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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

INFLUENCE OF VERMICOMPOST TEA ON SECONDARY METABOLITES IN *SOLANUM LYCOPERSICUM* WITHIN SOUTH FLORIDA

A thesis submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

in

ENVIRONMENTAL STUDIES

by

Daphne Kyoko Sugino Souffront

2019

To: Dean Michael R. Heithaus
College of Arts, Sciences and Education

This thesis, written by Daphne Kyoko Sugino Souffront, and entitled Influence of Vermicompost Tea on Secondary Metabolites In *Solanum lycopersicum* within South Florida, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this thesis and recommend that it be approved.

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Diego Salazar-Amoretti

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Krishnaswamy Jayachandran, Major Professor

Date of Defense: November 8, 2019

The thesis of Daphne Kyoko Sugino Souffront is approved.

_____
Dean Michael R. Heithaus
College of Arts, Sciences and Education

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Andrés G. Gil
Vice President for Research and Economic Development
and Dean of the University Graduate School

Florida International University, 2019

ACKNOWLEDGMENTS

I would like to express my highest esteem to all the members of my committee for guiding and encouraging me during my time at Florida International University: Dr. Krishnaswamy (Jay) Jayachandran (committee chair), Dr. Diego Salazar-Amoretti, and Dr. Amir Khoddamzadeh. Through my project, my committee members were always willing to help, listen, and advise; and I am forever grateful for your contributions in my project. I would also like to extend my gratitude to all the members of the Agroecology program, all your love, patience, guidance and support has made Miami and our office feel like home. I would like to specifically thank Eric Betancourt for always advising, listening, and encouraging me; and to Thomas "Tom" Beastly for always helping me and providing crucial materials for the execution of my project. I want to thank Claudia Garcia and Adrian Garcia for continuously assisting and supporting throughout the entirety of my field season. Thanks to all the undergraduates who assisted in site preparation and planting. I would also like to thank Mamiris and Papa and my mom and dad, life was a bit easier with some love, food, and encouragement. I would also like to offer my sincerest gratitude and appreciation to my partner, Jordan W. Prats. Your love, support, and continuous help was invaluable to the development and execution of my project. This research was possible thanks to the funding of funding of the United States Department of Agriculture NIFA Hispanic-Serving Institutions Higher Education Grants Program (2015-38422-24075).

ABSTRACT OF THE THESIS

INFLUENCE OF VERMICOMPOST TEA ON SECONDARY METABOLITES IN *SOLANUM LYCOPERSICUM* WITHIN SOUTH FLORIDA

by

Daphne Kyoko Sugino Souffront

Florida International University, 2019

Miami, Florida

Professor Krishnaswamy Jayachandran, Major Professor

Fresh Market Tomatoes provide a high revenue stream for Florida's agricultural sector. To attain profitable yields, farmers introduce high inputs of pesticides to suppress pest invasion/damage. Heavy usage of pesticides has adverse effects on human and environmental health. A possible solution might be the incorporation of vermicompost in pest management. Typically used as a fertilizer, vermicompost has pest suppressant properties. Mechanisms influencing enhanced pest resistance are unknown. To identify such mechanisms, a study was conducted to evaluate physical and chemical changes of the BHN589 tomato plant following the addition of varying vermicompost tea treatments (T5%, T10%, and T20%) . Results indicated that vermicompost tea positively affected various physical parameters such as biomass, chlorophyll content, yield, and soil pH. Moreover, the addition of vermicompost tea also influenced secondary metabolite production. Changes were mainly concentrated in compounds emerging from the mevalonic acid pathway, which regulates terpenoid production. Other metabolite groups were also affected.

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1. INTRODUCTION

The agricultural sector generates a high revenue stream for the state of Florida. The state's subtropical climate allows for year-round agricultural production. An economically important crop for Florida is the fresh market tomato. In 2017, Florida was ranked first nationally in the production value of fresh market tomatoes (Florida Department of Agriculture, 2017). According to the Florida Department of Agriculture, tomatoes generate approximately $262 million annually for the state (Florida Department of Agriculture, 2017). Although profitable, tomato cultivars require high inputs of labor, nutrients, and fertilizer to support the plant's life cycle (Letourneau & Goldstein, 2001).

To ensure crop success, farmers apply a wide array of chemical pesticides into their fields. Pesticides such as bifenthrin, chloropicrin, and paraquat are commonly used to control noxious weeds, harmful fungal associations, and herbivorous insect invasions that might decrease yield and quality of the fruit (NASS, 2007). A survey conducted by the USDA's National Agricultural Statistics Service (NASS) found that nearly all (94%) Floridian tomato fields contain some sort of pesticide (NASS, 2007).

Pesticide usage is associated with strong negative effects on human and environmental health. Indirect costs to pesticide application include soil and water pollution, the disruption of ecosystem services as a result of loss of pollinators and natural predators, and pest resistance. Due to harmful externalities and decreased effectiveness, farmers have reduced chemical pesticide usage through the implementation of integrated pest management practices (Leppla, 2007). Integrated pest management (IPM) is a combination of proactive measures that prevent pest infestations from reaching

damage thresholds (Leppla, 2007). Despite success, tomato farming still regularly relies on chemical pesticide usage.

To ameliorate issues surrounding agrochemical usage, scientists as well as farmers are seeking sustainable alternatives. A possible solution might be the incorporation of vermicompost and vermicompost tea/extractions within pest management practices. Vermicompost is a peat-like manure produced from the interactions between mesophilic bacteria and epigeic earthworms (Edwards et al., 2010; Joshi et al., 2015). Although vermicompost and vermicompost tea/extractions are typically applied as organic fertilizers, they exhibit pest repelling properties (Arancon et al., 2007; Chatterjee et al., 2013). Components such as organic acids, vitamins, free enzymes, soluble phenolic compounds and microorganisms in the vermicompost can alter the plant's physiology and chemical composition rendering it unpalatable or unattractive to herbivorous insects.

Several studies detail vermicompost's ability to control or limit pest invasion. A study by Arancon et al. (2005), tested the effects of commercially sold vermicompost on infestation and damage caused by the two-spotted spider mite (*Tetranychus urticae*), mealy bugs (*Pseudococcus* sp.), and aphids (*Myzus persicae*). Results indicate an overall reduction of leaf damage and population levels across all vermicompost treatments (Arancon et al., 2005).

Vermicompost by-products such as vermicompost extraction or tea are also being studied as a pest management practice. Vermicompost extract or tea is a liquid fertilizer made from vermicompost. Extractions/ teas can be produced through vigorous aeration or passive steeping for approximately 24 hours. The mixture contains all of vermicompost's

beneficial attributes and compounds and it is shown to deter pests in a similar fashion. Farmers and scientists have opted for its usage to ease application and target specific plant parts.

Edwards et al. (2010), different concentrations of vermicompost tea were added to cucumber and tomato plants to suppress invasion and attack from cucumber beetle (*Acalymna vittanum*) and tobacco hornworm (*Manduca sexta*). Edwards et al. (2010) concluded that all added concentrations of vermicompost tea significantly suppressed the establishment and damage inflicted by both pests (Edwards et al., 2010). Researchers also noted that the higher the vermicompost tea preparation used, the greater the pest suppression, which is likely caused by the accumulation or increase of secondary metabolites in plant tissues (Edwards et al., 2010).

As studies have shown, vermicompost and vermicompost extract/tea can decrease pest invasion and limit damage (Edwards et al., 2010; Arancon et al., 2005). Scientists speculate that increased resistance to pest invasion might be attributed to chemical changes occurring in the plant. These changes are likely due to the accumulation or elicitation of secondary metabolites such as phenolic acids, tannins, and flavonoids which are linked to chemical defense mechanisms in plants. Despite recent studies, mechanisms in which vermicompost and vermicompost extract/tea enhance suppression are poorly understood.

Therefore, the present research project was developed to understand the mechanisms behind the effect of vermicompost in economically important crops. Specifically, I will assess chemical defense compound changes in a popular and widely consumed crop such as tomatoes through the addition of compost tea. I will also evaluate

alterations to the physical structure and resilience of the crop. Tomatoes, like most plants, are known to have hefty chemical defenses to deter insect infestation or pathogen caused diseases. Chemical defenses can be consecutive or induced, and can arise from phenolic acids, terpenoids, and other organic acids (Edwards et al., 2010). Understanding induced change in phytochemical defenses from important food crops like tomatoes could lead to a reduction in pesticide application, while promoting agroecosystem health.

1.2 Objectives

The main goal of the present project is to assess the effects of vermicompost tea in tomato plants, taking special attention on changes in the phytochemical defense profile and other related physiological characteristics. Specific objectives include:

1. To assess the effects of vermicompost tea on secondary metabolites of BHN589 tomato plants and evaluate if change influences phytochemical defenses through plant secondary metabolite profiling.
2. To evaluate the effects of vermicompost tea on the tomato plant's physical structure.
3. To determine if enhanced pest resistance through the addition of vermicompost tea in the plant tomato is caused by chemical or physical changes within the plant, or a combination of both.

1.3 Hypothesis

1. The overall application of vermicompost tea on tomato seedlings across a 104-day planting period will have a significant effect on secondary metabolite production and phytochemicals linked to plant defense.

2. The overall application of vermicompost tea on tomato seedlings across a 104-day growth period will result in the significant difference of above and below ground biomass and yield/marketable yield as compared to the control treatment.

2. LITERATURE REVIEW

2.1 Tomato Production in Florida

Tomato (*Solanum lycopersicum*) is a vegetable-bearing crop belonging to the Solanaceae or tobacco family (Bai & Lindhout, 2007). Originating from the Andean region in South America, the tomato has become one of the world's most popular and consumed vegetable crop (Bai & Lindhout, 2007; Guan et al., 2017). In 2014, worldwide production of fresh and processed tomatoes surpassed 170 million tons (FAO, 2017). The United States ranks second worldwide in fresh market tomato production, generating around 1.35 million tons of tomatoes annually (Guan et al., 2017). Florida leads national production, with a total production value of $262 million (Florida Department of Agriculture, 2018).

Despite high market value, tomato cultivation requires substantial amount of labor, nutrients, and pesticides inputs. Field-grown tomatoes are cultivated from October to June and require a soil pH of 6.0- 7.0 (Guan et al., 2017). During the crop's life cycle, tomatoes are carefully monitored and harvested multiple times during the growing season. Tomato production is considered labor intensive, because of frequent hand-harvesting, nutrient/pest monitoring, and pruning (Guan et al., 2017).

Accounting for a major part of production expenses, fertilizer use is also an important part of tomato cultivation (Hochmuth & Hanlon, 2000). The right balance of nutrients is

crucial for crop success and high-quality fruit. The recommended target nutrient rates are 200-150-225 lb/acre N, P_2O_5, K_2O depending on the type of soil being farmed (Hochmuth & Hanlon, 2000). Given the high variation in soil types and pH, farmers must adjust their fertilizing practices to avoid volatilization or leeching (Wang et al., 2015).

Finally, tomato crops are highly susceptible to pest damage and invasion (Schuster & Smith, 2003; Webb et al., 2001). For this reason, pesticide inputs during production and harvest are necessary to maintain profitable yields. A report released by USDA's National Agricultural Statistics Service (NASS) revealed that nearly all (94%) Floridian tomato farmers apply pesticides into their farm fields. Common pesticides include paraquat, chlorothalonil, and bifenthrin (NASS, USDA, 2007). Pesticide application deters invasion and limits damage inflicted by weeds, herbivorous insects, and fungi. Although pesticide use has been effective in the past, pesticide resistance and the emergence of new/ exotic pests has rendered agrochemical-only management tactics obsolete. To mitigate damage or invasion, farmers have opted for integrated pest management practices.

2.1.1 Integrated Pest Management of Tomato Crops

Depending on the variety, tomato crops can be highly susceptible to pest infestation and damage (Picanco & Marqini, 1999; Pianco et al., 2007). In the past, agricultural success was heavily dependent on agrochemical application. Frequent and excessive pesticide applications have decreased chemical effectiveness, and decimated natural enemy populations. Presently, pest populations and crop damage cannot be controlled solely by chemical pesticides. For this reason, Florida Department of Agriculture and the

University of Florida IFAS Extension have encouraged tomato farmers to utilize integrated pest management practices (IPM) (Leppla, 2007).

IMP is a combination of low risk and cost-effective pest control methods that combine available pest and environmental information to prevent pest from reaching damage thresholds (Leppla, 2007). Common practices among tomato farmers include early pest detection through scouting, crop rotation or cover cropping, usage of resistant varieties, plasticulture, organic amendments, and other biological controls (Leppla, 2007).

Numerous studies conducted detail the relationship between the adoption of IPM practices and its effectiveness at controlling economic threshold levels. A study conducted by Picanço et al. (2007) studied the effect of IPM practices on tomato production and conservation of natural enemies. Picanço et al. (2007) observed that integrated pest management practices efficiently controlled different species of aphids, moths and harmful weeds. Moreover, the integration of IPM drastically reduced the number of insecticide applications and increased the population of natural enemies (Picanço et al., 2007). Similarly, a study lead by Demirozer et al. (2012) assessed the effect of integrated pest management programs for thrips on fruiting vegetables like tomato in Florida. The study concluded that effective and sustainable control of thrips and topoviruses is accomplished through IPM with a special focus on resistance management (Demirozer et al., 2012). Demirozer et al. (2012) also noted that farmers from Palm Beach County, who shifted from calendar chemical applications to scouting, saw a significant reduction in yield. Total savings amassed to $28.8 million from reduced damage and decreased pesticide applications (Demirozer et al., 2012).

Non-chemical use IPM practices reduce pest population through the conservation of natural enemies and overall improvements in agroecological biodiversity. The conservation of natural enemies supplements agrochemical usage. Many studies such as Letourneau & Goldstein (2001) suggest that non-agrochemical pest management increases natural predator population while maintaining the comparable economic damage thresholds of conventional farms. Limited agrochemical usage and informed fertilizer applications enhance overall biodiversity. Many studies detail the benefits or positive correlations between biodiverse agroecosystems and pest management. For example, greater microbial activity in organic soils contributes to root pathogen suppression through microbial antagonism or competition (Drinkwater et al., 1995). Diverse microbial communities may be able to compensate for synthetic chemical inputs (Drinkwater et al., 1995).

2.1.2 Common Pests of Tomato Crops in Florida

An agricultural pest can be defined as an arthropod or insect that causes economic loss through a decrease of marketable yield (Lange & Bronson, 1981). Tomato crops cultivated in Florida are prone to insect infestation and damage (Schuster & Smith, 2003). Infestation and damage inflicted by pests is directly correlated with the life cycle of the plant, environmental conditions, and the control methods employed by growers. Common tomato pests in Florida include whiteflies, aphids, thrips, multiple species of larvae, and leafminers (Webb et al., 2001). These pests are attracted to the tomato plant's fruit and sap (Webb et al., 2001). Additionally, leaves and other parts of the plant are used by pest to lay their eggs and provide a stable food source for larvae (Webb et al., 2001; Schuster & Smith, 2003).

The silverleaf whitefly or *Bemesia tabaci* biotype B is a key pest for tomato plants in Florida (Schuster, 2001). *B. tabaci* is a polyphagous phloem feeder, which means they feed on the sap of a wide array of plants (Schuster et al., 2009; Webb et al., 2001). Through its indiscriminate feeding habits, *B. tabaci* can serve as a vector for diseases, viruses, deformities, and mold development (Webb et al., 2001; Schuster et al., 2009). Severe ailments such as the tomato yellow leaf curl virus and tomato mottle virus are commonly transmitted by this pest (Webb et al., 2001; Schuster et al., 2009). Whitefly infestations are also associated with irregular ripening disorder which causes early ripening of the fruit and the build-up of internal white tissue affecting optimal yield (Schuster, 2001; McCollum et al., 2004).

Other pests such as aphids and thrips also act as vector for viruses, diseases, and mold. The green peach aphid or *Myzus persicae* is the most common aphid species in Florida tomatoes (Webb et al., 2001; Lange & Bronson, 1981). *Myzus persicae* feeds on the blossoms of the plant. Consumption of blossoms reduces fruit set, while facilitating mold colonization and virus transmission to neighboring plants (Webb et al., 2001; Lange & Bronson, 1981). Viruses commonly spread by aphids are the tobacco etch virus, pepper mottle virus, and destructive streak disease and cucumber mosaic virus (Webb et al., 2001; Lange & Bronson, 1981; Lapido & Roberts, 1977).

Invasive thrips such as the Western flower thrip (*Frankliniella occidentalis)* are considered one of the most damaging pests to tomato crops in southern and central Florida (Funderburk et al., 2011; Webb et al., 2001). *Franklinella occidentalis* is the most efficient vector of the topovirus tomato spotted wilt virus (Funderburk et al., 2011; Demirozer et al., 2012). The virus is spread through the consumption of epidermal cells

in the plant (Funderburk et al., 2011; Webb et al., 2001; Demirozer et al., 2012). Moreover, preferred oviposition of *F. occidentalis* eggs are inside of the tomato fruit. Burrowing of eggs onto the fruit set causes dimples and discoloration in the tomato (Funderburk et al., 2011; Webb et al., 2001).

Similarly, the Tomato pinworm larvae (*Keiferia lycopersicella*) and the Tomato fruitworm larvae (*Heliocoverpa zea*) cause severe damage to the foliage and fruit of the tomato plant (Webb et al., 2001). Both species of larvae are avid feeders, and their consumption patterns can lead to complete defoliation of the plant and damaged fruit through rot, contamination or the introduction of secondary pathogens (Webb et al., 2001). Finally, leafminers such as the Vegetable leafminer (*Liriomyza sativae, L. trifolli*) also cause foliage damage. Leafminers decrease photosynthetic area by burrowing in the foliage (Webb et al., 2001). Burrows can serve as entry points for other pathogens leading to necrotic tissue and sunscald (Webb et al., 2001).

2.2 Secondary Metabolites in Plants

Plant secondary metabolites are defined as low molecular weight compounds that have no recognized role in fundamental life sustaining processes (Oksman-Caldentey & Inzé, 2004). Secondary metabolite production or accumulation does not contribute to growth, development, or reproduction of the plant. Secondary metabolites large and diverse class of compounds are characterized through their biosynthetic origins and can be divided into four major groups: alkaloids, isoprenoids, glucosinolates, and phenylpropanoid**s** (Table 1 and Table 2) (Oksman-Caldentey & Inzé, 2004). Although major groups and compounds may differ in function, it is generally understood that the

production of secondary metabolites aids the plant in survival and adaptation to their ecosystem (Delgoda & Muray, 2016; Oksman-Caldentey & Inzé, 2004).

2.2.2 Roles of Secondary Metabolites

Secondary metabolites are often produced through the synthesis of primary metabolites (Ramakrishna & Ravishankar, 2011). The production of these compounds is a response to abiotic or biotic stress, and therefore play a major role in adaptation of plants to their environment (Borgaud et al., 2001). Many compounds contain antiviral, antifungal, antibiotic, anti-feeding, and toxic properties that protect plants against pathogens, pests, and competition (Borgaud et al., 2001). Moreover, they constitute important UV absorbing compounds, preventing leaf damage.

Abiotic stress, such as drought or temperature, significantly affect plant growth and development. Blanch et al. (2008) studied the effects of drought and temperature stress on leaf volatile terpene concentrations in *Pinus halepensis* and *Quercus ilex*. During their study, Blanch et al. (2008) monitored concentrations of α-pinene and Δ^3-carene, two common terpenes. Results demonstrated that total terpene concentrations in both plant species increased significantly under drought treatment (Blanch et al., 2008). Drought conditions induce oxidative stress in plants. Accumulation of monoterpenes in water-stressed plants may help with oxidative stress, protecting the plant from predation or aid with storage (Blanch et al., 2008; Peñuelas & Estiarte, 1998). Jaleel et al. (2008) conducted a similar study involving the effects of soil salinity and secondary metabolite accumulation in *Catharanthus roseus*. Jaleel et al. (2008) also observed greater total indole alkaloid content in plants treated with salt compared to control treatments.

Biotic stresses such as herbivory attack or pathogen infection can also trigger for secondary metabolite production. For instance, Tian et al. (2012) observed a significant increase in jasmonic acid after caterpillar saliva was added to a wound in a tomato plant. Jasmonic acid plays a major role in plant defense against pests and pathogens (Srivastava, 2002). The compound is responsible for inducing the synthesis of proteinase inhibitors, volatile aldehydes, and chitinases (Srivastava, 2002). Levels in treated plants remained 13 to 40 times higher as compared to control plants suggesting secondary metabolite accumulation (Tian et al., 2012). Induced chemical defenses have also been observed in rice. Rice produces fifteen phytoalexins or defense compounds such as orzyalexins A to F, sakuranetin, or phytocassanes A to E (Hasegawa et al., 2010; Contreras-Cornejo et al., 2014). These compounds are known to accumulate and exhibit antibiotic properties in response to rice-blast pathogens *Magnaporthe grisea* and *Rhizoctonia solani* (Kuc, 1995; Koga et al., 1995; Dillon et al., 1997; Contreras-Cornejo et al., 2014).

Table 1 Secondary Metabolite Groups and Description

Secondary Metabolite Type	*General Description*	*Function*	*Examples*	*References*
Nitrogen Containing: Alkaloids	A diverse group of heterocyclic nitrogen compounds that are typically alkaline. The main role of these compounds is defense. Some defense mechanisms triggered by alkaloids includes bitter or unpalatable taste of biomass; and disruption of protein function and central nervous system alteration in pests.	Defense	Cocaine Morphine	(Matsuura & Fett-Neto, 2015)
Nitrogen and Sulfur Containing: Glucosinolates	Sulfur containing compounds are typically found in the Brassicaceae family. When these compounds undergo hydrolysis, they form diverse products processing different biological activities. Some by-products have antinutritional or toxic effects, aiding in herbivore and pathogenic defense.	Defense Flavoring	Singrin Glucobrassicin	(Holst & Fenwick, 2003) (Ishida et al., 2014)
Isoprenoids or Terpenoids	Terpenes are formed by the condensation of two or more isoprene units. They form the largest family of compounds found in nature. Although their major role is in defense against herbivory, not all terpenoids act as secondary metabolites. Some may have crucial roles in photosynthesis or are a source of plant hormones. They are classified by the number of isoprene units that comprise the parent terpene. Classes of terpenoids includes: hemiterpenoids, monoterpenoids, sesquiterpenoids, diterpenoids, triterpenoids, etc.	Defense Photosynthetic Pigment Growth Regulator	Phytol Linallol	(Srivastrava, 2002) (Tholl, 2015)
Sterols	Phytosterols are steroid alcohol compounds that are biosynthetically derived from squalene and other triterpenes. They play a crucial role in cellular and developmental processes, as well as regulate membrane fluidity.	Regulate membrane fluidity	Sitosterol Campesterol	(Piironen et al., 2000) (Tholl, 2015)
Carotenoids	Isoprenoid pigments that provide color and aroma to fruit, vegetables, flowers, and leaves. Coloration associated with carotenes are usually yellow, orange or red. Carotenoids also play role in light absorption, serving as photoprotective compounds.	Photosynthetic Pigment Pigmentation Aroma	Lycopene Alpha carotene	(Cazzonelli, 2011)

Table 2 Secondary Metabolite Groups and Descriptions Continued

Secondary Metabolite Type	*General Description*	*Function*	*Examples*	*References*
Phenylpropanoids	A diverse group of secondary metabolites with an aromatic ring with a three-carbon substituent synthesized from phenylalanine or tyrosine. This group includes phenolic acids, flavonoids, monolignols, stilbenes, and coumarins.	Defense UV/ High light protection Cell wall component	Caffeic acid Eugenol	(Drifhout & Morgan, 2010) (Deng & Lu, 2017)
Phenolic Acids	A large group of aromatic acid compounds that are characterized by the presence of a benzene ring linked to one or more hydroxyl or methoxy group. Phenolic acids can be divided into polyphenols and simple phenols. Most phenolic acids are linked to structural components (lignin or cellulose), larger polyphenols, or smaller organic molecules. These compounds possess diverse functions influencing nutrient uptake, protein synthesis, enzyme activity, photosynthesis, allelopathy/ general defense, and structural components.	Defense Protein synthesis Cross-linking to act as cell wall component Pollinator Attractor	Vanillic acid Salicylic acid	(Deng & Lu, 2017) (Robbins, 2003) (Goleniowski et al., 2013)
Flavonoids	This subclass of polyphenols characterized for containing two or more aromatic rings. They are further categorized into flavones, flavanols, isoflavones, chalcones, flavanols, and anthocyanins. These compounds could exhibit color and can supply pigmentation to different plant parts and protect against excessive solar radiation.	Pigmentation Aroma Control of cellular enzyme activity Regulation of growth hormone transport	Rutin Quercentin	(Panche et al., 2016) (Pietta, 2000) (Treutter, 2006)

2.2.3 Tomato Secondary Metabolites

The tomato, *Solanum lycopersicum,* is known for secondary metabolite rich tissues and fruit. In the past, secondary metabolite profiling has been limited to tomato fruit. Fruit or edible parts of the fruit are usually tested to determine nutritional value of crop in question. Because of the focus of this study, the review will be limited to secondary metabolites found in tomato leaves.

Secondary metabolite profile from tomato leaves differs from those in the fruit. Secondary metabolites found in tomato fruits function as an attractant to encourage seed dispersal (Kim et al., 2014). Compounds such as α-tomatine (alkaloid) or lycopene (carotenoid) are responsible for preventing fruit rot while maintaining optimal color, aroma, and flavor to signal the presence of vitamins, sugars and amino acids to seed-dispersing herbivores (Kim et al., 2014). In contrast, metabolites produced in the leaves and internodes of tomato plants are noxious to insects and pathogens (Kim et al., 2014).

Tomato leaves are rich in alkaloids, terpenoids, and phenolic compounds. Kim et al. (2014) reported a high proportion dehydro-tomatine and α-tomatine, two alkaloids, in tomato leaf biomass. High concentrations of alkaloids were detected in young or sprouting leaves (Kim et al., 2014). Dehydro-tomatine and α-tomatine in plants serve as chemical defenses. For instance, α-tomatine disrupts cell membranes through lysing of the liposome (Morrissey and Osbourn, 1999). Moreover, Boulogne et al. (2012) found evidence of tomatine compounds acting as a fungicide and insect deterrent for *Leptinotarsa decemlineata*, *Melanopus bivittatus*, *Fusarium solani,* and *Fusarium oxysporum.*

Terpenoids are also a major component of tomato leaves (Buttery et al., 1987; Kim et al., 2014). Terpenoids are diverse, fulfilling roles in photosynthesis, cell membrane regulation or pigmentation. Buttery et al. (1987), Colby et al. (1993), and Kim et al. (2014) reported the presence of various monoterpenes and sesquiterpenes. Mono and sesquiterpenes such as β-Phellandrene and myrcene are considered volatile compounds. These volatile compounds are contained in leaf glandular trichrome and are released after leaves are damaged (Degenhardt et al., 2003). Once released, the aroma serves to attract natural enemies to herbivores attacking the plant (Degenhardt et al., 2003).

Carotenoids and phytosterols are other types of terpenoids also present in tomato leaf tissues. Carotenoids are isoprenoid pigments that supply color and aroma to various plant parts (Cazzonelli, 2011). They function as photoprotective compounds, preventing bleaching or damage from the sun in leaf tissues (Cazzonelli, 2011). Carotenoids such as β-carotene and lutein have been found in tomato leaves (Fraser et al. ,1994; Kim et al., 2014). Both compounds are extensively found in leafy greens and have strong antioxidant properties (Jiménez-Escrig & Sanchez-Muniz, 2000; Kim et al., 2014).

Phytosterols are steroid alcohols derived from squalene and other terpenes (Piironen et al., 2000). They play an important role in different cell processes, as well as regulate membrane fluidity (Piironen et al., 2000). Stigmasterol and β-sitosterol are predominant phytosterols in plant tissues (Hodzic et al., 2008; Wang et al., 2012). In tomato, these compounds may also function as defense from pathogens (Wang et al., 2012). Griebel & Zeier (2010) showed that stigmasterol synthesis was induced in plants upon virulent infection. To inhibit pathogen infection, plants reduce excess nutrient

efflux from their apoplasts (Wang et al., 2012). Manipulating sterol synthesis prevents nutrient efflux from apoplast through membrane permeability control (Wang et al., 2012).

Phenolic compounds are low molecular weight molecules widely distributed in plants. They fulfill several roles including defense, pigmentation, and protein synthesis (Drijfhout & Morgan, 2010; Deng & Lu, 2017). Depending on their chemical structure, phenolic compounds can be categorized into different groups, such as phenolic acids, flavonoids, lignans, and stilbenes (Gan et al., 2019). Chlorogenic, caffeic, and vanillic acids have all been detected in tomato leaves (Kim et al., 2014). Chlorogenic acid and its derivatives are the main phenolics in tomato leaves (Slimestada and Verheulb, 2009). Chlorogenic and caffeic acids are also oxidized by plants to create toxic quniones, which increase disease resistance (Kim et al., 2014). Additionally, caffeic acid has antimicrobial properties that can effectively suppress various fungi and bacteria (Kim et al., 2012; Rauha et al., 2000; Widmer and Laurent, 2006).

2.3 Vermicompost

2.3.1 Vermicompost Production

Vermiculture or vermicomposting is a non-thermophilic process that arises from the interactions between epigeic earthworms and mesophilic bacteria (Joshi et al., 2014). The process of vermilculture converts organic waste into a stable and nutrient-rich manure known as vermicompost (Joshi et al., 2014; Aira et al., 2000). Vermicompost can be made with diverse feeding stocks. Some organic feedstocks used include animal manure (Albanell et al., 1988; Wani & Rao, 2013), soft and alcoholic drink sludge (Orozco et al., 1996; Nogales et al., 2005), food waste (Arancon et al., 2004), and garden waste (Wani & Rao, 2013; Aremu et al., 2015). Vermicompost production can also range

from simple low-technology systems in boxes or windrows, to large and complex breeding facilities (Orozco et al., 1996). Overall, production requires low-energy and labor inputs, and transformation efficiency is maintained through environmental conditions such as oxygen levels, light, temperature, and moisture (Sinha et al., 2009; Orozco et al., 1996).

Vermiculture systems vary in size/ complexity, organic waste input, worm selection, and end-product. Although vermicomposting facilities can differ, the process can be divided in between two distinct phases defined by earthworm activity (Dominguez et al., 2010). The first phase, or the "direct function phase" occurs when earthworms consume, digest, and assimilate organic matter (Dominguez et al., 2010; Huang et al., 2014). This first phase alters the biochemical properties and microbial profiles in the system (Dominguez et al., 2010; Huang et al., 2014). The second phase, or the "indirect phase" happens when earthworms and bacteria coexist and cooperate to further decompose organic materials (Dominguez et al., 2010; Huang et al., 2014).

Earthworms are the main drivers of vermicomposting through the displacement, conditioning and digestion of the substrate (Suthar, 2009). With each movement, earthworms aerate and break down organic matter, altering its biological, physical, and chemical properties. Breakdown and conditioning of organic waste creates a favorable environment for microbial activity and decomposition (Suthar, 2009; Dominguez, 2004). Although feedstock, production system, and environmental conditions may affect the quality of the worm castings, earthworm species selection is the most important variable in vermiculture (Lim et al., 2016; Singh et al., 2011). Earthworm selection is crucial

because each species has different rates of waste stabilization and microbiota associations (Singh et al., 2011).

To choose the correct earthworm species for vermicomposting, the species must have: a) high rates of organic matter consumption; b) high tolerance to stress; c) high reproduction rate; and d) rapid growth/ maturation time (Singh et al., 2011; Lim et al., 2016). These characteristics align best with epigeic earthworm species. Epigeic earthworms are the most suitable for vermiculture because they live/ feed in the subsurface of the soil and they are highly effective decomposers (Lim et al., 2016). The most common species used in vermiculture are the *Eisenia fetida* and *Eisenia andrei.* Both species have a worldwide distribution, are resilient, and have a wide tolerance to temperature variations (Edwards et al., 2004; Lim et al., 2015; Suthar, 2009).

Environmental conditions must be heavily monitored to sustain a vermicomposting operation. Earthworms consume most organic materials in a pH of 5 to 8, moisture content must be held 40% to 55%, and C/N ratios must be approximately 30 (Singh et al., 2011; Lim et al., 2016). Since not all organic feedstock meet these parameters, organic waste is often mixed with bulking agents or undergone a pre-treatment process (Yadav & Garg, 2011; Lim et al., 2016). Different bulking agents can include cow/ chicken manure (Wani & Rao., 2013; Garg & Gupta, 2011), wheat straws (Suthar, 2009), garden/kitchen waste (Wani & Rao., 2013), and sawdust (Bustamante et al., 2013). Depending on the feedstock, bulking agents can a) help maintain pH at habitable levels; b) lower the concentration of noxious compounds/chemicals; c) increase nutritional value of waste; and c) enhance microbial activity (Suthar, 2009; Dominguez, 2004; Suthar, 2006; Flegel & Schreder, 2000; Suthar, 2008).

2.3.2 Biological Composition of Vermicompost

Microbial dynamics in vermicompost are shaped by earthworm activity. Differences in microbial species richness and diversity can be attributed to the special nutritional interactions and requirements of the earthworm employed (Gopal et al., 2010). Passage through the earthworm's gut also influences microbial communities. During gut transit, microbes are exposed to harsh anoxic conditions favoring an anaerobic and metabolically active microbial population (Gopal et al., 2010; Gomez-Brandon et al., 2011; Yasir et al., 2009).

Due to differences in vermiculture inputs, microbial communities may differ greatly in functional capacity and biodiversity. Several studies reveal the presence free-living nitrogen fixers (Gopal et al., 2009), filamentous actinomycetes (Elmer, 2009), antifungal bacteria (Mu et al., 2017), beneficial fungi (Zhang et al., 2014), plant growth promoting rhizobacteria (PGPRs) (Sahni et al., 2008), proteobacteria (Yasir et al., 2009) and fluorescent pseudomonas (Elmer, 2009; Gopal, 2009). The incidence of these organisms may be indicative of other beneficial properties vermicompost may provide. In a study conducted by Gopal et al. (2009) explored the potential amplification of plant beneficial microbial communities through vermicomposting. Gopal et al. (2009) observed an increase in free-living nitrogen fixing bacteria, microaerophilic *Azospirillum* sp., and phosphate solubilizers, which increased the available nitrogen and phosphorous content in the composted litter used.

Other studies such as Mu et al. (2017), Yasir et al. (2009), and Elmer (2009) found that an increase in beneficial microbial communities associated with anti-fungal and disease-suppressing properties. For example, Mu et al. (2009) was able to isolate a

broad-spectrum anti-fungal bacteria strain called *Bacillus subtilis* M29. Volatile compounds produced by the strain were extracted and identified (Mu et al., 2017). Purified compounds had antagonistic abilities against *Botrytis cinera* which causes gray mold disease (Mu et al., 2017). Similarly, Yasir et al. (2009) and Elmer (2009) found chitinolytic bacteria such as filamentous actinomycetes, florescent pseudomonas, streptomyces, and actinobacteria in vermicompost. The increase of chitinolytic activity is known to produce anti-fungal and anti-biotic compounds that may contribute to pest and disease suppression (Yasir et al., 2009). Compounds produced by actinobacteria are being researched and used to produce microbial antibiotics (Yasir et al., 2009; Yu et al., 2008).

2.3.3 Physical and Chemical Properties of Vermicompost

Through earthworm-bacteria interactions, organic waste is physically and chemically transformed into a stable nutrient rich manure (Zhang et al., 2014). During vermicomposting, characteristics such as pH, electrical conductivity (EC), carbon and nitrogen ratios (C:N), macro/micronutrient content are modified (Nath et al., 2009). Changes in physical and chemical structures of the organic feedstock are caused by break down or mineralization of compounds (Nath et al., 2009; Aquino et al., 2019; Albanell et al., 1988). In a study conducted by Nath et al. (2009), researchers monitored the change in physical and chemical characteristics pre-vermicomposting and post-vermicomposting. Overall, vermicomposted materials showed a significant increase in nitrogen, phosphorous, potassium, and calcium, and a significant decrease in total organic carbon (TOC), C:N, EC, and pH compared to original feedstock (Nath et al., 2009). A decrease in pH is attributed to nitrogen and phosphorous mineralization and the conversion of organic materials into organic acids. Changes in TOC and C:N ratio, and an increase in

EC which were caused by carbon loss through microbial respiration and mineralization (Nath et al., 2009; Suthar, 2009; Elvira et al., 1998; Wong et al., 1997). Albanell et al. (1988) also reported similar changes in vermicompost by-product. Results only differed in EC values, which can be attributed to mineralization and high rates of humidification in the vermicompost tested (Albanell et al., 1988). Differences between results could be caused by feedstock, earthworm selection, and mineralization rates of different compounds.

These physical and chemical properties combined with a high nutrient content make vermicompost an ideal organic fertilizer. Although nutrient concentrations are an important factor for any soil amendment agent/ fertilizer, it is the presence of non-hormonal plant growth promoters, phytohormones, and phenolic acids that separates vermicompost from other organic fertilizers. Several studies such as Aquino et al. (2019), Aremu et al. (2014), Ravidran et al. (2016), and Zhang et al. (2014) reported the presence of both compound types in vermicompost.

Non-hormonal plant growth promoters like humic substances are found naturally in soil, water and organic deposits (Wong et al., 2015; Piccolo et al., 2002). Humic substances can be classified as humic acids (HAs), fulvic acids (FAs), and humins (Piccolo et al., 2002). A study lead by Fernández-Gómez et al. (2011) explored the chemical composition of vermicompost. Fernández-Gómez et al. (2011) showed that vermicompost made from different feedstock could contain 11.2- 53.9 g kg^{-1} of humic acid and 8.7 – 44.5 g kg^{-1} of fulvic acid. Other studies, such as Albanell et al. (1988), reported 14.4- 21.8% of humic acid and 2.8- 5.7% of fulvic acid in dry matter tested. Humic substances are known to improve cell growth and nutrient uptake through the

formation of soluble complexes with numerous ions (Canellas et al., 2010; Pinton et al., 1999; Canellas et al., 2002). Furthermore, they act as root growth regulators by using cell auxin signaling to trigger growth (Canellas et al., 2010; Zandonadi et al., 2007; Dobbss et al., 2007).

Humic substances extracted from vermicompost and their benefits have been examined by various scientist. For instance, Atiyeh et al (2002) used humic acids derived from pig manure vermicompost as a growth medium for tomato and cucumber seedlings. Atiyeh et al. (2002) noted that all seedlings treated with humic acid had a significant increase in growth and fruit yield. In another study by Arancon et al. (2003), researchers extracted humic acids from cattle, food scraps and paper waste vermicompost. Humic acids were applied to marigold, peppers, and strawberry crops (Arancon et al., 2003). Arancon et al. (2003) also observed that all plants treated had higher plant height, leaf area, shoot dry weight, and root dry weight.

Unlike non-hormonal plant growth promoters, phytohormones are crucial for the growth and the regulation of physiological processes within the plant (Wong et al., 2015). Some regulatory functions include chlorophyll production, cell division and expansion, cell elongation, and the activation of bud growth and senescence (Wong et al., 2015). These compounds are grouped into classes: auxins, cytokinins (CKs), gibberellins (Gas), abscisic acid (ABA), salicylic acid (SA), jasmonates (JAs), brassinosteroids (BRs), strigolactones (SLs) and ethylene (Delatorre et al., 2017; Su et al., 2017). Although each phytohormone has a different function, they are effective through interactions among themselves through additive, synergistic, or antagonistic actions (Delatorre et al., 2017).

This mechanism is known as crosstalk (Delatorre et al., 2017; Kazan & Manners, 2012; Kohli et al., 2013).

Interactions among phytohormones determines the effects that each compound may have on the plant. To determine the full benefits of vermicompost, it is important to identify and quantify phytohormones within the composted materials. Despite the lack of research, Zhang et al. (2015) was able to develop of a method for phytohormone identification in vermicompost. Through and optimized ultrasound-assisted extraction (UAE) and liquid chromatography- mass spectrometry (LC-MS/MS), Zhang et al. (2015) was able to successfully identify and isolate various types of cytokinins and auxins. Ravindran et al. (2016) also found the presence of phytohormones. Ravindran et al. (2016) reported the presence of cytokinins, auxins, and gibberellins in the vermicompost tested. Lastly, Aremu et al. (2014) found evidence of phytohormones and phenolic acids in garden-waste derived vermicompost leachate. Aremu et al. (2014) was able to identify and quantify different types of cytokinins, auxins, gibberellins, brassinosteroids and phenolic acids.

2.3.4 Uses for Vermicompost

2.3.4.1 Organic Fertilizer and Soil Amendment Agent

Vermicompost and its by-products are typically used as an organic amendment for soil and plant growth. Its high nutritional value, diverse biological activity, and the presence of beneficial compounds such as humic acids renders it an ideal fertilizer. Numerous studies support vermicompost usage as an organic fertilizer and soil amendment agent. Singh et al. (2008) studied the effects of vermicompost on the growth,

physiological disorders, fruit yield/quality of strawberry crops. Results demonstrate that vermicompost had a significant positive effect on flowering, number of fruits per plant, total yield, biomass, and incidence of physiological disorders in strawberry crops (Singh et al., 2008). Atiyeh et al. (2000) observed similar results while evaluating the effects of vermicompost and compost on plant growth in media and soil. Atiyeh et al. (2000) noted that marigold, tomato, and raspberry seedlings had higher weights, lower mortality rate, and higher biological activity within growth media (Atiyeh et al., 2000). Enhanced growth is attributed to the gradual release of macronutrients and micronutrients, and the high microbiological activity occurring in vermicompost (Atiyeh et al., 2000).

The addition of nutrients/ beneficial compounds and a change in biological activity can also benefit physiochemical properties of soil. For example, Azarmi et al. (2008) reported changes to the structure and chemical composition of soil after the addition of vermicompost. Azarmi et al. (2008) observed that EC, pH, organic carbon and nutrient composition increased, while bulk density decreased. Changes in EC suggests that cation exchange sites have increased in the soil, therefore increasing the soil's potential to retain more nutrients. A decrease in bulk density and an increase in porosity, indicates a rise in the storage capacity and aeration of the soil (Azarmi et al., 2008). Manivannan et al. (2009) also observed an increase in porosity and cation exchange capacity, and a reduction in bulk density. Alterations in the physical structure of the soil may also arise from the presence of polysaccharides in worm castings (Lim et al., 2014). When present, polysaccharides act as a glue-like substance, increasing aggregation stability (Lim et al., 2014). In addition, mucus excreted from the worm's gut and

microbial exudates may also lead to enhanced aggregate stability, aeration, and porosity in soil (Lim et al., 2014).

2.3.4.2 Pest Suppressant and Biological Control Agent

Biological control is defined as the practice of using living organisms or "natural enemies" to suppress or stop pest infestation/damage. Recently, farmers and scientist have been investigating vermicompost's potential as a biological control agent because of its pest-resistant properties. Scientist speculate that pest-resistant properties are promoted by microbiological activity, the presence of plant growth regulators and non-hormonal plant growth regulators, and a balanced nutritional composition for plants (Arancon et al., 2005). Proposed mechanisms for pest suppression via the addition of vermicompost may be general and specific (Simsek-Ersahin, 2011). Enhanced suppression could be attributed to various factors such as microbial antagonism, nutrient release, induced host resistance, and other abiotic inhibitory factors (Simsek-Ersahin, 2011). For instance, Mu et al. (2017) found that vermicompost inhibited the development of different fungal pathogens such as *Botrytis cinerea* or Gray mold through the volatile organic compounds released by symbiotic microbes. Some of the volatile organic compounds produced found were 1-butanol, 3-methyl-3-hexanol, 1-heptylene-4-alcohol, and acetic acid butyl ester inhibited the growth of mycelia completely (Mu et al., 2017).

Arancon et al. (2005) also observed significant pest population and damage suppression after treating pepper, tomatoes, and cabbage crops. Results demonstrated that the addition of vermicompost to a soilless medium resulted in major suppression of all three types of insect attacks from aphids, mealy bugs, and cabbage caterpillars. Arancon et al. (2005) attributes pest suppression to nutrient availability. Vermicompost is

composed of biologically available nutrients that are slowly released as decomposition advances (Arancon et al., 2005). The “slow-release” process affects nutrient availability, offering the plant a more balanced dietary intake as compared to traditional inorganic fertilizer treatments (Arancon et al., 2005). A change in nutritional intake could affect morphological and physiological aspects of a plant, altering senescence, amino-N concentrations in phloem sap, sugar concentrations, and secondary metabolite production (Arancon et al., 2005; Patriquin et al., 1995).

Finally, Edwards et al. (2010) studied the effects of vermicompost extracts on cucumber beetles and tobacco hornworm attacks. Edwards et al. (2010) found that all vermicompost treatments significantly decreased the establishment/damage of both pests on cucumber and tomato crops. Edwards et al. (2010) proposes that pest suppression might be caused by the presence and/or increase of phenolic substances. It is well known that phenolic substances are unpalatable to some invertebrates and could function as insect anti-feedant (Koul, 2008; Edwards et al., 2010). Other studies such as QiTan (2004) and Haukioja et al. (2002) found evidence for pest deterrent properties in phenolic substances. QiTan (2004) used phenols and phenolic acids extracted from ginko trees to deter caterpillar attacks. QiTan (2004) concluded that the extracts were as effective as several pesticides used to control caterpillar populations. Likewise, Haukioja et al. (2002) found that the incidence of phenolic substances in plant tissues lowered the rate of tissue consumption by caterpillars.

2.3.4.3 Bioremediation

Bioremediation is defined as the usage of microorganisms or plants to consume and break down environmental pollutants. To combat soil and organic waste toxicity,

vermicomposting technologies are being studied to evaluate its effectiveness as a remediation tactic. Utilizing vermicompost as a bioremediation practice is called "vermiremediation" (Suthar et al., 2014). Earthworms used in vermiculture have high tolerance to heavy metals and can easily absorb them into their tissues (Pattnaik & Reddy, 2011; Morgan & Morris, 1982). Additionally, earthworm- bacterial interactions create humic substances which could fractionate heavy metals reducing the amount of soluble or exchangeable fractions of metals (Pattnaik & Vikram Reddy, 2011; Edwards & Bohlen, 1996).

Chemical and metabolic process produced through earthworm-microbial interactions retain or immobilize heavy metal fractions. Various studies such as He et al. (2016), Suthar et al. (2014), and Pattnaik & Reddy (2011) have tested vermiremediation as a viable bioremediation practice. Suthar et al. (2014) examined the remediation through vermiculture of heavy metals in paper sludge waste produced from the paper and pulp industry. Results from their study suggest that all four heavy metals studied (Pb, Cu, Cd, & Cr) experienced a significant decrease across observed time. Although all metals decreased over time, Cadmium (Cd) and Lead (Pb) decreased faster over time. This observation indicated that heavy metal speciation is different for all metals, and is dependent on different abiotic factors produced through earthworm-microbial interactions such as pH, organic matter content, electrical conductivity, etc. Suthar et al. (2014) study also evaluated bioaccumulation of heavy metals in earthworm tissues. High concentrations of heavy metals in tested tissues indicates that heavy metals were accumulated. Thus, the decrease of heavy metals was correlated to earthworm action.

Pattnaik & Reddy (2011) evaluated heavy metal remediation through the vermiculture of three different earthworm species. Five different heavy metals (Cd, Pb, Zn, Cu, & Mn) were observed. Results showed that tissue accumulated heavy metal concentrations were significantly different and could be negatively correlated with heavy metal concentrations in the treated waste. Moreover, results indicate that different earthworm species have a high rate of accumulation, which means that accumulation and heavy metal speciation can be a species-specific action.

2.3.5 Vermicompost Tea and Extracts

Vermicompost tea or extract refers to brewed water extracts of previously composted materials (Gomez-Brandon et al., 2015). During the brewing process, most valuable aspects such as mineral nutrients, biologically active metabolites, and beneficial microbes of vermicompost are transferred to vermicompost tea (Mishra et al., 2017). Because of their proven effectiveness and enhanced application control, many have opted for their usage. Vermicompost tea is usually applied as a soil/foliar drench or spray (Simsek-Ersahin, 2011). Although commercial production and usage of vermicompost tea has skyrocketed, effects in plant growth and pest suppression are poorly understood.

2.3.5.1 Vermicompost Tea Production

Vermicompost tea is categorized through two different production methods, aerated and non-aerated (Gomez-Brandon et al., 2015; Mishra et al., 2017). Aerated vermicompost teas are produced by aerating the compost-water extracts continuously during the brewing process, whereas non-aerated teas allow vermicompost to steep passively with little to no agitation (Gomez-Brandon et al., 2015; Litterick and Wood,

2004). Both methods require incubation and filtration prior to application (Gomez-Brandon et al., 2015).

Aerated production methods are associated with several advantages such as shorter brewing time, greater microbial diversity, lower phytotoxicity, and lower levels of human pathogen reproduction as compared to non-aerated tea production (Ingham and Alms, 2003; Gomez-Brandon et al., 2015). Despite these observed advantages, non-aerated teas are preferred over aerated teas (Gomez-Brandon et al., 2015). Non-aerated production methods are easier and require less energy and specialized materials as compared to aerated tea production (St. Martin and Brathwaite, 2012). While both production methods yield the same by-product, scientist disagree which produces the best results.

Differences between the benefits of aerated and non-aerated teas might be directly influenced by the brewing time. Vermicompost teas are usually brewed for 24 hours in shaded and temperate areas (Kiyasudeen et al., 2015). With enough brewing time, non-aerated tea could be as effective as aerated teas at enhancing plant growth and deterring pests. Koné et al. (2009) reported that non-aerated compost teas brewed for two weeks were able to inhibit foliar fungal pathogens in tomatoes. Welke (2005) observed no significant difference between growth and yield increase between aerated and non-aerated tea. Although statistical differences were not observed, overall yields were higher in aerated tea. Welke (2005) suggests that this is likely caused by the concentration of plant growth promoting hormones and micronutrients from aerobic microorganisms.

Contrasting results were also observed when the dilution rate of vermicompost tea was tested. For example, Welke (2005) did not observe any significant difference

between high and low ratios of vermicompost tea. Whereas Edwards et al. (2010) observed that the higher water-vermicompost ratio in tea, the higher the increase in overall plant parameters measured. Results demonstrated that out of the three vermicompost concentrations (5%, 10%, and 20%), 20% suppressed pest damage/incidence and significantly increased biomass.

Other chemical and biological characteristics in vermicompost tea may vary due to differences in inputs and production processes. High nutrient levels and biological activity are needed in each mixture to ensure beneficial results. To strengthen microbial populations, additives such as molasses, kelp, humic acids, or fish emulsion might be included in the mix (Duffy et al., 2004; Kiyasudeen et al., 2015). Although additives are an attractive option to boost tea production, the presence of carbohydrate-rich materials might encourage the growth of human pathogens (Kiyasudeen et al., 2015).

2.3.5.2 Beneficial Aspects of Vermicompost Tea

Vermicompost tea and extracts share all the beneficial properties vermicompost has on plant growth, enhanced pest suppression, and disease incidence. For this reason, many studies support the usage of vermicompost tea as liquid fertilizer or as a biological control agent. For example, Pant et al. (2009) observed that all vermicompost treatments implemented enhanced plant production, mineral nutrients, and total carotenoids in pak choi compared to the control treatment. Growth enhancement was attributed to the considerable amount of soluble mineral nutrients present in vermicompost tea (Pant et al.; 2009).

Similar results were also observed by Renčo and Kováčik (2015) when studying the effects of vermicompost tea on potato crops. Researchers observed that all

vermicompost treatments significantly affected growth compared to the control treatment. Renčo and Kováčik (2015) also noted that vermicompost tea treatments successfully suppressed nematode population and egg hatchings. Mishra et al. (2017) also observed a decrease in root penetration and egg viability in nematode populations on cucumber crops after the addition of vermicompost tea. Finally, Singh et al. (2003) tested the efficacy of vermicompost tea extracts to control powdery mildews on balsam and pea plants. Singh et al. (2003) recorded that even very low concentrations of the tea had a significant decrease in disease intensity over time and concluded that the usage of vermicompost tea as a foliar spray could be a cost-effective disease prevention practice.

2.3.6 Vermicompost and Chemical Elicitation

Vermicompost is composed of natural plant growth regulators, phenolic compounds, and other phytohormones that contribute to growth stimulation, yield, as well as a change in the chemical composition of the plant (Aremu et al., 2014). Various studies have recorded vermicompost's effect on secondary metabolites and/or the essential oil content in different crops. Elicitation of change could arise from the improvement of soil structure and biological activities, higher retention of mineral nutrients and the presence of diverse phytohormones and compounds (Darzi et al., 2015). Increase of mineral uptake and retention has a positive effect on biomass production, subsequently enhancing the essential oil content (Darzi et al., 2015).

Heidarpour et al. (2019) observed an improvement in essential oil content of *Satureja hortensis L.* following several vermicompost treatments. Heidarpour et al. (2019) noted that the higher the vermicompost application, the higher the oil content in the plant. Plants treated with 30% vermicompost, the highest percentage, had a greater

amount monoterpene and sesquiterpene compounds (Heidarpour et al., 2019). Increased secondary metabolite content was also related to the combination of abiotic stress endured by the plant. Anwar et al. (2004) yielded similar results when studying the effect of organic manures on the essential oil quality and yield in French basil. Anwar et al. (2004) concluded that the larger the amount of vermicompost added, the greater the essential oil content in the plant. Despite having the highest percentage of essential oil content and principal chemical compounds found in basil oil, the lower treatment of vermicompost performed the best in terms of combined effects in plant growth, yield, and essential oil content and quality.

Darzi et al. (2015) also observed an increase in essential oil compound following the application of vermicompost. The maximum percentage of essential oil content was obtained by adding 6 tons of vermicompost per hectare, further addition decreased oil content (Darzi et al., 2015). Similar results were also seen by Javanmardi & Ghorbani (2012) when comparing the effects of vermicompost tea and chicken manures on secondary metabolite production in lemon basil. The highest essential oil content was recorded in 1:10 vermicompost tea treatment, which was 3.12 times higher than the control plants (Javanmardi & Ghorbani, 2012). Total phenolics was also higher in 1:10 vermicompost tea treatment, although total flavonoids and total antioxidants were greater in 1:5 vermicompost tea treatment, a lower ratio of water and tea (Javanmardi & Ghorbani, 2012).

2.3.7 Effects of Vermicompost in Tomatoes

2.3.7.1 Effects on Biomass and Yield

Vermicompost has a positive and significant effect on the growth, quality and productivity of crop plants (Singh et al., 2010). Research on medical and vegetable-producing crops show evidence to support vermicompost's effects on seed germination, biomass production, root development, yield, and marketable yield (Singh et al., 2008; Atiyeh et al., 2000; Azarmi et al., 2008; Lim et al., 2014). Positive effects have also been recorded in different tomato cultivars.

For instance, Singh et al. (2010) studied the effect of vermicompost and NPK fertilizer in the hybrid tomato cultivar Avinwash-2. Results demonstrated that vermicompost treatments had a significant effect on plant height and leaf biomass. Overall, yield was unaffected by the addition of vermicompost except fruit weight (Singh et al., 2010). Elevated fruit weights might be due to the stimulation of production or accumulation of naphthalene acetic acid, an auxin hormone, which plays a crucial role in flowering and fruit setting (Singh et al., 2010). Gutierrez-Miceli et al. (2007) also saw a significant increase in plant sizes and marketable yield. The greatest plant heights and leaf numbers were obtained after treating plants with a 1:4 soil-vermicompost mixture (Gutierrez-Miceli et al., 2007). Results were alike those observed in Singh et al. (2010) study, highlighting that despite no significant increase in yield after the addition of vermicompost, the marketability of the fruit increased (Gutierrez-Miceli et al., 2007). A 1:1 soil-vermicompost mixture increased marketable yield by 1.8% as compared to control yield (Gutierrez-Miceli et al., 2007).

Bahrampour & Ziveh (2013) observed similar yet contrasting results while studying the effects of vermicompost in tomato fruit. Results revealed that the highest concentration of vermicompost in soil significantly increased growth and yield compared to control plants. Tomato plants treated with vermicompost had higher leaf area, shoot dry weight, and total yield (Bahrampour & Ziveh, 2013). Additionally, fruit weight and elemental leaf content of the plant was also significantly increased.

2.3.7.2 Effects on Pest and Disease Suppression

Vermicompost is known to suppress pest populations and pathogen infection in tomato plants. Various studies such as Sedaghabaf et al. (2017), Szczech (1998), and Mohamadi et al. (2016) showcase enhanced plant defenses after vermicompost treatment. For instance, Sedaghatbaf et al. (2017) tested the effects of four types of vermicompost on whitefly populations in tomatoes. Sedaghatbaf et al. (2017) observed that all vermicompost treatments had a significantly lower number of whiteflies established compared to plants treated with conventional fertilizer. Pistachio waste derived vermicompost treatment outperformed all other treatments (Sedaghatbaf et al., 2017). Enhanced pest resistance can be correlated with an increase in total phenolic compounds (Sedaghatbaf et al., 2017). A bioassay on the tomato leaves revealed that pistachio waste vermicompost contained a greater amount of total phenolic compounds compared to other treatments (Sedaghatbaf et al., 2017). Phenolic compounds are known to play a major role in herbivory and pathogen defense (Bhattacharya et al. 2010). The addition of vermicompost could have elicited change in secondary metabolite production, resulting in enhanced pest resistance.

Increased pest and pathogen resistance can likely be attributed to a combination of physical and chemical changes to the plant's physiology. Mohamadi et al. (2016) found that moths reared on plants treated with 40% vermicompost and 2g/kg of humic fertilizer had lower fecundity and shorter lifespans as compared to plants fertilized with inorganic agrochemicals. The combination of vermicompost and humic fertilizer also had higher biomass compared to the other treatments (Mohamadi et al., 2016). This result suggests that pest invasion might be tied to plant nutrition (Mohamadi et al., 2016). Organic amendments such as vermicompost provide a more balanced source of nutrition for plant growth which might deter pest invasion in tomato plants (Mohamadi et al., 2016).

Different external factors such as the presence of beneficial microorganisms can also contribute to pathogen and pest suppression. High microbiological activity in vermicompost is a major contributing factor in pathogen resistance. Szczech (1998) observed that the addition of vermicompost in potting media inhibited *Fusarium oxysporum f.* sp. infection in tomato plants. Treatments above 30% entirely inhibited infection (Szczech, 1998). Moreover, vermicompost also strongly inhibited the growth of *F. oxysporum* in agar medium (Szczech, 1998). Hyphae treated with vermicompost were destroyed and colonized by microbes (Szczech, 1998). *Phytophthora infestans* or Late-Blight, another fungal pathogen, is also inhibited by the presence of vermicompost. Zaller (2006) reported that plants treated with vermicompost foliar spray had significantly lower incidence of late-blight compared to control treatments which are also accredited to the presence of beneficial microbes producing antagonistic compounds (Zaller, 2006).

3. METHODOLOGY

3.1 Site Description

The study was conducted at Florida International University's (FIU) Organic Garden in Miami, Florida. The one-acre farm is home to student/faculty-run projects, including a food forest, ornamental and vegetable beds, as well as other experimental plots. No synthetic pesticides are used. Organic fertilizers such as fish emulsion and chicken manure are applied throughout the garden. The garden is considered transitioning and does not yet have proper organic certification. (SW 17th St, Miami, FL 33174 Coordinates: 25.754169, -80.345069).

3.2 Experimental Design

A potted study was conducted at an enclosed field at FIU's Organic Garden. Within the enclosed field, two hoop houses (Dimensions: 26ft x 10ft) were installed (Figure 1). Planting began on March 20, 2019 and ended July 2, 2019, which equals to 104 days of growth. Four tomato seeds of BHN589 variety were sowed into 430 five-gallon pots (Dimensions: 10.25" L x 11.8" W) which were filled with 70% sandy loam acquired from the FIU Organic Garden and 30% Sungro Professional Potting Metro-Mix® 830 potting soil. A month after planting, seedlings were thinned and assigned a treatment/ID number. Subsequently, pots were grouped by treatment and arranged using a randomized block design. The treatments used within the experiment were three different vermicompost tea treatments ranging from 1:20 (T5%), 1:15 (T10%), and 1:5 (T20%) ratios of vermicompost and water, and a control group. To avoid bias, all plants were treated with 100ml of Miracle-Gro® Water Soluble Tomato Plant Food fertilizer

(18-18-21 NPK) every two weeks. Preparation and application of vermicompost tea treatments occurred every week. Treatments were applied through soil and leaf drench.

	1	2	3	4	5	6	7	8	9	10
1	1	2	3	4		232	233	234	235	236
2	5	6	7	8		237	239	240	241	242
3	348	349	350	351		116	117	118	119	120
4	352	353	354	355		121	122	123	124	125
5	243	244	245	246		9	10	11	12	13
6	247	248	249	250		14	15	16	17	18
7	126	127	128	129		356	357	358	359	360
08	130	131	132	133		361	362	363	364	365
9	19	20	21	22		251	252	253	254	255
10	23	24	25	26		256	257	258	259	260
11	366	367	368	369		134	135	136	137	138
12	370	371	372	373		139	140	141	142	143
13	261	262	263	264		27	28	29	30	31
14	265	266	267	268		32	33	34	35	36
15	144	145	146	147		374	375	376	377	378
16	148	149	150	151		379	380	381	382	383
17	37	38	39	40		269	270	271	272	273
18	41	42	43	44		274	275	276	277	278
19	384	385	386	387		152	153	154	155	156
20	388	389	390	391		157	159	160	161	162
21	279	280	281	282		45	46	47	48	49
22	283	284	285	286		50	51	52	53	54
23	163	164	165	166		392	393	394	395	395
24	167	168	169	170		397	398	399	400	401
25			55	56		287				
26			402	403		171				

Plant Quantity: 225
Plants per Treatment: 56
Treatment Legend:
- Control- Orange
- T5%- Red
- T10%- Blue
- T20%- Pink

a

b

c

Figure 1 Arrangement of Experimental Plots. a) Pots arranged inside hoop house used for germination stages of the plant. b) Hoop house construction. c) Example of how treatments were arranged within the infrastructure.

3.2.1 Preparation of Vermicompost Tea

To prepare vermicompost tea, 100kg of organic SimpleGrow® Worm Castings was purchased. To brew the vermicompost tea, three EcoAir ™ Commercial Air pumps produced by EcoPlus (California, USA) with a maximum capacity to pump 94L and 10-gallon plastics bins were used. Vermicompost was homogenized to ensure even distribution of nutrients and other beneficial compounds. To homogenize the vermicompost, all purchased vermicompost was mixed before tea preparation. Vermicompost tea production followed Edwards et al. (2010) brewing methods. Varying

amounts of vermicompost were placed in a canvas sheet and immersed in water (Table 3). The mixture was aerated for 24hrs at FIU's Soils Lab in a shaded and temperate environment. Once brewing time elapsed, the mixture was transported to research plots and dispersed. Vermicompost and fertilizer treatments began four weeks after planting and was repeated every week for the duration of the experiment.

Table 3 Proportion of Ingredients Used for Vermicompost Tea Production

Treatment	Ratio (Vermicompost: Water)	Water (L)	Vermicompost (kg)
Control	N/A	N/A	N/A
T5%	1:20	22.7	1.08
T10%	1:10	22.7	2.17
T20%	1:5	22.7	4.35

3.2.2 Field Sampling and Data Collection Methods

3.2.2.1 Leaf Chlorophyll Concentration

To monitor and compare plant health across all treatments, the average leaf chlorophyll concentration of each plant was measured using the Soil-Plant Analyses Development (SPAD) 502 Plus Chlorophyll Meter every month, for a total of three readings. Twenty randomly selected plants from each treatment were sampled following Freidenreich et al. (2019)'s sampling protocol. Each leaf selected was sampled three different times. The concentration values were averaged for each leaf measured. A SPAD-502-meter measures the transmittance of red and infrared light through the leaf, calculating a value that is relative to the concentration of chlorophyll present in the sample (Uddling et al., 2007). Chlorophyll concentrations are generally a good indicator for plant health (Freidenreich et al.,2019). Low SPAD values are an indication of

yellowing or chlorosis in leaves, which are symptoms associated with nutrient deficiency and a multitude of diseases in plants (Freidenreich et al.,2019).

3.2.2.2 Soil pH, Yield and Plant Height

Soil pH measurements were taken during the first and last week of the experiment, utilizing a Fieldscout pH 400-meter probe. Twenty plants were randomly selected at the beginning and end of the experiment. pH values were averaged and analyzed across all treatments. Weeks prior to termination, ripe tomatoes were counted, harvested and weighed using a digital kitchen scale. Harvesting occurred five different times during the month of June and July to ensure tomatoes were collected while they were ripening. Lastly, plant height was measured at termination of the experiment. Twenty randomly selected plants from each treatment were measured following Nagashima and Hikosaka (2011) height measuring protocols. To guarantee accurate results, tomatoes were measured from the lowest stem node to the uppermost shoot.

3.2.2.3 Above and Below Ground Dry Biomass

Following termination (104 DAP), twenty randomly selected samples of shoot and root mass per treatment were collected. Shoots samples collected were composed of stem and leaf biomass and were harvested from the first node up. Roots were carefully washed, ensuring all soil particles and debris was removed from the biomass. After sample collection, shoot and root samples were dried at 70 °C for 72hrs in a Thermo Scientific Precision drying oven. Once samples were completely dried, they were weighed to the second decimal point of a gram.

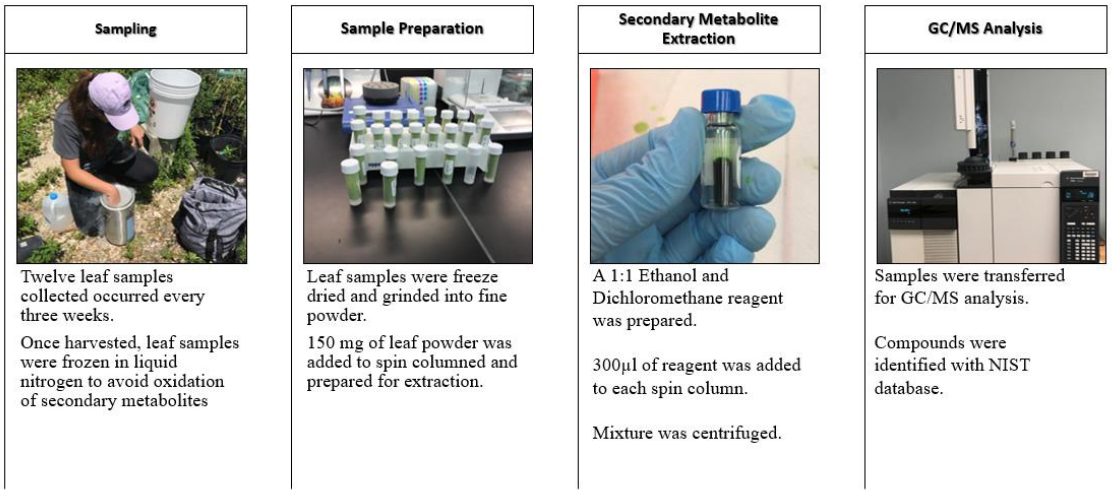

Figure 2 Sample Preparation for GC/MS Analysis

3.2.2.4 Biomass Sampling for Secondary Metabolite Profiling

To conduct secondary metabolite profiling via GC/MS analysis and Total Phenolics Bioassay, leaf samples were collected every three weeks. Prior to sampling, twelve plants from each treatment were randomly selected. Following Dong et al. (2011) biomass collection method for secondary metabolite testing, three leaf samples were randomly collected from the upper, middle, and lower part of the crop. Immediately after harvesting, biomass samples were placed in paper tea bags and frozen in liquid nitrogen for 10 seconds. Subsequently, samples were placed in dried ice and were stored in a -80 ◦C freezer awaiting chemical testing.

3.3 Secondary Metabolite Profiling

3.3.1 Sample Preparation for GC/MS Analysis

Previously collected leaf samples were sorted and freeze-dried at -65ºC. After freeze drying, samples were homogenized and ground into a fine powder using a Fischer

Scientific ™ Bead Mill 24. All samples were grinded for 1 minute at a speed of 5,000 rpm. After samples were ground, 150 mg (+/- 1) was subsampled for analysis. To accurately subsample the ground powder, an analytical balance was used. Subsamples were placed in a spin column to complete extraction. This protocol was developed and modified by Dr. Diego Salazar- Amoretti.

3.3.2 Secondary Metabolite Extraction

Following Lisec et al. (2006) secondary metabolite extraction protocols, a 250 ml reagent with an Ethanol and Dichloromethane solved 1:1 mixture was made. To ensure pipetting and GC/MS injection errors could be detected and normalized throughout all chemical analysis, 45mg Piperine and 100µl Citronelle were added as internal standards to the mixture. Before adding the mixture, spin columns containing the samples were attached to glass vial insert and placed inside a microcentrifuge tube. Once the inserts were assembled, two aliquots of 150µl of solvent were added to each sample. Prepared microcentrifuge vials were then placed in a Sorvall Legend Micro 21 Centrifuge. Samples were centrifuged for four minutes at 5,000 rpm. Once the extraction process was completed, glass vial inserts were removed with tweezers from microcentrifuge vials and placed into 2 mL glass vials for GC/MS analysis. This secondary metabolite extraction protocol is only viable for acidic metabolites. For this reason, alkaline metabolites such as glucosinolates and alkaloids were not detected.

3.3.3 GC/MS Analysis

The GC/MS analysis of various organic extracts of *Solanum lycopersicum* leaves was performed on an Aligient Technology 5977A GC/MSD system fitted with a glass

column (30 m x .25 mm x .25 µm, maximum temperature, 350º C). Ultra-high purity helium (99.9%) was used as a carrier gas at a constant flow rate of 1.2 mL/min. Injector was programed to uptake 3 µL of each sample with a split ratio of 1:1. Before and after injecting the sample for analysis, the needle was washed with Hexane and 99.9 % Ethanol. The initial oven temperature was set to 65º C to 250º C at a rate of 15º C/min. All data were obtained by collecting the full-scan mass-spectra and evaluating/ identifying peaks of high proportion (peak area above $5.4x10^7$). The peak area is presented as a percentage normalized using citronellol internal standard which was calculated by dividing the relative area of each compound by the citronellol's area. Internal standards were added to account for pipetting or injection errors and to ensure that data obtain could be comparable to each other.

Identification and characterization of chemical compounds detected was based on GC retention time and m/z (mass to charge ratio). To identify the compounds, GC/MS spectra was analyzed in Openchrom through peak identification and integration capabilities. Subsequently, AMDIS and NIST (National Institute of Standards and Technology) database was used to identify individual compounds.

3.3.4 Total Phenolics Bioassay

Grinded samples were subsampled for 100 mg (+/- 1) and prepared for extraction. Extraction was performed using 1 ml of 70% Methanol solvent and 2 ceramic beads. Mixture was homogenized in a Fischer Scientific ™ Bead Mill 24. Samples were centrifuged in Sorvall Legend Micro 21 Centrifuge for 5 minutes at 14,800 rpm to separate cell debris and supernatant. Subsequently, 40 ml of the supernatant was transferred into 2.0 ml snap vials. A blank standard and a positive control were prepared.

The positive control added was Gallic acid. Gallic acid is a simple phenol found in most plants.

Once the extraction is ready, 750 ml of 10% Methanol is added to the supernatant. 100 ml is transferred into three separate 2.0 ml snap vials. This means that each sample will be tested three separate times. This is to ensure that no major errors were committed during the extraction and transfer process. Following transfer, 200 ml of 10% (v/v) Folin reagent and 1 ml of Na_2CO_3 (0.7M) were added to the samples. The final mixture was placed in an IKA Trayster for 5 minutes to be homogenized. Additionally, the samples were placed in a 30º C dry bath for 15 minutes. Samples are transferred one last time into cuvettes. Finally, the supernatant was analyzed using a Thermo Scientific Genesys 30 Visible Spectrophotometer. Total phenolics were quantified using a gallic acid equivalent. The light absorbency value provided by the spectrophotometer for each sample was plugged into the regression formula below. The final value for total phenolics is expressed in grams of Gallic acid equivalent. The regression formula was developed using Gallic acid serial dilutions tested in the spectrophotometer.

$$\textbf{Gallic Acid Equivalent } (\mathbf{g}) = \mathbf{70.80994\,x - 1.7056}$$

Equation 1 Regression Formula for Gallic Acid Equivalent Values

3.4 Statistical Analysis

Data analysis was performed utilizing R version 3.5.3 (Great Truth) and JPM14. One-way and two-way ANOVAs were performed to test for significance across treatment type and time. To determine which treatment groups were different from each other, a TukeyHSD test was also conducted. $P \leq 0.05$ was considered statistically significant.

4. RESULTS AND DISCUSSION

4.1 Physical Results

4.1.1 Above and Below Ground Biomass, Stem Height, Leaf Chlorophyll Concentration, and Soil pH

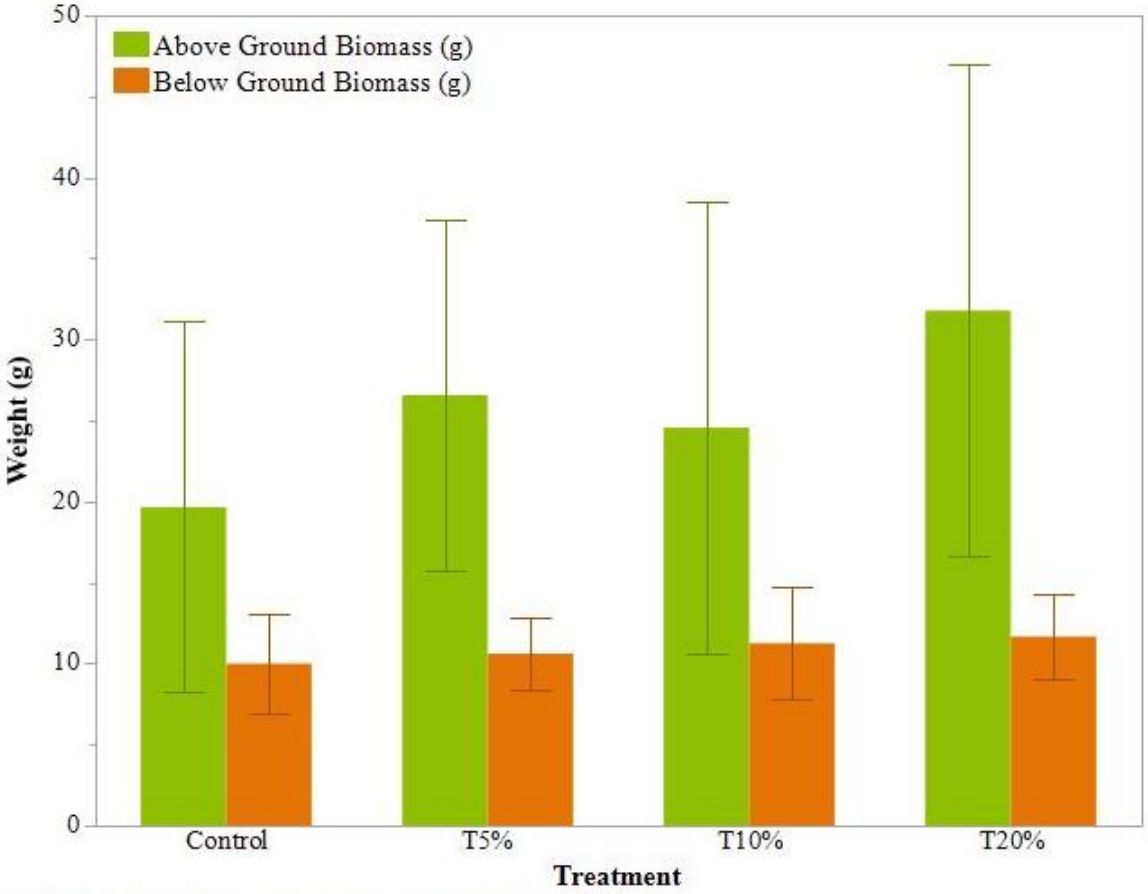

Figure 3 Bar graph depicting differences in above and below ground biomass (g) across treatment type. Treatments consisted of various vermicompost treatment types (T5% =1:20, T10%= 1:10, and T20%= 1:5). Values are expressed as mean and each treatment consisted of n=20. Error bars are one standard deviation from the mean.

Plant growth response to varying vermicompost tea treatments was evaluated by monitoring and measuring physical parameters such as dry above and below ground biomass (Figure 3), stem height and leaf chlorophyll concentrations. During termination, stem height was measured. The greatest mean heights were observed in T20% treated

plants (82.83 ± 19.9 cm), followed by T5% (78.02 ± 20.6 cm), T10% (74.02 ± 21.3 cm), and Control (64.53 ± 25.8 cm). None of the treatments were significantly different to each other ($p > .05$). Although differences were not significant, T20% and Control were the most different with a $p = .0505$.

After termination, all above and below ground biomass samples were harvested and dried. The highest mean dry above ground biomass observed was T20% (31.78 ± 14.5g), followed by T5% (26.57 ± 10.7 g), T10% (24.56 ± 13.9 g), and Control (19.62 ± 11.4 g) (Figure 3). A One-way ANOVA determined that above ground weight was significantly different throughout the treatments ($p < 0.05$). A TukeyHSD test was conducted to assess differences between the treatments. T20% treatment was significantly higher ($p \leq 0.05$) than Control treatment. Although the remaining vermicompost tea treatments had greater mean dry above ground biomass as compared to the Control, the treatments were not significantly different from each other. Therefore, T5% and T10% did not have any noticeable effects on above ground biomass weight. The greatest dry below ground biomass was T20% (11.58 ± 2.6 g), then T10% (11.25 ± 3.4 g), T5% (10.61 ± 2.2 g), and Control (10.0 ± 3 g). A one-way ANOVA determined that there was no significance between treatments ($p.> 0.05$). Vermicompost tea treatments had no significant effect on below ground biomass.

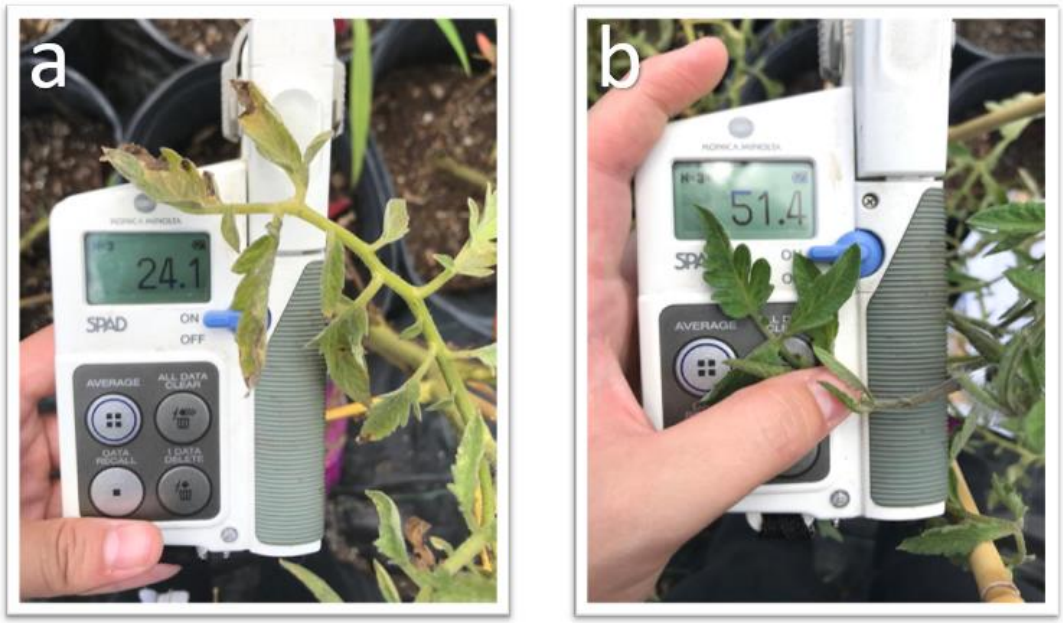

Figure 4 Example of SPAD readings taken during the experiment. a) Low SPAD reading taken on tomato plant experiencing chlorosis. b) Moderate-high reading taken from healthy tomato plant.

To determine the effect of vermicompost tea treatments on leaf chlorophyll concentration, Soil Plant Analytical Development (SPAD) readings were taken monthly (Figure 4). SPAD values varied slightly overtime. From April (1D) to May (2D), all treatments experienced higher SPAD values. SPAD values once again decreased in June (3D) for all treatments. The highest mean SPAD value was T20% (46.52 ± 9.9), subsequently T5% (44.52 ± 8.4), T10% (44.03 ± 12), and Control (41.93 ± 9.6). Statistical analysis revealed that there was no significant different between any of the treatments and collection dates. Chlorophyll concentrations were unaffected by vermicompost tea treatments.

Finally, soil pH was measured before cultivation, and after termination of tomato plants. Soil pH readings were taken to determine if a change in acidity might enhance growth. Initial pH for all treatments was slightly alkaline soil (pH 7.24). At termination, the mean lowest soil pH was T20% (pH 6.75 ± 0.3), followed by T5% (pH 7.03 ± 0 .4), T10% (pH 7.20 ± 0.2) and Control (pH 7.19 ±). A One-way ANOVA revealed that the treatments are significantly different ($p \leq 0.05$). To determine how the treatments differed from each other a TukeyHSD test was conducted. Results indicate that soil pH in T20% treated pots was significantly lower ($p \leq 0.05$) than T10% and Control treatments. T20% treatment had a significant effect on soil pH.

Figure 5 Tomatoes. A) Healthy tomato during early growth stage. b) Tomatoes damaged due to inclement weather.

4.1.2 Fruit Weight and Yield

Tomato fruits were harvested multiple times during the month of June. The harvesting process was affected by continuous inclement weather, damaging and deforming tomato fruits (Figure 5). To assess yield, only marketable tomatoes were

counted and weighed. Total yield produced was 302 tomatoes. The greatest yield was produced by plants treated with T20% vermicompost tea (89), closely followed by T10% (88), then T5% (70) and Control (55). After harvest, fruits were weighed in scale. The largest fruits were produced under T10% (76.86 g), followed by T20% (68.33 g), Control (64.11 g) and T5% (55.17 g). Each treatment presented high variability around the mean. There was no significant difference found among the treatments ($p > 0.05$).

4.1.3 Mortality

Mortality rate was assessed during termination. Only plants with >70% dried and discolored leaves/ stem were considered. Overall, 19.76% of tomato plants perished. The highest mortality rate was quantified by dividing individual deaths per treatment and total individuals per treatment. The highest mortality rate was experienced under treatment T5% (23.4%), followed by Control (20.5%) and T20% (20.5%), and T10% (14.9%). Mortality rate was significantly affected by leaf harvesting, 65% of all sampled seedlings perished.

4.1.4 The Effect of Vermicompost Tea Treatments on *Solanum lycopersicum*

Plants treated with T20% vermicompost tea had greater above ground biomass, SPAD values, yield, and lower pH. Physical parameters indicated that the highest concentration of vermicompost tea did affect the growth and development of the plant. Results with physical parameters are consistent with previous findings. For instance, Singh et al. (2010) and Bahrampour & Ziveh (2013) reported that vermicompost treatments had a significant effect on plant height and biomass. Although there was no significant difference in plant height and below ground biomass, T20% treated plants did

have the greatest total biomass and height compared to any other treatment. Plant height and biomass was lowest in Control treated plants. All plants were fertilized with equal amounts of fertilizer biweekly, which indicates that vermicompost tea treatments, specifically T20% enhanced developmental parameters in BHN589 tomato plants. Enhanced growth might also be attributed to pH of the soil. Tomato cultivation requires a slightly acidic soil pH. T20% had a significant lower soil pH than Control treated plant which would have also contributed to increased growth.

SPAD values indicating chlorophyll values was also highest in T20%. SPAD-503- meter measures the transmittance of red and infrared light through leaf tissues, calculating relative concentration of chlorophyll for the plant (Udding et al., 2007). Chlorophyll concentrations are an indicator for plant health. Low chlorophyll concentrations could indicate nutrient deficiency or a pathogen infection through symptoms of chlorosis (Freidenreich et al., 2019). The lowest chlorophyll concentrations were found in Control treated plants, which might be an indicator for dwindling plant health.

Results for fruit yield and weight (g) were affected by inclement weather during the harvesting period. For this reason, fruits that were viable and marketable were only considered. Tomato counts were highest in T20% treated plants, and lowest throughout the Control group. Despite differences, there was no significant difference across treatments. Guitirrez-Miceli et al. (2007) and Singh et al. (2010) both reported no significant difference in yield across all vermicompost treatments. However, both studies reported a significant increase in fruit weights in tomato plants treated with vermicompost. Contrasting results were found in this study. The highest fruit weights

were observed in T10% treated plants, and lowest in T5% treated plants. There was no significant difference between any treatment. Differences among results may be attributed to vermicompost tea components. Singh et al. (2010) credited the increase in fruit weights to production or accumulation of naphthalene acetic acid, an auxin hormone, which plays a crucial role in flowering and fruit setting.

Mortality rate across treatment was affected by sampling. Due to weight and size of leaf biomass, multiple sampled had to be harvested from each plant. Decreasing photosynthetic area and wounding stem could have facilitated sunlight deficiency or pathogenic infection.

4.2 Chemical Profiling Results

To determine chemical change across treatment and time, 64 tomato leaf samples were tested using GC/MS analysis. Fifty-eight compounds were detected and identified using the NIST database and sixteen compounds were selected to be further analyzed. Compounds found were classified by their secondary metabolite type: phenolic compounds, terpenoids, fatty acids and alkanes (Table 4). Furthermore, leaf samples were also analyzed using a Total Phenolics bioassay through a spectrophotometer.

Table 4 List of Secondary Metabolites Detected by GC/MS Analysis

Secondary Metabolite Type	*Compound Detected*
Phenolic Compounds	Eugenol
	α -Tocophenol
	Phenol, 4-ethenyl-2,6-dimethoxy-
	Triphenyl phosphate
Terpenoids	
Monoterpene	β-Phellandrene
Diterpene	Phytol
Triterpenes/ Sterols	γ-Sitosterol
	Stigmasterol
	Campesterol
	Lupeol
	β-Amyrin
	Stigmasta-5,24(28)-dien-3-ol, (3.beta.,24Z)-
	Squalene
Steroid Precursors	9,19-Cyclolanost-24-en-3-ol, (3β)-
	9,19-Cyclolanostane-3,7-diol
	9,19-Cyclolanost-24-en-3-ol, acetate, (3β)-
Tetraterpenes/ Carotenoids	Lycopersene
	Rhodopin
Alkanes/Paraffins	Dotriaconte
	Hentriaconte
	Heptacosane
	Nonacosane
	Tritriaconte
Fatty Acids	Linolic acid
	Docosanoic acid
	Octadecanoic acid
	Trans-3- Hexenoic acid
	Hexadecanoic acid
	Dimethylaminoethyl palmitate
Glycosides	Stevioside
Other	Cyclononasiloxane, octadecamethyl-
	Ethyl iso-allocholate
	Ergosta-5,24(28)-dien-3-ol, (3β)-
	Lup-20(29)-ene-3,21,28-triol, 28-acetate, (3β,21β)-
	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate
	Octadecane, 3-ethyl-5-(2-ethylbutyl)-
	Unknown
	Unknown
	2-Methyltriacontane
	6a,14a-Methanopicene, perhydro-1,2,4a,6b,9,9,12a-heptamethyl-10-hydroxy-

Table 5 Relative Proportion for Secondary Metabolites Chosen for Analysis

Compound Detected	*Treatment*			
	Control	T5%	T10%	T20%
Phenolic Compounds				
a- Tocopherol	$3.2964 \pm 1.15_{a}$	$3.0245 \pm 0.89_{a}$	$3.0351 \pm 0.86_{a}$	$2.460 \pm 0.06_{a}$
Eugenol	$0.0261 \pm 0.05_{a}$	$0.0169 \pm 0.04_{a}$	$0.0298 \pm 0.05_{a}$	$0.0237 \pm 0.04_{a}$
Phenol, 4-ethenyl-2,6-dimethoxy-	$0.0207 \pm 0.05_{a}$	$0.0362 \pm 0.08_{a}$	$0.0358 \pm 0.06_{a}$	$0.0105 \pm 0.04_{a}$
Terpenoids				
Monoterpene				
β-Phellandrene	$0.0955 \pm 0.14_{a}$	$0.1820 \pm 0.16_{a}$	$0.2281 \pm 0.19_{a}$	$0.3024 \pm 0.19_{b}$
Diterpene				
Phytol	$0.3005 \pm 0.13a$	$0.2896 \pm 0.11_{a}$	$0.2139 \pm 0.06_{ab}$	$0.2066 \pm 0.06_{b}$
Sterols				
Stigmasterol	$0.8648 \pm 0.24_{a}$	$0.7781 \pm 0.21_{a}$	$0.7226 \pm 0.22_{a}$	$0.7595 \pm 0.19_{a}$
γ-Sitosterol	$0.3608 \pm 0.10_{a}$	$0.3398 \pm 0.09_{a}$	$0.3324 \pm 0.07_{a}$	$0.3419 \pm 0.07_{a}$
Lupeol	$0.2633 \pm 0.22_{a}$	$0.2540 \pm 0.21_{a}$	$0.2214 \pm 0.17_{a}$	$0.1976 \pm 0.13_{a}$
β-Amyrin	$0.6319 \pm 0.39_{a}$	0.6359 ± 0.33^{a}	$0.5876 \pm 0.27_{a}$	$0.4987 \pm 0.22_{a}$
Carotenoids				
Lycopersene	$0.1328 \pm 0.09_{a}$	$0.1820 \pm 0.09_{a}$	$0.1817 \pm 0.06_{a}$	$0.2066 \pm 0.11_{a}$
Fatty Acids				
Hexadecanoid acid	$0.8107 \pm 0.38_{a}$	$0.7723 \pm 0.26_{a}$	$0.6942 \pm 0.28_{a}$	$0.6661 \pm 0.22_{a}$
Linolenic acid	$0.1845 \pm 0.25_{a}$	$0.2384 \pm 0.24_{a}$	$0.1463 \pm 0.16_{a}$	$0.1872 \pm 0.18_{a}$
Octadecanoic Acid	$0.4007 \pm 0.32_{a}$	$0.4958 \pm 0.24_{a}$	$0.3362 \pm 0.17_{ab}$	$0.1722 \pm 0.03_{b}$
Alkanes				
Hentriacontane	$0.3695 \pm 0.25_{a}$	$0.3626 \pm 0.17_{a}$	$0.4254 \pm 0.21_{a}$	$0.4160 \pm 0.13_{a}$
Nonacosane	$0.4104 \pm 0.10_{a}$	$0.4305 \pm 0.09_{a}$	$0.3864 \pm 0.09_{a}$	$0.8375 \pm 0.73_{b}$
Dotriacontane	$0.2692 \pm 0.17_{a}$	$0.2105 \pm 0.10_{a}$	$0.2243 \pm 0.13_{a}$	$0.2118 \pm 0.09_{a}$

Compounds with high frequency and relative proportions were chosen for further analysis. Treatments consisted of varying vermicompost tea ratios (T5%= 1:20, T10%= 1:10, and T20%= 1:5). Values were expressed as mean ± Standard Deviation. Each treatment consisted of n = 16. One-way ANOVAs were performed on the Relative Proportion of compounds detected across time and treatment. Means within a column followed by the same letter are not significantly different at $p \leq .0500$. Significantly different treatments were highlighted by adding a letter to mean relative proportion values.

4.2.1 Phenolic Compounds

Table 6 Mean Relative Proportions of Phenolic Compounds Detected through GC/MS Analysis

	Harvesting Period			
Compound *Treatment Type*	1H	2H	3H	4H
a- Tocopherol				
Control	2.207 ± 0.15	3.896 ± 1.45	3.3670 ± 1.28	3.715 ± 0.76
T5%	1.946 ± 0.24	2.868 ± 0.10	3.831 ± 0.80	3.453 ± 0.77
T10%	2.129 ± 0.17	2.526 ± 0.35	3.668 ± 0.52	3.816 ± 0.74
T20%	1.718 ± 0.13	2.382 ± 0.62	3.118 ± 0.66	2.787 ± 0.32
Eugenol				
Control	0.027 ± 0.05	n/d	0.769 ± 0.09	n/d
T5%	n/d	n/d	0.039 ± 0.07	0.029 ± 0.06
T10%	n/d	n/d	0.119 ± 0.01	n/d
T20%	n/d	n/d	0.086 ± 0.074	0.024 ± 0.33
Phenol, 4-ethenyl-2,6-dimethoxy-				
Control	n/d	n/d	0.083 ± 0.10	n/d
T5%	n/d	n/d	0.145 ± 0.11	n/d
T10%	n/d	n/d	0.143 ± 0.04	n/d
T20%	n/d	n/d	0.053 ± 0.09	n/d

Treatments consisted of varying vermicompost tea ratios (T5%= 1:20, T10%= 1:10, and T20%= 1:5). Harvesting periods are mean to represent sampling time. Each harvesting was completed every three weeks after treatments began. Values were expressed as mean ± Standard Deviation. Each treatment consisted of n = 16. One-way ANOVAs were performed on the Relative Proportion of compounds detected across time and treatment. Means within a column followed by the same letter are not significantly different at $p \leq .0500$. Significantly different treatments were highlighted by adding a letter to mean relative proportion values. Compounds that were not detected were denoted as "n.d".

Phenolic compounds comprised a large proportion of BHN589 tomato leaves. Specifically, a-Tocopherol or Vitamin E, which had the highest relative proportion out of all other detected compounds. Other phenolic compounds such as Eugenol and Phenol, 4-ethenyl-2,6-dimethoxy- were also detected, but at substantially lower quantities (Table 5; Table 6). Relative proportions for a-Tocopherol were greatest in Control group. Relative proportion in Eugenol was highest in T10% treated plants. Relative proportion for

Phenol, 4-ethenyl-2,6-dimethoxy- was highest in T5% treatment. Although none of the treatments were significantly different from each other ($p > 0.05$), concentrations for all phenolic compounds varied over time.

The relative proportion of all phenolic compounds was the highest during harvesting period 3H. For example, the mean relative proportion of a-Tocopherol nearly doubled from harvesting period 2H to 3H (2.612 to 3.831 mean relative proportion; Figure). T10% and T20% followed an identical trend, experiencing large increases in the mean relative proportions during harvesting period 3H. Similarly, Eugenol and Phenol, 4-ethenyl-2,6-dimethoxy- also saw significant ($p \leq 0.05$) increases in all treatments during harvest period 3H. For instance, the mean relative proportion of Eugenol during harvesting period 3H was 4 (.1190) and 3.6 (0.0863) (Table 6) times greater than the mean relative proportion in T10% and T20% treated plants respectively (Table 6). Moreover, Phenol, 4-ethenyl-2,6-dimethoxy- was only detected during harvesting period 3H. Relative proportion values for Phenol, 4-ethenyl-2,6-dimethoxy- was highest in T5% and T10% treated plants.

4.2.1.1 Total Phenolics Bioassay

Table 7 Concentration of Total Phenolics (g) in Tomatoes Treated with Vermicompost Tea

	Harvesting Period				
Treatment	1H	2H	3H	4H	Average
Control	37.523 ± 4.3	31.817 ± 2.5	43.264 ± 3.6	32.389 ± 6.7	$36.248 \pm 8.2_{a}$
T5%	44.680 ± 9	36.048 ± 4.8	52.971 ± 13.3	40.090 ± 7.4	$43.447 \pm 8.6_{ab}$
T10%	43.075 ± 8.9	46.392 ± 7.1	59.314 ± 7.8	43.046 ± 3.5	$47.957 \pm 10.3_{b}$
T20%	42.249 ± 6.6	33.481 ± 8.9	54.116 ± 6.8	17.395 ± 2.4	$36.810 \pm 14.6_{a}$

Total Phenolics are expressed in Gallic acid equivalent (g). Treatments consisted of varying vermicompost tea ratios (T5%= 1:20, T10%= 1:10, and T20%= 1:5). Values were expressed as mean total phenolics concentrations ± standard deviation. Each treatment consisted of n = 16. One-way and two-way ANOVAs were performed on the total phenolics concentration values across time and treatment. Means within a column followed by the same letter are not significantly different at $p \leq .0500$. Significantly different treatments were highlighted by adding a letter to mean total phenolics concentration values.

High concentrations of phenolic compounds were detected in tomato leaves across all treatments. Total concentration of phenolic compounds was significantly different across time and treatment type. The greatest concentration of phenolic compounds was detected during the third harvesting period, 3H (Table 7). During this harvesting period, phenolic compound concentrations significantly increased ($p \leq 0.05$) in all treatments. The greatest increase was experienced by T20% treated plants. This sharp increase in the concentration of phenolic compounds coincided with tomato ripening and fruit harvest. Total phenolic concentration also significantly differed across treatment type (Table 7). T10% treated plants had a significantly higher ($p \leq 0.05$) concentration of phenolic compounds compared to the remaining vermicompost tea treatments and control group. The lowest phenolic compound concentration was observed in the Control group.

4.1.2 Terpenoids

Eight terpenoids were detected and identified in BHN589 tomato plants. Terpenoids identified were highly diverse and included a multitude of functional isoprenoid groups such as monoterpenes, diterpenes, triterpenes (sterols), and tetraterpenes (carotenoids). Stigmasterol, a sterol, had the highest relative proportion of all the terpenes registered. Overall, there is a significant difference ($p \leq 0.05$) between the relative proportion in treatments T20% and Control (Figure 6, Table and Table). Significant differences between both groups might be attributed to inverse concentrations of four different terpenes. Control group plants had high mean relative proportion values for Phytol, Lupeol and β-Amyrin, and low relative proportion values for β-Phellandrene and Lycopersene. An inverse of this pattern was observed in T20% treated plants. An example of this inverse pattern can be observed in Figure 6.

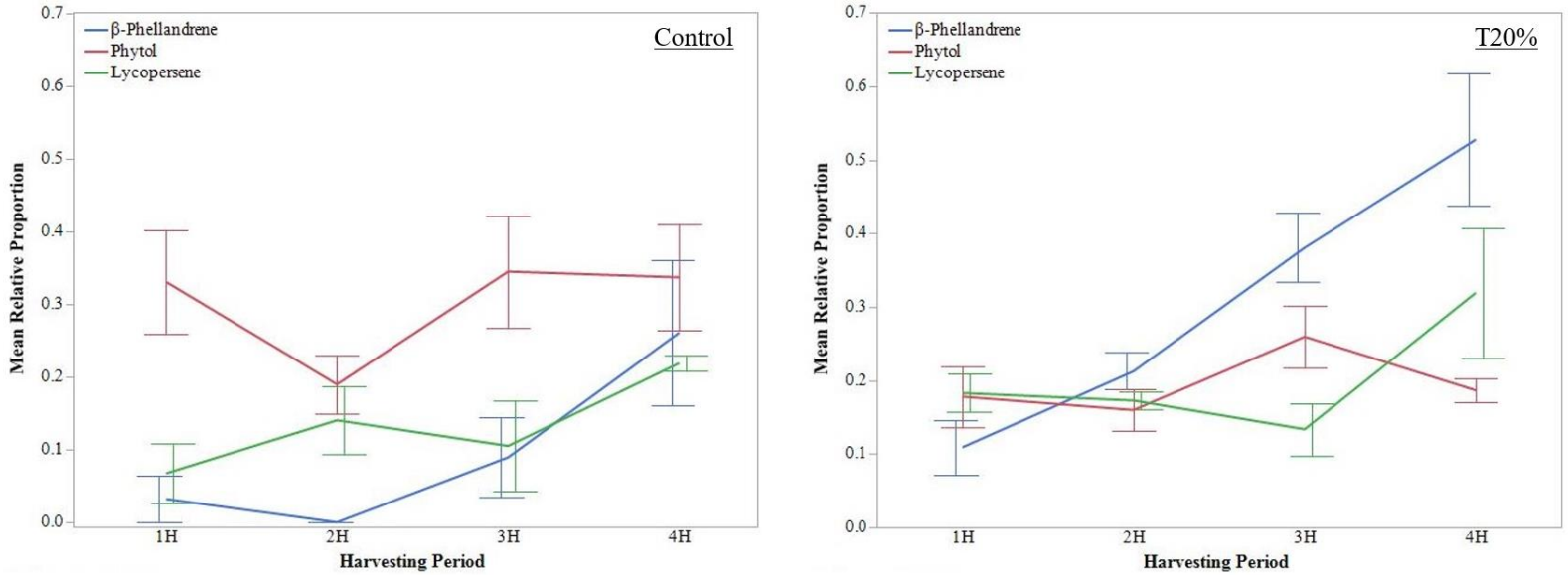

Figure 6 Line Graph of Diterpenes, Monoterpenes and Carotenoids depicting a) Mean relative proportion over time and b) Inverse pattern between β-Phellandrene, Phytol and Lycopersene concentration in Control and T20% treated plants. Treatments consisted of varying vermicompost tea ratios (T5%= 1:20, T10%= 1:10, and T20%= 1:5). This graph only includes results for Control and T20% treated plants. Each color represents a different compound (blue= β-Phellandrene, red= Phytol, and green = Lycopersene). Values were expressed as mean ± Standard Deviation error bars. Each treatment consisted of n = 16. One-way ANOVAs were performed on the Relative Proportion of compounds detected across time and treatment. Means within a column followed by the same letter are not significantly different at $p \leq .0500$. Significantly different treatments were highlighted by adding a letter to mean relative proportion values.

4.1.2.1 Monoterpenes and Diterpenes

Table 8 Mean Relative Proportion of Monoterpenes, Diterpenes, and Carotenoids Detected through GC/MS Analysis Across Time and Treatment

	Harvesting Period			
Compound *Treatment Type*	1H	2H	3H	4H
β-Phellandrene				
Control	0.032 ± 0.06	n/d	0.089 ± 0.11	0.261 ± 0.20
T5%	0.071 ± 0.05	0.134 ± 0.16	0.139 ± 0.17	0.386 ± 0.07
T10%	0.056 ± 0.06	0.155 ± 0.20	0.206 ± 0.03	0.496 ± 0.09
T20%	0.109 ± 0.08	0.212 ± 0.05	0.380 ± 0.08	0.538 ± 0.18
Phytol				
Control	0.331 ± 0.14	0.189 ± 0.08	0.344 ± 0.15	0.337 ± 0.14
T5%	0.299 ± 0.10	0.157 ± 0.02	0.318 ± 0.12	0.385 ± 0.08
T10%	0.271 ± 0.07	0.202 ± 0.07	0.170 ± 0.05	0.170 ± 0.05
T20%	0.178 ± 0.08	0.159 ± 0.06	0.259 ± 0.07	0.184 ± 0.03
Lycopersene				
Control	0.067 ± 0.08	0.140 ± 0.09	0.105 ± 0.12	0.219 ± 00.02
T5%	0.096 ± 0.11	0.163 ± 0.03	0.195 ± 0.07	0.274 ± 0.04
T10%	0.174 ± 0.05	0.152 ± 0.12	0.172 ± 0.01	0.228 ± 0.02
T20%	0.183 ± 0.05	0.173 ± 0.03	0.134 ± 0.06	0.319 ± 0.18

Treatments consisted of varying vermicompost tea ratios (T5%= 1:20, T10%= 1:10, and T20%= 1:5). Values were expressed as mean ± Standard Deviation. Each treatment consisted of n = 16. One-way ANOVAs were performed on the Relative Proportion of compounds detected across time and treatment. Means within a column followed by the same letter are not significantly different at $p \leq .0500$. Significantly different treatments were highlighted by adding a letter to mean relative proportion values. Compounds that were not detected were denoted as "n.d".

β-Phellandrene and Phytol were the only monoterpene and diterpene respectively detected in BHN589 tomato plants (Table 8). The highest concentration of β-Phellandrene was observed in T20% treated plants (Figure 6 and Table 8), while the highest concentration of Phytol was recorded in the Control group (Figure 6 and Table 8). Mean relative proportions of both compounds varied over time and treatment type. Overall, all plants experienced an increase in β-Phellandrene across time. This positive trend could be observed across all treatment types. T20% treated plants had a

significantly higher ($p \leq 0.05$) proportion of β-Phellandrene as compared to the Control group and the remaining vermicompost tea treatments. Over the course of the experiment, mean relative proportion values for β-Phellandrene in T20% plants increased 4.8 times (from 0.109 to 0.538). The greatest accumulation of β-Phellandrene in T20% treated plants occurred during harvesting period 3H. Harvesting period 3H was characterized by tomato fruit ripening and harvesting. During this time, the plants allocated its resources to the production of monoterpenes. Relative proportions for Phytol were significantly different between T20%, and Control/T5% treatments. Relative proportions for Phytol were significantly lower ($p \leq 0.05$) in T20% as compared to Control and T5% treated plants (Table 5 and Table 8). Additionally, mean relative proportion values of Phytol decreased, as vermicompost ratios decreased.

4.1.2.2 Sterols

Table 9 Mean Relative Proportion of Sterols Detected through GC/MS Analysis across Time and Treatment

	Harvesting Period			
Compound *Treatment Type*	1H	2H	3H	4H
Stigmasterol				
Control	1.01 ± 0.39	0.759 ± 0.04	0.805 ± 0.28	0.885 ± 0.13
T5%	0.831 ± 0.31	0.883 ± 0.15	0.694 ± 0.23	0.704 ± 0.14
T10%	0.882 ± 0.09	0.509 ± 0.33	0.850 ± 0.11	0.649 ± 0.05
T20%	0.870 ± 0.08	0.834 ± 0.12	0.695 ± 0.38	0.676 ± 0.07
γ - Sisterol				
Control	0.476 ± 0.16	0.354 ± 0.05	0.299 ± 0.03	0.313 ± 0.04
T5%	0.396 ±0.14	0.405 ± 0.08	0.271 ± 0.45	0.288 ± 0.03
T10%	0.446 ± 0.04	0.304 ± 0.02	0.310 ± 0.05	0.270 ± 0.02
T20%	0.426 ± 0.06	0.357 ± 0.05	0.304 ± 0.04	0.301 ± 0.03
B-Amyrin				
Control	0.183 ± 0.12	1.038 ± 0.32	0.637 ± 0.41	0.668 ± 0.09
T5%	0.241 ± 0.06	0.974 ± 0.33	0.617 ± 0.18	0.712 ± 0.19
T10%	0.252 ± 0.07	0.850 ± 0.23	0.611 ± 0.21	0.638 ± 0.16
T20%	0.243 ± 0.02	0.789 ± 0.08	0.414 ± 0.16	0.541 ± 0.06
Lupeol				
Control	n.d	0.477 ± 0.14	0.309 ± 0.25	0.266 ± 0.10
T5%	0.026 ± 0.05	0.512 ± 0.15	0.326 ± 0.12	0.152 ± 0.10
T10%	0.066 ± 0.08	0.422 ± 0.17	0.264 ± 0.09	0.134 ± 0.10
T20%	0.054 ± 0.06	0.381 ± 0.04	0.193 ± 0.08	0.169 ± 0.04

Treatments consisted of varying vermicompost tea ratios (T5%= 1:20, T10%= 1:10, and T20%= 1:5). Values were expressed as mean ± Standard Deviation. Each treatment consisted of n = 16. One-way ANOVAs were performed on the Relative Proportion of compounds detected across time and treatment. Means within a column followed by the same letter are not significantly different at $p \leq .0500$. Significantly different treatments were highlighted by adding a letter to mean relative proportion values. Compounds that were not detected were denoted as "n.d".

Seven sterols and three isomers of a sterol precursor were identified and detected while testing BHN589 tomato plants. Only four compounds were chosen for an in-depth analysis given their frequency and relative proportion. Compounds chosen were

Stigmasterol, γ-Sitosterol, Lupeol and β-Amyrin. Overall, a significant ($p \leq 0.05$) downwards trend over time was observed for all sterols across all treatments (Table 9). Moreover, mean relative proportion values for sterols decreased as vermicompost concentration increased (Table 5 and Table 9). Finally, there was no significant difference in mean relative proportion values between any of the treatments. Despite a lack of significance, the same inverse pattern between Control and T20% treated plants was also observed. Mean relative proportions for Lupeol and β-Amyrin were highest in plants belonging to the Control group. Mean relative proportions for Lupeol and β-Amyrin were lowest in T20% treated plants.

4.1.2.3 Carotenoids

Only two carotenoids, Lycopersene and Rhodopin, were detected and identified in this study. Due to low frequency and relative proportion, Rhodopin was not considered. In general, concentrations for both compounds varied across time and treatment type. All treatments experienced a significant ($p \leq 0.05$) accumulation of Lycopersene over time. The greatest mean relative proportion for Lycopersene was observed in T20% treated plants (Table 5 and Table 8). T20% treated plants, experienced a sharp increase in Lycopersene concentration during harvest period 4H, doubling (from .134 to .319) in three weeks (Table 8 and Figure 6). Control, T5% and T10% treated plants also experience this increase at a lesser degree (Table 8 and Figure 6).

4.1.3 Fatty Acids

Table 10 Mean Relative Proportion of Fatty Acids Detected through GC/MS Analysis Across Time and Treatment type

Compound *Treatment Type*	***Harvesting Period*** 1H	2H	3H	4H
Hexadecanoic acid				
Control	1.107 ± 0.45	0.554 ± 0.34	1.028 ± 0.22	0.553 ± 0.10
T5%	0.896 ± 0.14	0.488 ± 0.06	1.01 ± 0.29	0.695 ± 0.16
T10%	1.049 ± 0.24	0.477 ± 0.08	0.805 ± 0.11	0.445 ± 0.54
T20%	0.824 ± 0.24	0.489 ± 0.07	0.827 ± 0.19	0.564 ± 0.11
Linolenic acid				
Control	0.399 ± 0.29	0.114 ± 0.22	0.225 ± 0.26	0.224 ± 0.81
T5%	0.188 ± 0.22	0.133 ± 0.09	0.412 ± 0.22	0.220 ± 0.44
T10%	0.995 ± 0.19	0.231 ± 0.10	0.254 ± 0.18	n/d
T20%	0.207 ± 0.24	0.143 ± 0.12	0.369 ± 0.05	0.059 ± 0.12
Octadecanoic acid				
Control	0.551 ± 0.41	0.336 ± 0.22	0.224 ± 0.08	0.492 ± 0.46
T5%	0.628 ± 0.20	0.250 ± 0.15	0.701 ± 0.21	0.404 ± 0.09
T10%	0.306 ± 0.17	0.284 ± 0.23	0.517 ± 0.08	0.238 ± 0.04
T20%	0.134 ± 0.05	0.179 ± 0.02	0.211 ± 0.01	0.175 ± 0.01

Treatments consisted of varying vermicompost tea ratios (T5%= 1:20, T10%= 1:10, and T20%= 1:5). Values were expressed as mean ± Standard Deviation. Each treatment consisted of n = 16. One-way ANOVAs were performed on the Relative Proportion of compounds detected across time and treatment. Means within a column followed by the same letter are not significantly different at $p \leq .0500$. Significantly different treatments were highlighted by adding a letter to mean relative proportion values. Compounds that were not detected were denoted as "n.d".

Six fatty acids were detected and identified throughout this study. Based on frequency and relative proportion values, three compounds were chosen for in-depth analysis. The compounds chosen were Hexadecanoic acid, Linolenic acid and Octadecanoic acid. Mean relative proportion of all the compounds fluctuated over time and treatment type. Generally, most compounds followed a similar trend across time. Hexadecanoic acid and Linoleic acid were highest during harvesting periods 1H and 3H, while intermittingly decreasing during 2H and 4H (Table 10). This trend is best observed

in Table 10. Changes throughout harvesting periods for all compounds and treatment types were significant ($p \leq 0.05$). Mean relative proportion values for Hexadecanoic acid and Octadecanoic acid decreased as vermicompost concentrations increased. Furthermore, relative proportion values for Octadecanoic acid were significantly lower ($p \leq 0.05$) in T20% as compared to Control and T5% treatments. Concentration of Octadecanoic acid in T20% treated plants remained relatively unchanged throughout each harvesting period as compared to the remaining vermicompost tea treatments (Table 10).

4.1.4 Alkanes

Table 11 Mean Relative Proportion for Alkanes Detected by GC/MS Analysis across Time and Treatment Type

	Harvesting Period			
Compound *Treatment Type*	1H	2H	3H	4H
Dotriacontane				
Control	0.512 ± 0.14	0.144 ± 0.10	0.187 ± 0.03	0.233 ± 0.12
T5%	0.303 ± 0.14	0.216 ± 0.04	0.169 ± 0.04	0.153 ± 0.10
T10%	0.412 ± 0.07	0.229 ± 0.04	0.108 ± 0.04	0.148 ± 0.02
T20%	0.326 ± 0.02	0.189 ± 0.19	0.198 ± 0.07	0.130 ± 0.09
Hentriacontane				
Control	0.702 ± 0.16	0.373 ± 0.09	0.251 ± 0.17	0.152 ± 0.20
T5%	0.533 ± 0.14	0.370 ± 0.14	0.206 ± 0.16	0.341 ± 0.06
T10%	0.739 ± 0.15	0.406 ± 0.09	0.311 ± 0.05	0.245 ± 0.03
T20%	0.595 ± 0.10	0.393 ± 0.76	0.336 ± 0.04	0.321 ± 0.07
Nonacosane				
Control	0.464 ± 0.10	0.426 ± 0.15	0.363 ± 0.09	0.389 ± 0.10
T5%	0.381 ± 0.09	0.473 ± 0.12	0.464 ± 0.08	0.404 ± 0.05
T10%	0.480 ± 0.11	0.369 ± 0.06	0.375 ± 0.09	0.322 ± 0.07
T20%	0.393 ± 0.06	0.475 ± 0.07	0.373 ± 0.05	1.993 ± 0.24

Treatments consisted of varying vermicompost tea ratios (T5%= 1:20, T10%= 1:10, and T20%= 1:5). Values were expressed as mean ± Standard Deviation. Each treatment consisted of n = 16. One-way ANOVAs were performed on the Relative Proportion of compounds detected across time and treatment. Means within a column followed by the same letter are not significantly different at $p \leq .0500$. Significantly different treatments were highlighted by adding a letter to mean relative proportion values. Compounds that were not detected were denoted as "n.d".

Five alkanes were detected and identified across all treatments. Based on frequency and mean relative proportion, three compounds were chosen for closer analysis. Compounds chosen were Dotriacontane, Hentriacontane and Nonacosane. Overall, mean relative proportion for all compounds varied across time. Dotriacontane and Hentriacontane followed a similar trend throughout every treatment, decreasing significantly ($p \leq 0.05$) over time (Table). Nonacosane remained relatively stable across all treatments except T20% (Table 11). Plants treated with T20% have a significantly higher ($p \leq 0.05$) mean relative proportion values for Nonacosane as compared to all other treatments (Table 5). High concentration of Nonacosane in T20% treated plants was registered during harvesting period 4H. Harvesting period 4H occurred during tomaot post-harvest.

4.1.5 Effect of Vermicompost Tea on Secondary Metabolites

Secondary metabolites changes to BHN589 tomato plants were monitored from seedling to harvesting stages. Results demonstrated that secondary metabolites fluctuated naturally through time and treatment types. Vermicompost tea treatments, especially T20%, had a noticeable effect on terpenoid metabolic pathways, upregulating the Mevalonic acid pathway. The Mevalonic acid pathway regulates all terpenoid production. The most notable change was produced on the monoterpene, β-Phellandrene. Monoterpenes are the one of the primary contributors to organoleptic properties like smell and taste (Davis, 2010). β-Phellandrene, a volatile monoterpene, produces the strong odors that characterize tomato plant aroma (Buttery et al., 1987). This volatile compound also functions as a chemical defense against pest. Chiu et al. (2017) reported

β-Phellandrene had moderate toxicity against *Dendroctonus ponderosae* or Pine beetles. Seo et al. (2014) also described β-Phellandrene's toxic properties, discovering its AChe (Acetylcholinesterase) inhibition capabilities. AChe regulates nerve impulse transmissions across cholinergic synapses (Siegfried & Scott, 1990; Lopez & Pascual-Villalobos, 2009). Inhibition of AChe causes neurotoxicity symptoms such as paralysis and can interfere with other physiological functions (Lopez & Pascual-Villalobos, 2009).

Production and accumulation of other terpenoids was also affected. For instance, relative proportion for Lycopersene increased with vermicompost concentration in administered treatments. The highest relative value for Lycopersene was observed during harvest period 3H, which was characterized by fruit ripening and harvesting. Greatest mean relative value for Lycopersene was observed in T20% treated plants. Ebadollahi et al. (2015) demonstrated that Lycopersene had arachnicide like properties, deterring *Tetranychus urticae* or spider mite attacks. Additionally, Li et al. (2015), found that Lycopersene had potent antibacterial properties against various bacterial groups. Enhanced production of carotenoids and other volatile compounds during fruit ripening might serve as an attractant for seed dispersal or as defense to warn off pest during fruit maturation.

Mean relative proportions for sterols Lupeol, Phytol and β-Amyrin were negatively affected by vermicompost tea treatments. The negative relationship between vermicompost and sterols could be attributed to the presence of gibberellic acid or indole acetic acid. Ravindran et al. (2016) and Aremu et al. (2014) reported the presence of gibberellins in vermicompost. Vermicompost tea's chemical composition could have induced change in sterol production. Jusaitis et al. (1981) reported that long-term

exposure of barley stems to gibberellic acid caused a decrease in sterol content. Furthermore, Heble et al. (1971) reported a decrease in sisterol following an indole acetic acid treatment.

Vermicompost tea applications also decreased fatty acid content. A negative relationship could be observed between mean relative proportions for fatty acids and vermicompost concentration in tea. Fatty acid production is influenced by abiotic/biotic stresses and developmental cues (Bigault Du Granrut & Cacas, 2016). For example, temperature induced stress causes changes in the plasma membrane's physico-chemical properties due to alteration in sterol concentration (Bigault Du Granrut & Cacas, 2016). Moreover, increased plant resistance to pathogens can provoke the consumption of fatty acids originating from chloroplast to supply an oxidative pathway that coordinates host cell dismantling (Bigault Du Granrut & Cacas, 2016). A decrease of fatty acids across vermicompost tea treatments could be a response to an enhanced mechanism for pest resistance. This could be considered antixenosis, which is an induced defense that modifies physical and chemical plant structures to deter pest infestation.

Long chain alkanes are produced as part of epicuticular waxes in terrestrial plants (Bliedtner et al., 2018). The primary role for cuticular waxes is to regulate the movement of molecules into and outside of the plants (Ziv et al., 2018). There is also evidence suggesting that cuticular waxes adapt to abiotic and biotic stress and are actively involved in plant defense and signaling pathways for plant growth and development (Raffaele et al., 2009; Javelle et al., 2011; Aragon et al., 2017; Ziv et al., 2018). The observed increase of Nonacosane in T20% treated plants might be attributed to chemical elicitation. Several plant hormones affect the development of plant cuticles and stress

tolerance. Xia et al. (2010) reported that gibberellic acid treatments increased cuticular waxes Arabidopsis plants. This increase improved plant immunity against pathogenic bacteria *Pseudomonas syringae* (Xia et al., 2010; Ziv et al., 2018). Reduced levels of abscisic acid increased cuticle permeability and resistance to fungal pathogen *Botrytis cinerea*.

Phenolic compounds detected by GC/MS analysis remained relatively unchanged by vermicompost treatments. Despite this observation, a total phenolics bioassay revealed that vermicompost tea treatments significantly affected phenolic compound production in tomato plants. Generally, vermicompost tea treated plants had a higher phenolic content than the control group. Additionally, T10% treated plants had significantly greater phenolic compound concentration than any other treatment. Similar results were found by Nur et al. (2013). Nur et al. (2013) evaluated the effects different fertilization regimes in total phenolics. Results demonstrated that phenolic content was significantly higher in vermicompost treated plants (Nur et al., 2013). Results from this experiment differed slightly. The highest concentration of phenolic compounds was observed in the moderate vermicompost tea treatment, and not the highest treatment (T20%). Moreover, Zhao et al. (2009) found that phenolic compounds increased with low nitrogen availability and considerable yield reduction. Different fertilization regimes could explain the differences between treatments. Plants treated with T20% vermicompost tea had reduced phenolic compound concentration. Low concentrations in T20% treated plants might suggest that greater amounts of vermicompost is needed to provide the plant with balanced nutrition. However, this does not explain why the control group and T20% treated plants have similar phenolic compound concentrations.

5. CONCLUSION

Vermicompost tea treatments, specifically T20% treatment type, induced physical and chemical changes in BHN589 tomato plants. T20% treated plants experienced significantly greater biomass and improved concentrations of chlorophyll, yield, and soil pH. Although significant differences were only observed in biomass and soil pH. Changes to secondary metabolite production were also observed within T20% treated plants. T20% vermicompost tea treatment had a strong influence in the mevalonic acid pathway. As a result, terpenoid production was altered. Changes in terpenoid production increased β-Phellandrene and Lycopersene accumulation, while decreasing sterol concentration. Vermicompost tea treatments also affected phenolic compound production, specifically in the phenylpropanoid pathway. Low and moderate (T5% and T20%) vermicompost tea treatments had the highest concentration of phenolic compounds. Differences between treatments might be associated to enhanced stress resistance or nutrient composition in the tea. Some fatty acids and alkanes were also influenced by vermicompost tea treatments. Decreases in fatty acid concentrations might be attributed to antixenosis. This would mean that plants are using a high concentration of fatty acids deter pest infestation. An increase in Nonacosane alkane might also be related to vermicompost tea. The addition of plant hormones such as gibberellins and abscisic acid found in vermicompost influences cuticular wax. Alterations to cuticular wax improves plant immunity to bacterial and fungal pathogens.

High variation and low influence on plant physical structures could be attributed to a multitude of factors such as inclement weather, low doses/concentrations of vermicompost, or any cultivation practices utilized. Furthermore, yield and mortality

were directly affected by leaf harvesting. Due to the size and weight of tomato leaves, samples had to be bulked for chemical analysis. A larger number of replications would have decreased variation between observed results.

Impact of inorganic fertilizers continues to threaten biodiversity, negatively affecting beneficial organisms such as pollinators. Due to increased growth and enhanced secondary metabolite production of defense compounds, vermicompost tea addition could be a viable and sustainable practice in pest resistance and fertilization. Further research must be conducted on the effects of vermicompost and vermicompost tea on chemical elicitation and secondary metabolite production to conclusively determine if pest enhanced pest resistance is due to a change in chemical or physical structures of the plant.

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