in synthetically-made conventional fertilizer. It has been shown that AD effluent increases the content of macro- and micro-nutrients in soil and plants (Chiew et al., 2015). Studies show that liquid digestate recycled as bio-fertilizer improves soil fertility and plant immunity to biotic and abiotic stressors, resulting in enhanced quality and yield of vegetables (Koszel and Lorencowicz, 2015; Kouřimská et al., 2012). Although the solid and liquid digestates have lower carbon to nitrogen ratio than in the initial feedstock, organic carbon levels will vary depending on the efficiency of the biogas digester itself, which is beyond the scope of this study (Sogn et al., 2018).

Koszel and Lorencowicz’s study (2015) revealed that liquid biogas digestate contains similar composition of macroelements as bovine liquid manure, and an increase in leaf macronutrient content is found in alfalfa after being fertilized with effluent versus those fertilized with inorganic fertilizer. Further, soil samples were taken before and after digestate application and revealed an 18.79% increase in phosphorous content in the soil [mg per 100 g of soil] after applying effluent (Koszel and Lorencowicz, 2015). This indicates that the effluent could have either added phosphorous to the soil or that the plants did not take up the nutrients. Additionally, from the first harvest, the alfalfa cultivated in soil fertilized with mineral fertilizer and digestate were examined respectively; alfalfa sown on soil fertilized with digestate had 0.55% more nitrogen and 0.29% more potassium in its biomass, demonstrating a positive response of crops towards biogas digestate (Koszel and Lorencowicz, 2015).

Another study by Sogn et al. (2018) assessed the NPK fertilizer value of biogas effluent in silt, loam, and sand soil types and also evaluated the risk of nutrient leaching. The results determined that applying digestates to silt and loam soils yielded the same biomass production of wheat as an application of mineral fertilizer (Sogn et al., 2018). A point of interest is that though phosphorous content was found to be four times higher in the digestate treatment than the mineral fertilizer, the leaching losses associated did not occur to the same degree, which is interpreted as a positive finding (Sogn et al., 2018).

Möller and Müller (2012) propose anaerobically digested crop residues and cover crops as a use-as-needed alternative to green manures, being “most beneficial in organic vegetable cultivation, where quick release fertilizers are lacking” (Möller and Müller, 2012). They found that digesting crop residues and cover crops leads to “an increase in total amounts of mobile organic manures in the farming system,” which in turn results in higher N-use efficiency. Another positive contribution of biogas digestate, is that digestates contain bioactive substances, such as phytohormones, that increase plant tolerance to biotic and abiotic stress while potentially promoting plant growth, thus likely promoting plant yield in a way that mineral fertilizer does not (Möller and Müller, 2012).

This research project focuses on examining the different effects that effluent solutions–relative to inorganic fertilizer–have by observing and comparing plant nutrient contents and subsequently make conclusions on the efficacy and substitution potential of AD effluent as a fertilizer. Successfully using the effluent in place of inorganic fertilizer would expand the applications of anaerobic digestion technology to environmentally, economically, and socially sustainable agriculture.

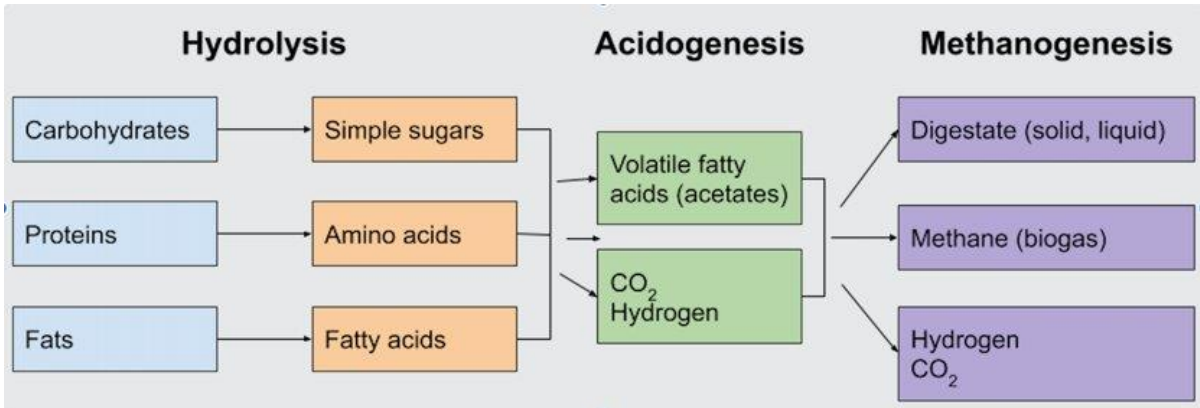


Figure 2: Flow diagram of the anaerobic digestion process and resulting products. [Adapted from Santibañez et al. (2011)]

3.2 Organic carbon

Organic carbon is found in organic matter and is the foundation of soil fertility. The presence of organic carbon marks a main difference between organic and inorganic forms of fertilizer. The benefits of supplying soils with organic material include the addition of nutrients, increased soil aggregation, permeability, water-holding capacity, and prevention of soil loss (Funderburg, 2001). Proper fertilization encourages shoot and root growth, which helps to maintain soil organic matter and thus reduces erosion through the aforementioned processes (Funderburg, 2001). Although the input of inorganic fertilizers can be an expensive practice, it is evident that additional supplementation is necessary to provide plants with essential nutrients in the Barbadian landscape. Effective and efficient nutrient management for optimal production of tomatoes and other crops must ensure adequate amounts of N, P, and organic C, the latter being present in effluent but not in inorganic fertilizers. Replacing traditional synthetic fertilizers with biogas effluent containing residual organic C has the potential to greatly reduce costs to smallholder farmers while promoting long term soil health and fertility.

3.3 Nitrogen

Digestate nitrogen content in the form of ammonium (NH_4^+) or ammonia (NH_3) is dependent on the original feedstock total N-content. Digestate derived from feedstocks of high degradability (such as starchy plants) result in effluent high in NH_4^+ relative to total N and narrow C:N ratios, while fibrous feedstocks that are low in N predictably lead to effluent low in NH_4^+ (Möller and Müller, 2012). Biochemical changes that occur during AD alter organic compounds containing the nutrients and enhance their plant availability. According to Möller and Müller (2012), several processes are triggered by field application of digestates including the partial immobilization of inorganic N in the presence of remaining organic compounds in effluent which induce soil biological activity. After a single digestate application, they found that soil organic N accumulation occurred, enhancing N mineralization (Möller and Müller, 2012). Lukehurst et al. (2010) express the fertilizer value of nitrogen in AD effluent as the "utilization percentage," which is defined as the relative quantity of mineral fertilizer N needed to obtain the same crop yield as total N supplied in effluent. Although theoretically, the utilization percentage of N in digestate should be equivalent to the amount of ammonium in digestate, ammonia volatilization following field application reduces the utilization percentage and suggests that effluent may better off as a supplement to fertilizer (Lukehurst et al., 2010).

Nitrogen is often the limiting factor for fruit crop growth: Because of its susceptibility to transformation through immobilization, (de)nitrification, leaching, and/or ammonia volatilization, availability of soil N fluctuates. Testing parameters for soil and effluent listed in the *Methodology* section, including plant-available nitrogen, will determine the critical amount of applied nutrients at which the tomato plants will achieve optimal growth. The form of nitrogen assessed using the indole phenol test is ammonia. Since NH_3 is the most reduced form of nitrogen expected to be present in the anaerobic environment of the digester and we are using the inoculum directly from the anaerobic digester without separation or maturation, the best thing to test for is ammonia since it should be the most prevalent form of nitrogen in the anaerobic inoculum. Ammonia toxicity can cause various plant injuries such as growth reduction and necrosis, and tomato plants are determined to be relatively sensitive to high concentrations of ammonia (Van der Eerden, 1982).

While a plant essential macro-element, toxicity from oversupply of nitrogen can occur. Through ammonification, ammonia (NH_3) converts to ammonium (NH_4^+)–one form of N the plants can uptake–in the soil. NH_3 contributes to the N supply for the plant as long as NH_3 is converted to NH_4^+ through the process of biological N-fixation. Contrasted with a nitrogen deficiency, excess NH_3 and/or NH_4^+ harms plants shown in leaves as chlorosis or necrosis. Ammonia toxicity occurs when the bond to H^+ (i.e., ammonification process) does not occur due to suboptimal conditions of high pH and excess NH_3, which inhibits conversion into ammonium (Van der Eerden, 1982). Therefore, only if ammonia is present in the effluent in *balanced* amounts would it could be beneficial to the tomato plants and promote healthy growth.

Möller and Stinner's (2009) study found that the utilization of effluents from biogas digestion of field residues resulted in lower nitrate leaching and lower nitrous oxide emissions, while providing the clear advantage of an additional source of energy in the form of CH_4^-Conducting research on the potential of feedstock-based biogas effluent as a soil amendment to release N at a slower rate for plant uptake as compared to mineral fertilizers has the potential to significantly benefit local farmers and the agricultural sustainability. The benefits are characterized as improving crop productivity and minimizing leaching losses of water-soluble N nutrients that occur especially during heavy rains common in the humid tropics. This could lower the costs of agriculture in Barbados, thereby making reliance on domestically produced food more viable reducing the island's exorbitant food import bill, which was over US$300M in 2011 (FAO, 2013).

Ross et al. tested three irrigation treatments on the growth of kale (which also has high N needs), as well as soil biochemical properties. The treatments applied were biodigester effluent, water-dissolved inorganic fertilizer, and water. The effluent was comprised of crop residues digested in an AD and was formulated to be as similar to the composition of inorganic fertilizer as possible. Although crop DM yields did not differ significantly between treatments, applying digester effluents kept soil biochemical properties at or above the levels found in inorganic fertilizer and water-only treatments. Lab tests determined that under the effluent treatment, significantly higher values of organic N are readily available in the soil for plant uptake and growth than for the non-effluent treatments. Results showed that crops watered with effluent *or* fertilizer were taller, leafier, and darker green than those receiving the water-only treatment. This result is consistent with the observation that plant N content was higher in both effluent and fertilizer treatments when compared to the water treatment (Ross et al., 1989). This suggests that biogas effluent could be interchangeable with fertilizer and has potential as a replacement for salt-based fertilizers. It should be noted, however, that the land found in Barbados is mainly comprised of residual soils that are clayey and rich in calcium carbonate and phosphates, in contrast to Ross et al.'s research which was performed on silty clay loam soil found in New Zealand. Assessing the possibility of using AD effluent as a fertilizer-type amendment for less-fertile soils to improve crop productivity could thus offer salient recommendations that present positive benefits for agriculture in Barbados. However, it is also probable that the performance of plants given AD effluent will not exceed those with conventional fertilizer treatments, similar to other published research by Ross et al. (1989) and Möller and Stinner (2009), that examine the possibility of AD effluent as a substitute for fertilizer in other crops.

3.4 Phosphorus

Phosphorus plays an important role in early plant development; as a component of nucleic acid structure, it regulates protein synthesis and, by extension, cell division and tissue formation (Grant et al., 2001). P deficiencies, on the other hand, can influence all energy-requiring processes in plant metabolism, restricting crop growth early on as well as final crop yield (Grant et al., 2001). Alternatively, over-fertilization of phosphorus is both an economic loss and environmental hazard, the latter being characterized by eutrophication in water bodies. However, if the amount of P provided by methanogenic digestate in the form of phosphate is adequate, it has the potential to replace synthetic fertilizers, thereby reducing externally-sourced inputs and potential for P leaching and runoff. In this study, the amount of phosphorus in the soil samples will be analyzed via standard phosphate tests.

3.5 Tomato growth requirements

Tomatoes are among the common horticultural crops cultivated in Barbados. The type of tomato grown in this study are of the HA3090 variety, which have a determinate growth pattern, meaning the main axis of the shoot ends in a flower bud and will stop growing after reaching a maximum height. Although well-adapted to a wide range of environmental and soil conditions, like other Solanaceae species they are heavy feeders, requiring a relatively high level of phosphorus during initial vegetative growth, nitrogen as they begin fruiting, and potassium during fruit ripening (UNCTAD, 2003; Lopez, 2019). Producing tomatoes in the tropics is limited by unfavorable climate conditions including high temperature, periods of drought and heavy rainfall, and high relative humidity (RH). High RH provides an environment for pests such as thrips and diseases like fusarium wilt or anthracnose to develop (Lopez, 2019). At too high a temperature, wilting occurs and pollen grains may melt, inhibiting the plant's capacity to produce fruit (Nicola et al., 2009). Additionally, with uneven rainfall patterns in Barbados, water management is important to ensure optimal fruit production. Water requirements of tomatoes differ at varying stages of growth, increasing from germination until the start of fruit setting and peaking during fruit development ("Crop Guide", 2018). Water shortage stunts growth but flooding with poor drainage–caused by poor management or heavy rainfall–is also detrimental, as anaerobic and compacted conditions in heavy clay soils lead to root death, delayed flowering, and fruit disorders (Lopez, 2019). Progress in agricultural development is largely measured by increased productivity, which is often largely achieved by using off-farm inputs such as chemical fertilizers, pesticides, and irrigation; however, increased productivity also has steep environmental and economic costs that could be avoided with more sustainable methods.

4. Hypothesis and Objectives

4.1 Hypothesis

We hypothesize that the median concentration of AD biogas effluent is effective as an approximately equal substitute for inorganic fertilizer in terms of its benefits to fruiting of tomato plants and will yield better growth and development than under the negative control. However, too high a concentration of effluent will lead to nutrient toxicity and hinder plant growth. Too low of a concentration will have little to no effect.

4.2 Objectives and Aims

The overarching objective is examining the potential of utilizing liquid AD digestate on agricultural fields in place of mineral commercial fertilizers and determining the optimal concentration of effluent. In most of the reviewed literature, specific concentrations of effluent were not emphasized. In determining a concentration level of effluent mixture that produced the most successful tomato plants, applying various known concentrations of AD effluent to tomato plants is followed by a comparison of the macroelement content after fertilization; several plant growth and health parameters of the plants are then measured every week over approximately two months and subsequently analyzed for nutrient content. These measurements are compared with those for plants fertilized with inorganic fertilizer commonly used by farmers in Barbados.

The second objective is to examine the mineral contents of the AD effluent and their nutritional and toxic effects. Each week, samples of each treatment solution are taken along with samples of soil from each pot before and after treatment application. The components of effluent mixture are compared with nutrients in the soil as well as aforementioned observations on plant growth, in order to examine the effects of specific nutrients (nitrogen, phosphorus, and organic carbon) in both the soil and on the plants.

After analyzing the recorded data from tomato plant measurements over time and soil assays, the potential of AD effluent is assessed for horticultural and agricultural use in Barbados. Recommendations on future studies that strengthen the argument for effluent use as fertilizer are made (with discretion) to farmers in Barbados who are looking to adopt an economically and ecologically sustainable fertilization alternative.

5. Experimental Design and Methodology

In order to determine what nutrient conditions will yield the most viable tomato plants, the growth of tomato seedlings were observed for their performance under different concentrations of the AD effluent and fertilizer. The effluent was obtained from UWI's single-stage batch-loaded anaerobic digester. The fertilizer used was Miracle-Gro® Water Soluble All Purpose Plant Food, which is an inorganic substance for a variety of plants, from shrubs and houseplants to vegetables and fruits. Its NPK nutrient ratio is 24:8:16, meaning that it is high in nitrogen. The tomato seedlings were of the HA3090 variety and were propagated from seed in a peat-based mix in a nursery.

5.1 Experimental Design

To conduct this experiment, 45 tomato seedlings were planted in individual plastic pots that each consist of 4.5kg of sand and 4.5kg of soil, both locally obtained with the exact compositions unknown. The 45 pots are organized using a randomized block design to account for variable sunlight, wind, and/or rainfall exposure received by the plants, which are grown outside under shade on the University of The West Indies Cave Hill campus. Each pot is labeled with a number (1-9) to indicate the associated treatment group and a letter (A-E) to specify the replicate. In total, there are 9 treatments (labeled 1 through 9) with 5 replicates within each treatment group (labeled A through E). A1-9 is considered one block in the randomized block design method.

From 3 June 2019 to 29 July 2019, each pot was watered to excess (i.e., until water begins dripping out of the bottom) every Monday with their respective treatments as outlined in Table 1. The treatment solutions were prepared by mixing the assigned volumes of AD digester effluent or fertilizer with tap water. As shown in the table, AD effluent in concentrations ranging from 0-100% or Miracle-Gro® Water Soluble All Purpose Plant Food, a conventional mineral-based fertilizer, was applied to the pots according to assigned treatments. To ensure consistency in the procedure, samples of all treatment solutions were taken immediately after being mixed to be lab-analyzed.

Table 1: Treatments applied to tomato plants. For example, a pot labeled "A2" indicates Treatment Group 2 (receiving 1% effluent) and replicate A.

Plant (Treatment Group	**Treatment Type**	**Effluent Concentration**	**Contents in 5000ml**

and Replicate)			solution mix
Treatment 1 (A-E)	Control	0%	5000ml water
Treatment 2 (A-E)	Treatment	1%	50ml effluent + 4950ml water
Treatment 3 (A-E)	Treatment	2%	100ml effluent + 4900ml water
Treatment 4 (A-E)	Treatment	5%	250ml effluent + 4750ml water
Treatment 5 (A-E)	Treatment	10%	500ml effluent + 4500ml water
Treatment 6 (A-E)	Treatment	25%	1250ml effluent + 3750ml water
Treatment 7 (A-E)	Treatment	50%	2500ml effluent + 2500ml water
Treatment 8 (A-E)	Treatment	100%	5000ml effluent
Treatment 9 (A-E)	Control	0%	5000ml water + 5 tsp fertilizer

5.2 Measuring qualitative plant growth

A variety of measurements were taken for each potted plant each week of experimentation prior to the application of the designated treatment. For each plant, 9 SPAD readings of both older and new leaves were taken with a Konica Minolta SPAD-502 meter and averaged to determine the chlorophyll content per plant. Using a digital caliper, stem thickness was measured at the thickest part of the stem. Stem height was also measured from the soil up to the shoot apex just before the apical leaf. Petiole count and base leaf count were also recorded for each plant. Lastly, fruit numbers are measured and recorded.

5.3 Soil Testing

In addition to field measurements, laboratory analyses of soil and effluent samples were performed in accordance to protocols presented by Holder (2019). Two soil samples from every pot were collected each week before and after respective treatments were applied. Samples were stored in the freezer until ready for individual testing and analysis for phosphorus and ammonia content, PH, and chemical oxygen demand. The tests on the soil and effluent samples were performed as follows.

5.3a Determining Chemical Oxygen Demand

To test the chemical oxygen demand on the substrate, a small sample of the desired soil, between 50µg-200µg, was placed into a labelled assay tube and weighed using an analytical balance. After weighing the sample and recording the mass reading, 100 µL of distilled water and 100µL of chromic acid digestion solution were added to the assay tube using a pipette. Next, the sample solution was heated in the oven for 90 minutes at 90ºC. Once heated, the samples were cooled and 2.5mL of distilled water added. If there is residue present, we will remove 1mL of the solution, centrifuge, and then read the absorbance of the supernatant. In order to test the chemical oxygen demand in the effluent samples, we will first place 250µL of the sample 750µ of water into an assay tube. The effluent samples will then follow the same procedures outlined above for determining the chemical oxygen demand in the soil samples. Give the reference for this protocol

5.3b Determining Phosphorus Content

In order to determine the phosphorus content in the soil samples, between 50µg and 200µg of soil, 1mL of distilled water, and 100 µL of reagent was placed into an assay tube. Then, we will incubate samples at 37C for 1.5 hours. Once the samples have been incubated, we will measure the absorbance at 820nm. A procedure for testing the effluent samples will be determined at a later date. Reference for this protocol

5.3c Determining Ammonia Content

To test for the ammonia content in our soil and effluent samples we will begin by preparing the following reagents:

1. Phenol-alcohol reagent: Dissolve 10g of phenol in 95% ethyl alcohol to 100ml.

2. Alkaline complexing reagent: Dissolve 100g of trisodium citrate and 5g of sodium hydroxide in distilled water to a volume of 500ml.

3. Sodium hypochlorite: Commercial bleach (i.e., Clorox).

4. Oxidizing solution: add 100ml of the alkaline solution (step 2) to 25ml of the sodium hypochlorite (step 3). Prepare this solution fresh daily before using it to test the samples.

Once the reagents are prepared, approximately 250µg of the desired soil sample is placed into a labelled test vial. In each tube 10µL of phenol reagent and 25µL of the oxidizing solution are added to the soil sample. The tubes are placed into a dark room to react for 3 hours, and the sample solutions in the tubes are mixed periodically while the development is occurring. After 3 hours, the tubes are taken out to be analyzed by colour.

6. Results

Over 6 weeks of the experiment, all of the tomato plants have entered in the flowering and fruiting stage as shown in the photos below, except for those that have died.

Figure 1(a&b). A tomato plant entering the flowering stage pictured on July 8th and a plant entering fruiting stage pictured on July 15th.

As the tomato plant seedlings developed more fully over the 6 weeks, collected data from different treatment groups have demonstrated the influence of each treatment in plant growth. Line graphs were constructed to demonstrate a trend in the data indicating the influence of different treatments on average SPAD readings and plant heights over time.

Plant Death

Throughout the 6 weeks of experiment, plant death were only observed in groups applied with Treatment 5, Treatment 7, and Treatment 8. Treatment 5 and Treatment 7 groups have only one plant dead in each group. Treatment 8, with 100% effluent, was seen with 3 plants dead in Week 4, and 5 plants dead in Week 5 and 6.

Figure 2. Replicate B and C in Treatment group 8 are dead in Week 4. Photos were taken on July 8th (Week 4).

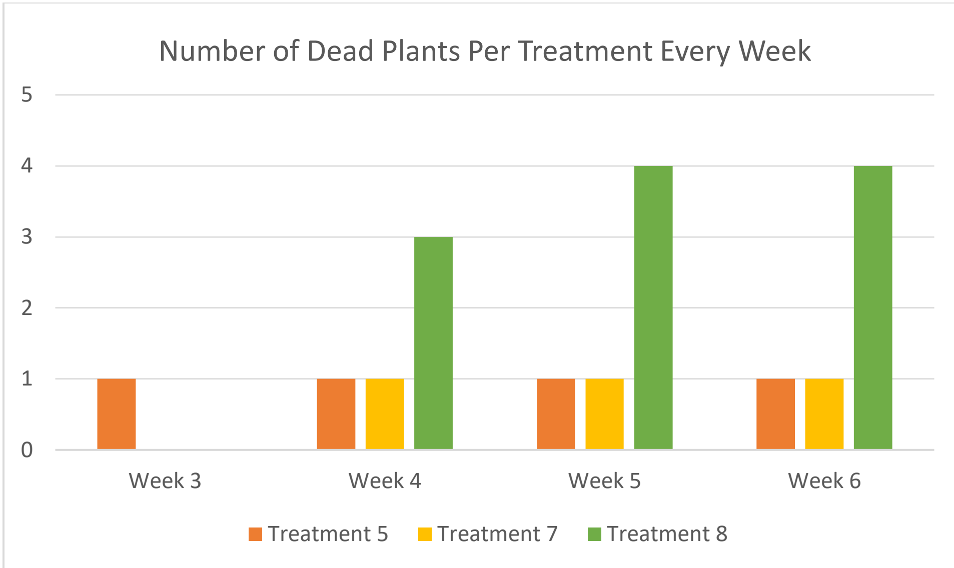


Figure 3. Number of dead plants of each treatment group over time.

SPAD Readings

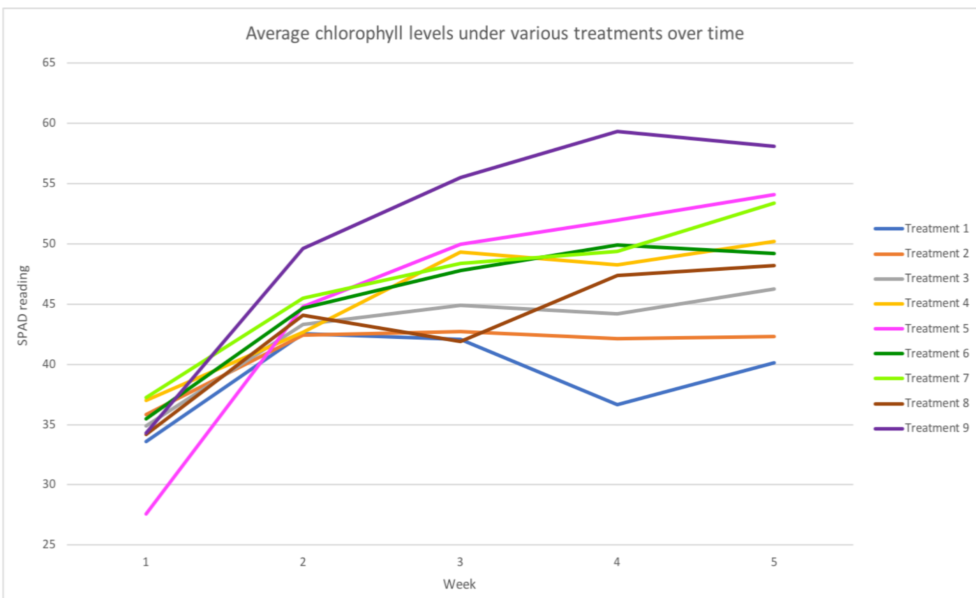
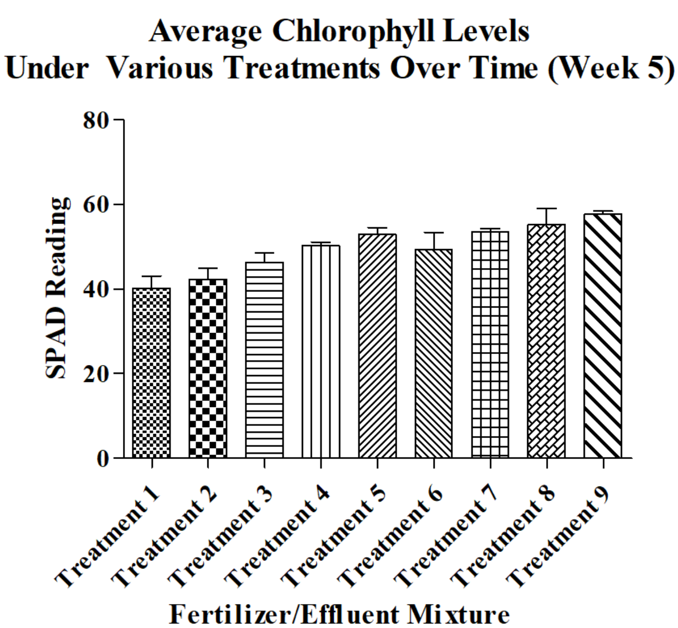


Figure 4. Average SPAD readings, indicating average chlorophyll content of each individual tomato plant over time.

We collected the SPAD reading of each plant every week, and we have averaged the value per treatment groups from its replicates. Depicted in Figure 4, throughout the 5 weeks, all the treatment groups showed a general increasing trend in SPAD reading, while some treatment groups had more significant change in value than others. Over Weeks 1-2, most plants demonstrated similar average chlorophyll content, which was expected due to initial establishment of development. All trendlines became much more separated as weeks go by. Treatment 9 being the inorganic fertilizer, showed the highest value in SPAD reading amongst all the treatments, and there is a relatively big gap between Treatment 9 trendline and Treatment 5 trendline. This indicates that inorganic fertilizer contributed more significantly towards the overall chlorophyll content of plants than the other treatments involving effluent or water. We can observe a general trend of higher SPAD readings with treatment groups of high concentration of effluent and inorganic fertilizer, and lower SPAD readings with treatment of low concentration of effluent and water. Treatments 2, 3, 5, 6, and 7 showed consistent gradual increases in SPAD values, while Treatments 1, 4, and 8 showed some fluctuations in their values. In Weeks 4 and 5, Treatment 1 (negative control) produced the lowest SPAD reading among all treatment groups.



Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Treatment 1 vs Treatment 2	-2.140	0.9503	No	ns	-12.77 to 8.489
Treatment 1 vs Treatment 3	-6.100	2.709	No	ns	-16.73 to 4.529
Treatment 1 vs Treatment 4	-10.06	4.467	No	ns	-20.69 to 0.5693
Treatment 1 vs Treatment 5	-12.82	5.693	Yes	**	-23.45 to -2.191
Treatment 1 vs Treatment 6	-9.235	3.866	No	ns	-20.51 to 2.039
Treatment 1 vs Treatment 7	-13.26	5.551	Yes	*	-24.53 to -1.986
Treatment 1 vs Treatment 8	-15.09	5.804	Yes	**	-27.37 to -2.820
Treatment 1 vs Treatment 9	-17.53	6.740	Yes	**	-29.80 to -5.253
Treatment 2 vs Treatment 3	-3.960	1.758	No	ns	-14.59 to 6.669
Treatment 2 vs Treatment 4	-7.920	3.517	No	ns	-18.55 to 2.709
Treatment 2 vs Treatment 5	-10.68	4.743	Yes	*	-21.31 to -0.05071
Treatment 2 vs Treatment 6	-7.095	2.970	No	ns	-18.37 to 4.179
Treatment 2 vs Treatment 7	-11.12	4.655	No	ns	-22.39 to 0.1541
Treatment 2 vs Treatment 8	-12.95	4.981	Yes	*	-25.23 to -0.6797
Treatment 2 vs Treatment 9	-15.39	5.917	Yes	**	-27.66 to -3.113
Treatment 3 vs Treatment 4	-3.960	1.758	No	ns	-14.59 to 6.669
Treatment 3 vs Treatment 5	-6.720	2.984	No	ns	-17.35 to 3.909
Treatment 3 vs Treatment 6	-3.135	1.312	No	ns	-14.41 to 8.139
Treatment 3 vs Treatment 7	-7.160	2.998	No	ns	-18.43 to 4.114
Treatment 3 vs Treatment 8	-8.993	3.459	No	ns	-21.27 to 3.280
Treatment 3 vs Treatment 9	-11.43	4.394	No	ns	-23.70 to 0.8470
Treatment 4 vs Treatment 5	-2.760	1.226	No	ns	-13.39 to 7.869
Treatment 4 vs Treatment 6	0.8250	0.3454	No	ns	-10.45 to 12.10
Treatment 4 vs Treatment 7	-3.200	1.340	No	ns	-14.47 to 8.074
Treatment 4 vs Treatment 8	-5.033	1.936	No	ns	-17.31 to 7.240
Treatment 4 vs Treatment 9	-7.467	2.871	No	ns	-19.74 to 4.807
Treatment 5 vs Treatment 6	3.585	1.501	No	ns	-7.689 to 14.86
Treatment 5 vs Treatment 7	-0.4400	0.1842	No	ns	-11.71 to 10.83
Treatment 5 vs Treatment 8	-2.273	0.8742	No	ns	-14.55 to 10.00
Treatment 5 vs Treatment 9	-4.707	1.810	No	ns	-16.98 to 7.567
Treatment 6 vs Treatment 7	-4.025	1.599	No	ns	-15.91 to 7.859
Treatment 6 vs Treatment 8	-5.858	2.154	No	ns	-18.69 to 6.978
Treatment 6 vs Treatment 9	-8.292	3.049	No	ns	-21.13 to 4.544
Treatment 7 vs Treatment 8	-1.833	0.6741	No	ns	-14.67 to 11.00
Treatment 7 vs Treatment 9	-4.267	1.569	No	ns	-17.10 to 8.569
Treatment 8 vs Treatment 9	-2.433	0.8370	No	ns	-16.16 to 11.29

Figure 5. Average SPAD reading of every treatment group in Week 5 and analysis of significant difference between treatment groups (“Yes” highlighted)

Average SPAD readings of each treatment group in Week 5 were put through an ANOVA test, to determine whether if the difference between data points are significant. Those significant differences are highlighted in yellow in Figure 5. Significant difference in SPAD data is found between Treatment 1 and Treatment 5, Treatment 7, Treatment 8, and Treatment 9. This hints that higher concentration of effluent as well as inorganic fertilizer significantly elevates SPAD reading of tomato plants from negative control. Significant difference in SPAD data is also found between Treatment 2 (1% effluent) and Treatment 5, Treatment 8, and Treatment 9. This shows that there is much higher SPAD in plants treated with high concentration of effluent and inorganic fertilizer than ones treated with effluent mixture of as low as 1% concentration. There is no significant difference in SPAD readings between any other treatment groups.

Plant Height

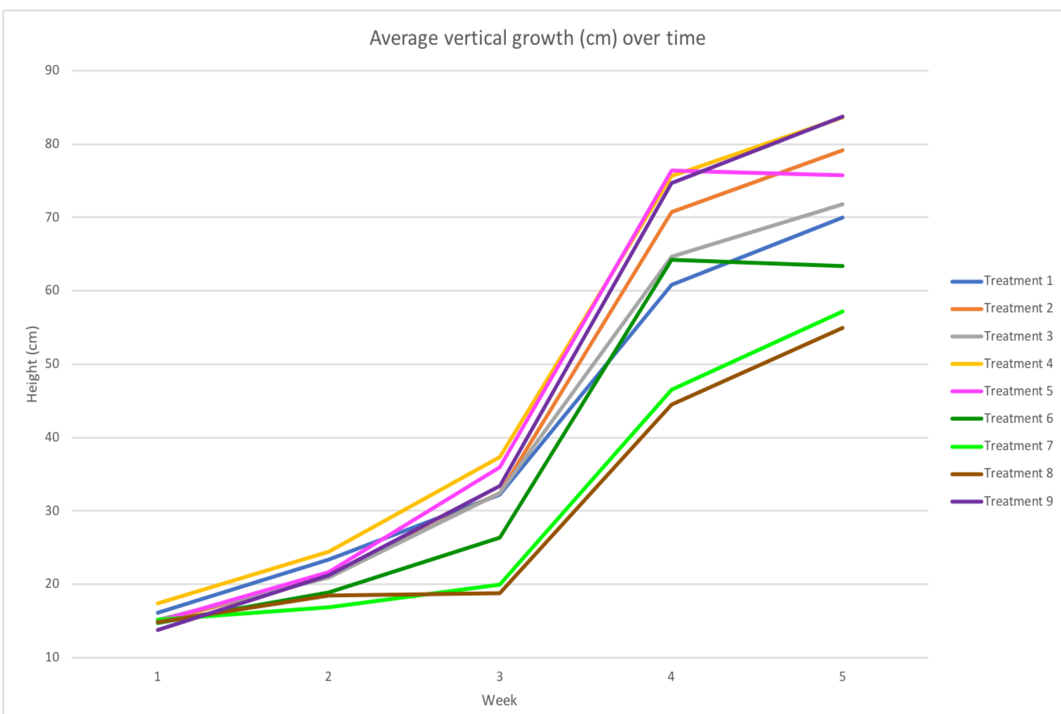
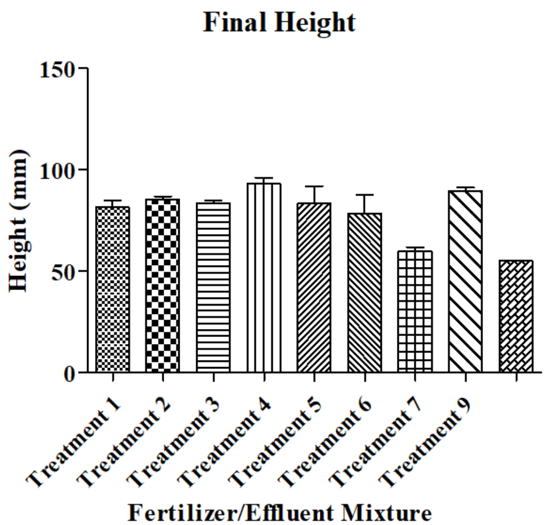


Figure 6. Average vertical growth of different treatment groups of tomato plant over time.

The height of the plant was also chosen to be a measure of vegetative growth of the tomato plants. As shown in Figure 6, over Weeks 1-2, most plants demonstrated similar average height, which is expected due to initial establishment of development. However, Treatment 9 (inorganic fertilizer) and Treatment 2 showed the highest levels of increase in average height overtime. In Week 4, we can observe a significant increase of height in all plants, indicating a growth spurt before the measurements were taken that week.

Throughout the 5 weeks, we observed an overall increase of tomato plant height in all treatment groups. Aligning the Treatment 9 trendlines closely, are the trendlines of Treatment 4 and 5, meaning that the 5% effluent and 10% effluent organic fertilizer treatment mimic effects on tomato plant height closely with inorganic fertilizer. It is worth noting that Treatment 7 and Treatment 8 (50% effluent and 100% effluent) had the lowest height growth among all treatment groups, as we observe a gap between the two trendlines and the rest of the trendlines on the line graph. As Treatment 1 is water and serves as the negative control, it was projected that it would result in the lowest height growth of plants in this treatment group. However, the height of plants in this treatment group has been higher than the height of plants with Treatment 6, Treatment 7, and Treatment 8 between Week 1 and Week 4. This observation suggests the trend of effluent concentrations of 25% and higher having a negative effect on tomato plant growth, since the negative control trendline was higher than those treatment groups.



Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Treatment 1 vs Treatment 2	-4.200	0.9125	No	ns	-25.38 to 16.98
Treatment 1 vs Treatment 3	-2.200	0.4780	No	ns	-23.38 to 18.98
Treatment 1 vs Treatment 4	-11.70	2.542	No	ns	-32.88 to 9.482
Treatment 1 vs Treatment 5	-1.850	0.3790	No	ns	-24.32 to 20.62
Treatment 1 vs Treatment 6	3.000	0.6518	No	ns	-18.18 to 24.18
Treatment 1 vs Treatment 7	21.65	4.435	No	ns	-0.8164 to 44.12
Treatment 1 vs Treatment 9	-8.200	1.782	No	ns	-29.38 to 12.98
Treatment 2 vs Treatment 3	2.000	0.4345	No	ns	-19.18 to 23.18
Treatment 2 vs Treatment 4	-7.500	1.629	No	ns	-28.68 to 13.68
Treatment 2 vs Treatment 5	2.350	0.4814	No	ns	-20.12 to 24.82
Treatment 2 vs Treatment 6	7.200	1.564	No	ns	-13.98 to 28.38
Treatment 2 vs Treatment 7	25.85	5.295	Yes	*	3.384 to 48.32
Treatment 2 vs Treatment 9	-4.000	0.8691	No	ns	-25.18 to 17.18
Treatment 3 vs Treatment 4	-9.500	2.064	No	ns	-30.68 to 11.68
Treatment 3 vs Treatment 5	0.3500	0.07169	No	ns	-22.12 to 22.82
Treatment 3 vs Treatment 6	5.200	1.130	No	ns	-15.98 to 26.38
Treatment 3 vs Treatment 7	23.85	4.885	Yes	*	1.384 to 46.32
Treatment 3 vs Treatment 9	-6.000	1.304	No	ns	-27.18 to 15.18
Treatment 4 vs Treatment 5	9.850	2.018	No	ns	-12.62 to 32.32
Treatment 4 vs Treatment 6	14.70	3.194	No	ns	-6.482 to 35.88
Treatment 4 vs Treatment 7	33.35	6.831	Yes	***	10.88 to 55.82
Treatment 4 vs Treatment 9	3.500	0.7604	No	ns	-17.68 to 24.68
Treatment 5 vs Treatment 6	4.850	0.9935	No	ns	-17.62 to 27.32
Treatment 5 vs Treatment 7	23.50	4.567	No	ns	-0.1817 to 47.18
Treatment 5 vs Treatment 9	-6.350	1.301	No	ns	-28.82 to 16.12
Treatment 6 vs Treatment 7	18.65	3.820	No	ns	-3.816 to 41.12
Treatment 6 vs Treatment 9	-11.20	2.433	No	ns	-32.38 to 9.982
Treatment 7 vs Treatment 9	-29.85	6.114	Yes	**	-52.32 to -7.384

Figure 7. Final Height of every treatment group in Week 6 and analysis of significant difference between treatment groups ("Yes" highlighted)

The final height of each treatment group in Week 6 were put through an ANOVA test, to determine whether if the difference between these height data points are significant. Treatment 8 height data was omitted because the lack of data points available in Week 6 (4 out of the 5 plants died). The presence of significant differences is highlighted in yellow in Figure 7. Significant difference in final height was only found between Treatment 7 and other treatment groups including Treatment 2, Treatment 3, Treatment 4, and Treatment 9. Treatment 2, 3, and 4 are lower concentrations of effluent mixture (1%, 2%, and 5%). Treatment 7 was 50% effluent and the final height of plants with this treatment was significantly lower than plants with effluent treatments of lower concentration. Treatment 9 is inorganic fertilizer and the final height of plants with this treatment was significantly higher than plants with Treatment 7. There is no significant difference in SPAD readings between any other treatment groups. It is particularly worth noting that there is no significant difference in final plant height between Treatment 1 and other treatments.

Fruit Count

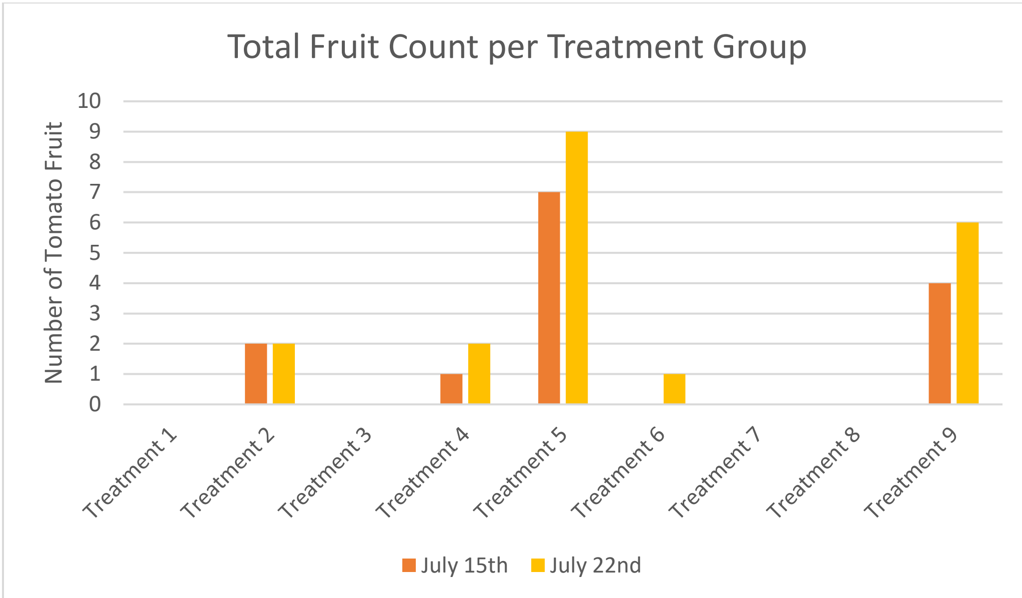


Figure 8. Total fruit count of different treatment groups of tomato plant over Week 5 and Week 6 (July 15th and July 22nd).

The tomato plants started fruiting in Week 5 and the total number of tomato fruits was collected for each treatment group in both Week 5 and Week 6. Tomato plants with Treatment 2, Treatment 4, Treatment 5, Treatment 6, and Treatment 9 have all produced fruits. Treatment 5 (10% effluent) group has clearly more tomato fruits produced than other treatments. Inorganic fertilizer treatment group produced about half of the amount of fruits that Treatment 5 produced. There are no fruits produced in Treatment 1 (negative control), as well as in groups with treatments with high concentration of effluent applied (Treatment 7 and Treatment 8).

Symptoms of Diseases and Toxicity

There has also been evidence of deficiency or toxicity of certain nutrients, as well as symptoms of diseases in tomato plants, observed as yellowing and browning of leaves in several plants in Treatment 6,7, and 8 (shown in Figure 9). These symptoms were not observed in plants irrigated with lower concentration of effluent mixture or with inorganic fertilizer. The particular patterns of circular browning correspond to symptoms of early blight, which is a fungal infection common in tomato plants, and this disease is often associated with crops suffering from a lack of nitrogen (Kemmitt, 2002). As the symptoms of plants are documented in photos, further investigation into the cause will be done by assessing the correlation between the soil nitrogen content of these pots and their observed symptoms. Additionally, the white squiggly lines seen on several tomato leaves are indicators of the presence of leaf miners, a major tomato (and general agricultural) pest. There were no further effects of this pest observed since the plants are just entering the fruiting stage, but heavily infested plants may eventually lose most of their leaves, while defoliation can reduce yield and fruit size and expose fruit to sunburn (Xu et al. 2007). In Figure 11, curling leaves could be the physiological responses of plants to environmental factors such as excessive moisture and nitrogen, heat, drought, severe pruning, root damage, and/or transplant shock (Scott and Williamson, 2019).

Figure 9. Tomato plant with yellow and browning of leaves pictured on July 8th.

Figure 10. Tomato plant with white squiggle pattern on leaves pictured on July 8th.

Figure 11. Tomato plant with curled leaves (a common trait among the plants that surfaced this past week), pictured on July 15th.

Sample Laboratory Analysis

There were three essays performed on all soil samples and data was collected via qualitative measures and observations in colour.

- Chemical Oxygen Demand

  In the chromic acid assay, we determined the relative amount of available organic carbon in each sample based on the appeared colour. Lighter the colour is, less available organic carbon in the sample. Darker the colour is the sample, more available organic carbon in the sample. As seen on the left side of Figure 12, there is only one significantly darker sample in the plate of July 15^{th} after treatment, which corresponds to 8E. In the plate of July 15^{th} before treatment on the right of the figure, the few samples that are significantly darker correspond to 1E, 2C,9E.

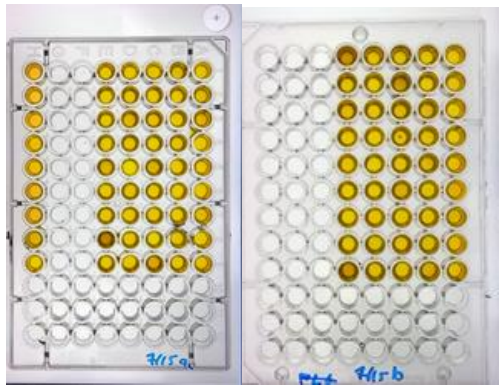


Figure 12. Chromic acid assay results of soil and treatment samples from July 15th before and after treatment application in microtiter plates.

- Phosphate Content

  In the phosphate content assay, we wanted to determine the relative amount of phosphate content in each sample based on the appeared blue colour. We assigned the three gradients of blue colour with values 1, 2, 3. All samples appeared to be dark blue, with assigned value of 3.

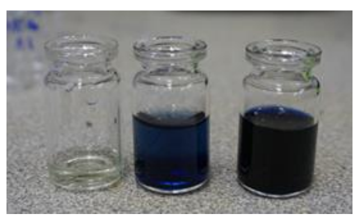


Figure 13. The three gradients of blue colour in phosphate content assay.

- Ammonia Content

  In the ammonia content assay, we wanted to determine the relative amount of ammonia content in each sample based on the appeared yellow and green colour. The green or harder yellow would indicate the presence of high ammonia. All samples appeared to be yellow as shown in the figure, with two showing green colour.

Figure 13. The three gradients of blue colour in phosphate content assay.

7. Economic Analysis

A cost-benefit analysis is a practical use of welfare economics to evaluate the economic viability and efficiency of potential projects. The net present value (NPV) criterion will be used to conduct the economic analysis for this project as it is considered a reliable evaluation guide (reference). A key feature to the NPV method is that indicates how much value a project will create for its stakeholders. The NPV is the sum of the present values (PVs) of all the outflows (costs) and inflows (benefits). A positive NPV indicates that that the inflows are greater than the PV of the outflows, thus the benefits of the project are worth more today than the costs. A negative NPV indicates that the PV of the inflows is less than the PV of the outflows, thus the benefits of the project are worth less today than the costs. Calculating the NPV of a project is a useful tool to help determine whether a project will result in a net profit or a loss. In order to keep values consistent, all data is calculated using real values (nominal – inflation) of BBD. It is clear that the search for economically viable technologies that function to alleviate and reduce the environmental impact of agriculture has undoubtedly become a concern and a priority. The aim of this economic analysis is to demonstrate the benefits generated by installing an anaerobic biodigester as an economically viable alternative in production and cultivation of tomato plants. According to the NPV calculations using the 2018 Barbados real interest rate at r=7%, the sum of the NPV for the AD effluent fertilizer ($6181799.54) is greater than the sum of the NPV for the inorganic fertilizer ($5815952.64), and is therefore a more viable economic option for the cultivation of tomato production in Barbados. Aside from being an alternative source of plant fertilizer, our findings suggest that using AD effluent as a substitute to inorganic fertilizer can maintain soil health without jeopardizing the health of our environment.

Economic Data Set

Data Set	**Value**	**Year**	**Source**
Barbados Tomato Production Quantity	357 tons (714000 lbs)	2017	FAOSTAT
Area harvested	18 hectares	2017	FAOSTAT
Market Value of Tomato (Benefit)	$1.75 BBD/lb	2019	Cheapside Market
Capital Cost of AD Biodigester (Cost)	$500 BBD	2019	Nikolai Holder

Maintenance Cost of AD Biodigester (Cost)	$0	2019	Nikolai Holder
Cost of Technical Skill and Know-how (Cost)	$0	2019	Nikolai Holder
Cost of Miracle – Gro Fertilizer 1.1lb (Cost)	$26 BBD	2019	Nikolai Holder

Calculations

Inorganic Fertilizer Costs	**AD Effluent Fertilizer Costs**	**Benefits**
500g (1.1lb) feeds 41m2 of garden area (Miracle- Gro ® Website)	In order to supply enough effluent for tomato production on the island, 350 biodigesters are needed given that 40 gallons of effluent are produced every cycle (3 months).	Market value of tomatoes $1.75BBD/lb * 714000lbs = $1249500 BBD
2.2 tons of Miracle-Gro * 3 (applications per growth period) = 6.6 tons	Each tomato plant needs 50ml of effluent. One hectare yields 11000 tomato plants, for each plant you need 50ml of effluent (5% treatment).	
6.6 tons(3000lbs) * 4 growing cycles per year =12000 lbs	50ml * 110000 plants*18 hectars=9900000mls. (2178 gallons). To irrigate the 18 hectars of tomato plants every week, approximately 2000 gallons of effluent are required 2178 gallons * 7 weeks of irrigation every cycle = 15246 gallons. Each biodigester yields 40 gallons per cycle.	
12000 lbs/Miracle-Gro 1.1lb bag = 10909 Miracle-Gro bags	15246gallons/40gallons per cycle with the AD biodigester = 381.15 biodigesters	

10909 Miracle-Gro * 10BBD = $109,090 BBD per year to fertilize Barbados tomato production	Approximately 381 biodigesters would be required to produce effluent on the island for every cycle. 381 biodigesters * $500 BBD per biodigester = $190500BBD	***all assumptions are

Net Present Value Calculations

Net Present Value: Inorganic Fertilizer with real interest rate r= 7%						
Year	**0**	**1**	**2**	**3**	**4**	**5**
Benefits	$1249500	$1249500	$1249500	$1249500	$1249500	$1249500
(-)Cost	$109090	$109090	$109090	$109090	$109090	$109090
Net (B-C)	$1140410	$1140410	$1140410	$1140410	$1140410	$1140410
Discount Factor	(1+0.07)^0 =1	(1+0.07)^1 = 1.07	(1+0.07)^2= 1.1449	(1+0.07)^3=1.225	(1+0.07)^4=1.311	(1+0.07)^5=1.403
Discount Cash Flow	$1140410	$1065803.7	$996,078.26	$930,946.94	$869,877.96	$812,836.78
Sum NPV	$5815953.64					
Net Present Value: AD Effluent Fertilizer with real interest rate r=7%						
Year	**0**	**1**	**2**	**3**	**4**	**5**
Benefits	$1249500	$1249500	$1249500	$1249500	$1249500	$1249500
(-)Cost	$190500	$0	$0	$0	$0	$0
Net (B-C)	$1,059,000	$1249500	$1249500	$1249500	$1249500	$1249500
Discount Factor	(1+0.07)^0 =1	(1+0.07)^1 = 1.07	(1+0.07)^2=1.1449	(1+0.07)^3=1.225	(1+0.07)^4=1.311	(1+0.07)^5=1.403

Discount Cash Flow	$1059000	$1167757.01	$1091361.69	$1020000	$953089.25	$890591.59
Sum NPV	$6181799.54					

8. Discussion

We hypothesize that the median concentration of AD biogas effluent is effective as an approximately equal substitute for inorganic fertilizer in terms of its benefits to fruiting of tomato plants and will yield better growth and development than under the negative control. Examining the data we have collected, different parts of our hypothesis can be re-evaluated.

Firstly, we have hypothesized that the optimal concentration of effluent treatment applied to the tomato plants will yield better growth and development than under the negative control. Regarding general aspects of the tomato plants in negative control group (Treatment 1), they grew in a good condition with no symptoms of disease or deficiencies. Looking at the SPAD data, the Treatment 1 trendline in the SPAD over time line-chart was the lowest line amongst treatment groups. As well, significant difference in SPAD data is found between Treatment 1 and treatment groups applied with high concentration of effluent mixture (Treatment 5, Treatment 7, and Treatment 8). Therefore, the SPAD data hints that higher concentration of effluent significantly elevates SPAD reading of tomato plants from negative control. From the height data, we can see several trendlines of effluent treatment groups above the Treatment 1 trendline, suggesting that effluent application improves the vegetative growth of tomato plants compared to plants with no treatments applied. However, there is no significant difference in final plant height between Treatment 1 and other treatments. One plausible explanation to the lack of this significant difference is that the negative control group still accessed nutrients from the soil to support its plant growth. The soil in the pots was consisted of half sand and half soil from someone's backyard, which means that there were some nutrients to begin with in the pot. For future studies, we would recommend either taking initial soil tests on what nutrients existed originally, or using half sand and half clay to make the soil base to ensure the negative control did not have any nutrients to begin with. Another possibility for this result is that the height of tomato plants may not be the best parameter to examine tomato plant growth because tomato plants height varies inherently. From the fruit count data, we draw the comparison of no fruit produced from Treatment 1 and fruits produced in other treatments (highest in Treatment 5), and this suggests the positive effect of effluent treatments in fruiting compared to no treatment.

Another part of our hypothesis is that a median concentration of AD biogas effluent will lead to its effectiveness as a fertilizer. A low concentration of effluent mixture, seen in Treatment 2 and Treatment 3, have shown not to be effective as a fertilizer. For example, in the final SPAD data, there is no significant difference between Treatment 1 and Treatment 2, while there is between Treatment 1 and Treatment 5. It is also interesting that there is significant difference between Treatment 2 and high concentration of effluent treatment groups (Treatment 5 and Treatment 8). This serves as a strong indication that the concentration of effluent mixture application is critical to the chlorophyll content of leaves which supports the growth of tomato plants. In both the height over time graph and SPAD over time graph, trendlines of Treatment 2 and Treatment 3 are lower than trendlines of Treatment 4 and Treatment 5. We can also observe from the fruiting data that plants in Treatment 2 and Treatment 3 did not produce as many fruits as plants in Treatment 4 and Treatment 5. Thus, it can be concluded that a very low concentration (1-2%) of effluent treatment is not effective as a fertilizer for tomato plants. On the other hand, nutrient toxicity was observed in tomato plants with highly concentrated effluent treatments (Treatment 6,7, and 8). An obvious evidence of this is most of the plants with Treatment 8 (100% effluent) dying during the experiment. As well, photos of plants in these treatments showed symptoms such as leaf miner trails and leaf yellowing. One explanation of this is that the high concentration of effluent mixture may lead to water stress on the plants with not enough water in the treatment. There have been studies that demonstrated water stress may restrict the ability of plants to reduce and assimilate nitrogen through the inhibition of enzymes implicated in nitrogen metabolism, shown in symptoms such as yellowing of leaves (Sanchez-Rodríguez et al., 2013). We conducted the ammonia content assays to support this reasoning, however the results of the assay were not indicative of any claims. Future studies should be conducted to adjust the method of examining ammonia content of soil samples, and a correlation test between ammonia content and low SPAD readings (chlorophyll content) would be helpful to make the connection. Looking at the plant height chart, the trendlines in treatment groups of Treatment 6, Treatment 7, and Treatment 8 were lower than the Treatment 1 (negative control) plants, which is an indication of the negative effect of high concentration effluent application on plant growth. Treatment 4 and Treatment 5 (5% and 10% effluent concentration) showed the greatest plant growth overall with SPAD, height, and fruits produced, with the 5% treatment having the highest tomato yield of all treatments. From these results, we concluded that the optimal effluent concentration ranges between 5-10%.

Lastly, we wanted to compare the effect of applying a median concentration of effluent application to tomato plants with the commercial inorganic fertilizer. No significant difference was found between plant height or SPAD of plants applied with optimal concentration of effluent and inorganic fertilizer. Though, trendlines of Treatment 9 (inorganic fertilizer) were superior of those with Treatment 4 or 5 in the SPAD and plant height growth graphs. With fruiting, Treatment 5 (10% effluent) resulted in more fruit production than inorganic fertilizer. It can only be concluded that the possibility of the effluent application as a complete substitute for inorganic fertilizer needs to be further examined. There could be studies focused on comparing only the optimal concentration of effluent application and inorganic fertilizer, examined with a larger diversity of parameters. Our literature review shows the consensus that inorganic fertilizer provides nitrogen to the soil that is taken up by the plant to support its growth. The ammonia content assay conducted did not provide results that showed a difference between inorganic fertilizer and effluent sample. It is recommended in the future to conduct other assays to substitute for the ammonia test. However, researchers should keep in mind that due to the wide range of feedstock used in biogas production, assessing the efficiency and predictability of the fertilizer N value of the effluent is difficult (Sogn et al., 2018).

Conclusion

As soil fertility declines and natural resources become more and more limited around the world, strategies must be developed and implemented to produce enough food without negatively impacting the environment or farmers' livelihoods. One approach is viewing the farm as a system where organic matter and thus nutrients from cover crop production for instance can be recycled to be used in other ways. Anaerobic digestion of crop residues and locally available organic materials can positively contribute to the current energy and greenhouse gas emission issue through generation of renewable energy. Concurrently, as indicated in the results, there is a general increasing trend of plant growth, chlorophyll content, and fruiting corresponding to application of low concentrations of effluent of around 5-10%. Despite the lack of a statistically significant difference against the control, we found that overall the previously mentioned treatments performed better and are therefore promising fertilizer alternatives or supplements for agricultural production in Barbados.

Although expectedly inorganic fertilizer resulted in the best-performing plants, a low concentration of AD effluent in tomato production in Barbados is a viable sustainable alternative to use as a substitute for inorganic fertilizer because of its low environmental impact, economic advantage, and competitive performance.

This suggests that partly substituting traditional fertilizers with biogas digestate may have success in addressing challenges of poor soil conditions and costly off-farm inputs faced by farmers.

Future considerations

This study was conducted as a pilot project that examines the possibility of applying biodigester effluent to Barbadian soil to better grow tomato plants. As the scope of this study is limited, further studies are needed to improve current knowledge on the appropriate dose of effluent and subsequent responses of tomatoes or other crops, from which recommendations can be made to farmers in Barbados. For future studies similar to this one, changes could be made in parameters measured, base soil content, and methods of sample essays for nutrient content as mentioned above. Future studies should explore the effect of treatments supplementing mineral fertilizer with different proportions of effluent, such as substituting 50% of fertilizer with effluent. Moreover, because exact compositions of the effluent were unknown when carrying out this study, performing a multitude of biochemical tests on the effluent itself to determine nutrient contents would be greatly beneficial to making specific objectives of future studies of the same topic. Similarly, determining soil pH as well as whether soil type has an impact on tomato plants' access to nutrients would provide better insight into post effluent-application soil processes. If this study were carried out until all plants had fruited, comparison of fruit biomass and taste test under the various treatments would be conducted because fruit quality is the main priority of growing tomatoes.

The cost-benefit analysis of this practice was done with a few uncertainties, especially relating the process of anaerobic digestion. Thus, by determining appropriate digestate application rate based on nutrient composition and plant needs, liquid biogas digestate may be integrated into a fertilization plan in order to reduce the use of mineral fertilizers; this will thereby maximize farmers' profits and minimize environmental damage caused by excess inorganic fertilizer leaching into the ecosystem.

9. References

Atalay, A., & Blanchar, R. W. (1984). Evaluation of methane generator sludge as a soil amendment [Sludge obtained from Festuca arundinacea, maize, sorghum, soybeans, wheat, carbon dioxide evolution, Missouri]. *Journal of Environmental Quality (USA)*.

Atalay, A., & Blanchar, R. W. (1988). Urease activity in soil amended with methane-generator sludge. *Biological wastes, 26*(1), 49-58.

Barbados Official Meteorological Services. (2019). Barbados weather climate data. Retrieved from http://www.barbadosweather.org/barbados-weather-climate-data.php

Caribbean Agricultural Research and Development Institute [CARDI]. (2019). Barbados country profile. Retrieved from https://www.cardi.org/country-offices/barbados/

Chiew, Y. L., Spångberg, J., Baky, A., Hansson, P. A., & Jönsson, H. (2015). Environmental impact of recycling digested food waste as a fertilizer in agriculture—A case study. *Resources, Conservation and Recycling*, *95*, 1-14.

Comparetti, A., Febo, P., Greco, C., & Orlando, S. (2013). Current state and future of biogas and digestate production. *Bulgarian Journal of Agricultural Science, 19*(1), 1-14.

Crop Guide: Growing Tomato. (2018). Retrieved from https://www.haifa-group.com/articles/crop-guide-growing-tomato

[FAO] Food and Agriculture Organization of the United Nations. (2013). *CARICOM food import bill, food security and nutrition.* (Issue brief No. 5). Barbados: Subregional Office for the Caribbean. Retrieved from http://www.fao.org/3/a-ax740e.pdf

[FAO] Food and Agriculture Organization of the United Nations. (2016). Food Balance Sheets. Retrieved 17 July 2019, from http://www.fao.org/faostat/en/#data/FBS

Funderburg, E. (2001). What Does Organic Matter Do In Soil? Retrieved from https://www.noble.org/news/publications/ag-news-and-views/2001/august/what-does-organic-matter-do-in-soil/

Grant, C. A., Flaten, D. N., Tomasiewicz, D. J., & Sheppard, S. C. (2001). The importance of early season phosphorus nutrition. *Canadian Journal of Plant Science*, *81*(2), 211-224.

Holder, N. (2019). Methodology [protocol]. Unpublished internal document, University of the West Indies, Cave Hill Campus.

Kemmitt, G. (2002). Early blight of potato and tomato. *The Plant Health Instructor.* DOI: 10.1094. PHI-I-2002-0809-01.

Koszel, M., & Lorencowicz, E. (2015). Agricultural use of biogas digestate as a replacement fertilizers. *Agriculture and Agricultural Science Procedia*, *7*, 119-124.

Kouřimská, L., Poustková, I., & Babička, L. (2012). The use of digestate as a replacement of mineral fertilizers for vegetables growing. *Scientia Agriculturae Bohemica*, *43*(4), 121-126.

Lopez, F. (2019). *AEBI421: Tomato production* [PowerPoint slides]. University of the West Indies, Cave Hill Campus.

Lukehurst, C. T., Frost, P., & Al Seadi, T. (2010). Utilisation of digestate from biogas plants as biofertiliser. *IEA bioenergy*, *2010*, 1-36.

Madramootoo, C. A. (2000). An integrated approach to land and water resources management in the Caribbean. *Food and Agricultural Organization*, *1*.

Miracle-Gro Water Soluble All Purpose Plant Food - Plant Food - Miracle-Gro. (2019). *Miraclegro.com.* https://www.miraclegro.com/en-us/products/plant-food-care/miracle-gro-water-soluble-all-purpose-plant-food

Möller, K., & Stinner, W. (2009). Effects of different manuring systems with and without biogas digestion on soil mineral nitrogen content and on gaseous nitrogen losses (ammonia, nitrous oxides). *European Journal of Agronomy, 30*(1), 1-16.

Nicola, S., Tibaldi, G., & Fontana, E. (2009). Tomato production systems and their application to the tropics (International Symposium on Tomato in the Tropics). *ISHS Acta Horticulturae* 821, 27-33.

Sahlström, L. (2003). A review of survival of pathogenic bacteria in organic waste used in biogas plants. *Bioresource Technology*, *87*(2), 161-166.

Santibáñez, C., Varnero, M. T., & Bustamante, M. (2011). Residual glycerol from biodiesel manufacturing, waste or potential source of bioenergy: a review. *Chilean journal of agricultural research*, *71*, 469-475.

Scott, J., & Williamson, J. (2019). Tomato Leaves Rolling?. *Home & Garden Information Center | Clemson University, South Carolina.* Retrieved from https://hgic.clemson.edu/factsheet/tomato-leaves-rolling/

Sogn, T. A., Dragicevic, I., Linjordet, R., Krogstad, T., Eijsink, V. G., & Eich-Greatorex, S. (2018). Recycling of biogas digestates in plant production: NPK fertilizer value and risk of leaching. *International Journal of Recycling of Organic Waste in Agriculture*, *7*(1), 49-58.

Ross, D. J., Tate, K. R., Speir, T. W., Stewart, D. J., & Hewitt, A. E. (1989). Influence of biogas-digester effluent on crop growth and soil biochemical properties under rotational cropping. *New Zealand Journal of Crop and Horticultural Science*, *17*(1), 77-87.

United Nations Conference on Trade & Development [UNCTAD]. (2003). In United Nations (Ed.), *Organic fruit and vegetables from the tropics*. Geneva: United Nations. Retrieved from https://unctad.org/en/docs/ditccom20032_en.pdf

Van der Eerden, L. J. M. (1982). Toxicity of ammonia to plants. *Agriculture and environment*, *7*(3-4), 223-235.

Vernon, K. C., & Carroll, D. M. (1965). *Barbados.* (Soil and Land-Use Surveys No. 18). Trinidad: The Regional Research Centre of the British Caribbean at The Imperial College of Tropical Agriculture, University of The West Indies. Retrieved from http://library.wur.nl/isric/fulltext/isricu_i2826_001.pdf

Xu, H. R., Ying, Y. B., Fu, X. P., & Zhu, S. P. (2007). Near-infrared spectroscopy in detecting leaf miner damage on tomato leaf. *Biosystems Engineering*, 96(4), 447-454.