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Safety Assessment of Hydroponic Closed System

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SAFETY ASSESSMENT OF HYDROPONIC CLOSED SYSTEM

By

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A THESIS

Submitted in Partial Fulfillment of the

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An Abstract of the Thesis Presented
in Partial Fulfillment of the Requirements for the
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The hydroponic system is an increasing sector for horticultural production. It is used mostly for fruit and vegetable production. Lettuce (*Lactuca sativa*) is one of the most cultivated types of produce in the hydroponic system. It runs on nutrient solution and in some cases substrates. Water serves as the backbone for hydroponic production, mainly utilized for nutrient solution preparation. Substrates are sometimes added to provide support for plants root systems. Selection of substrate depends on the type of crop and the availability of the substrate. A good substrate should be able to balance the oxygen - water ratio around the root system and have a high-water retention ability. Peat moss is an organic substrate mostly used by growers due to its sustainability and additional ability to retain nutrients on its surface. The hydroponic system is classified as open or closed system depending on the nutrient solution usage. The closed system reuses spent nutrient solution and is economical with less water wastage. There is, however, a high rate of pathogen build-up in this system.

The assurance of food security, food safety, and high yield has made the hydroponic system a widely accepted mode of production many vegetable horticultural commercial growers. Due to less to no contact of growing media to edible portions, the system is believed to provide a relatively safe, healthy, and clean product. However, the isolation of pathogens such as *Salmonella,*

Eshericheria coli O157:H7*, Listeria monocytogenes,* and *Campylobacter* spp. from hydroponically grown crops has created awareness about the potential risk of foodborne illnesses from this system. Research is geared toward screening of source of irrigation water and other potential sources of contamination in the hydroponic production. However, little is known about the possible source of contamination in the hydroponic system.

The objectives of this study were to: (i) identify possible sources of contamination in the hydroponic system; (ii) evaluate the efficacy of behavior modification and/or sanitization in the reduction of microbial count on harvested produce throughout expected shelf life; and (iii) evaluate the microbial load on different peat moss substrates as well as heat-treated peat moss substrates.

Water, leaf, root, and substrate samples were collected from an actively growing, closed hydroponic system. Water samples included 'water outlet', 'water inlet', tap water and 'water reservoir'. The leaf samples consisted of onsite leaf and harvested leaf while the substrates were onsite substrate and fresh substrate. Substrate used in this study was of peat moss origin. Samples were enumerated for aerobic plate count (APC), coliform bacteria (CB), and yeast and mold (YM). Detection of *Listeria* was carried out and none was detected on any of the samples. Enumerated count for all microbes was highest in the onsite substrate samples. Interestingly, onsite lettuce leaves had the lowest count for all counts. The harvested leaves were relatively higher in APC and YM count compare to the onsite leaves. The time of contact of the other samples with the onsite substrate significantly increased the microbial count on these samples, raising the possibility of the substrate being the source of contamination.

Reduction in the microbial load on the substrate was carried out by combining sanitizers, storage time, and packaging method. Sanitizers consisted of chlorine (Cl-200 ppm), peroxyacetic acid (PAA-80 ppm), and sterile distilled water (SDW). Microbiological and sensory quality measures

were carried out on harvested substrate (plug), roots, and leaves. The harvested lettuce maintained its appearance and color after sanitizer application. Storage time and sanitizer significantly reduced APC and yeast count. PAA was most effective against APC and YM while chlorine was effective against CB. Sensory quality measurement indicated that dipping the harvested lettuce substrate in a solution before packaging aided in maintaining the lettuce color and fresh appealing look.

Other peat moss substrates and heat-treated substrates were examined for microbial populations. A difference in microbial load was found on substrates due to difference in rate of decomposition, chemical, and physical properties.

Overall, this research shows that substrate is a possible source of contamination in the hydroponic closed system. This research demonstrates that sanitizer wash could effectively help reduce microbial load on lettuce leaves and different compositions of substrates influence their ability to host microbes

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ABSTRACT

Lettuce (*Lactuca sativa*) is a vegetable which is mainly consumed in its raw state and is also used with other vegetables in making salads. Evidence suggests that it can serve as a vehicle for foodborne pathogen transfer. The hydroponic system of production is believed to produce clean, healthy, and safe produce. However, *Salmonella, Eshericheria coli* O157:H7*, Listeria monocytogenes,* and *Campylobacter* spp. have been isolated from hydroponic produce which has heightened an interest to evaluate the system for its safety, sources of contamination, and strategies to reduce microbial load.

The hydroponic system is grouped into the open or closed system. Many commercial growers utilize the closed hydroponic system which recycles and reuses a nutrient solution. However, these closed hydroponic systems are more prone to harboring pathogens due to the reutilization of spent nutrient solution. Little is known about the source of pathogen build up in the closed hydroponic systems. This research therefore evaluates possible points of entry of pathogens in a closed hydroponic system of the lettuce production. This chapter provides a general overview of the closed hydroponic system, possible sources of contamination and ways to control, reduce and/or eliminate microbial contamination in the hydroponic system.

CROP PRODUCTION

Plant Growth and Development

Plant growth and development requires certain fundamental elements; namely water, air, light, and mineral salts (Taiz & Zeiger, 2002). Plants manufacture food through the process of carbon assimilation or photosynthesis by absorbing air, inorganic salts from soil solution, transporting minerals dissolve in water through the xylem and intercepting light energy through their palisade cells (Taiz & Zeiger, 2002).

Plants consist of about 80% water in fresh weight (Resh, 2013). The cell and tissue type determine the distribution of water in the plant. Water builds turgor pressure for cell structure maintenance, enlargement, and gaseous exchange in the leaves (Resh, 2013). Furthermore, it also serves as the medium of transport for minerals and other solutes. Plants utilizes about 90% of the water absorbed to cool the plant and create air space for absorption of carbon dioxide from the atmosphere (Taiz & Zeiger, 2002). Water is required for plant growth and development, yet, the often limited in supply for agricultural production (Silber, 2018). Water scarcity is the leading factor for plant growth impairment and accounts for crop stress, reducing productivity (Srivastava, 2002)

Plants absorb about 60 different mineral elements, with about 16 being classified as essential for plant growth and development (Taiz & Zeiger, 2002). The essential elements are subclassified as micronutrients and macronutrients. Macronutrients needed in large quantities for production include carbon, hydrogen, oxygen, nitrogen, potassium, phosphorous, magnesium, and calcium (Jordan et al., 2018; Ferreira Domingues et al., 2015). Mineral elements after absorption are transported as ions through two major transport systems: passive transport, which consists of diffusion and mass movement of molecules; and active transport, which requires utilizing energy

to move solutes against a concentration gradient (Taiz & Zeiger, 2002). The correct proportion of nutrients must be available to plants for growth order to thrive.

Soilless Production

Soilless production is dated as far back as 4000 years ago and has been used by many plant botanists and physiologists in their laboratory experiments in understanding plant nutrition and physiology (Treftz, 2015; Resh, 2013). Evidence of early soilless production includes the migration of 'container plants' by the Egyptians, the hanging gardens of Babylon, and the floating garden of the Aztecs of Mexico (Resh, 2013). Soilless production is documented to have been born out of the lab work of Theophrastus (372-287 B.C.) to better understand plant nutritional requirements for growth and development (Resh, 2013). In 1600, Jan van Helmont, a Belgian, determine that the soil provided less than 1% of a plant's needs for growth and development (Christie, 2014). This finding drove further scientific research into the primary source of plant nutrients for growth. Sachs and Knop (1859 – 1865) later developed the "nutriculture"; a water solution that contained nitrogen, phosphorous, potassium, calcium, sulfur, and magnesium (Douglas, 1959). Crop production in nutriculture remained a laboratory technique until 1929 when W.F. Gericke successfully grew a twenty-five-foot tomato crop outdoors whose fruits were ladder harvested (Resh, 2013; Douglas, 1959). He commercialized the cultivation of vegetables and ornamental plants using this same technique (Resh, 2013). He coined the word 'hydro-ponics' to describe his nutriculture crop production system. The term hydroponic is of Greek origin ('hydro'-water, ponos - 'labor') which laterally translates as 'water-working'. Large scale hydroponic production has subsequently been employed in most developed countries (Christie, 2014; Resh, 2013; Abd-Elmoniem et al., 2006).

General Overview of Hydroponic Production

Hydroponic production has been in existence for a long time, however, its acceptance and commercialization started about 70 years ago (Resh, 2013; Abd-Elmoniem et al., 2006). Hydroponic production, by strict definition, is the growing of crops in water culture without any solid substrate (Raviv and Lieth, 2008). However, most hydroponic systems incorporate solid substrates such as coconut fiber, sand, gravel, or Rockwool® (mineral wool) for anchorage, stabilization, and as an inert water support matrix for the crop root system (Sikawa & Yakupitiyage, 2010; Abd-Elmoniem et al., 2006). Therefore, the typical commercial hydroponic of production grows plants in a soilless condition with water, nutrients, and an inert medium (Douglas, 1959)

Hydroponic Substrates

The ancient hydroponic growers incorporated sand and gravel as growing media. Today hydroponic substrates come in form such as loose soilless media or pot mix, and plugs. Selection of either of these depends on the type of crop, its availability, and grower's choice (Lopez-Galvez et al., 2014; Sikawa & Yakupitiyage, 2010; Abd-Elmoniem et al., 2006). Substrates are designed to provide plant with support and to serve as small nutrient reservoirs for plant use. Substrates are grouped as organic or inorganic based on their material composition, physical, and chemical properties (Jordan et al., 2018).

Organic substrates are primarily made up of sphagnum peat moss, coir and/or composted milled pine bark (Jordan et al., 2018). Organic substrates have a buffering capacity somewhat similar to soil, which enables them to serve as reservoirs of nutrients for plant use (Sikawa & Yakupitiyage, 2010).Organic substrates are porous, have a high water holding capacity, and are lightweight

(Lopez-Galvez et al., 2014; Sikawa & Yakupitiyage, 2010). To improve these characteristics, inorganic materials such as sand perlite and vermiculite may be added.

Hydroponic Nutrient Solution

Plants grown in the hydroponic systems derive their nutrients from a solution of dissolved fertilizer salts. Concentrated stock nutrient solutions are diluted in the water and dispensed using an injector or blending system. Nutrient solutions are adjusted and replaced over the growing cycle based on changes in pH, electrical conductivity (EC), and/or water consumption (Ding et al., 2018; Douglas, 1959). A pH and EC sensor meter are often attached to the dispenser systems and monitored to determine if and when adjustments is needed to be made (Jordan et al., 2018; Walters, 2015; Avila-Vega et al., 2014; Christie, 2014). Calcium nitrate is the most widely used hydroponic fertilizer in North America. Potassium nitrate, monopotassium phosphate, and magnesium sulfate supply the other macronutrients including phosphorus and potassium (Resh, 2013). Micronutrient are supplied through premixes that are added to the formulation.

Through these nutrient solutions, hydroponic growers try to provide optimum nutrient formulations to meet specific crop needs. Nutrient solutions are adjusted based on the plant type, its growth stage, and the time of year (Ding et al., 2018; Walters, 2015). The primary nutrients for all plants are nitrogen, phosphorus, and potassium. However, the correct proportions of these are important in order to meet the requirements of each plant. Leafy crops such as lettuce, require higher nitrogen contents for good growth while fruit forming crops such as tomatoes require a higher amounts of potassium, phosphorus, and calcium (Strayer, 1994). Therefore, tomato fruits require a lower nitrogen content of about 140 ppm but higher potassium content of about 300 ppm, while lettuce (*Lactuca sativa*) requires a low potassium amount of about 150 ppm.

Classification of the Hydroponic System

There are several groups of hydroponics systems. Generally, hydroponic systems are classified based the water culture, the nutrient culture, and the soilless (substrate or container) culture (Christie, 2014). Most classifications are based on either one of these or a combination of all groups depending on the crop, its growing cycle, and the planting method. For short growing cycles such as leafy vegetation, water culture classification is mostly used (Resh, 2013). For fruits and vegetables (such as the members in the solanancea and cucurbit families) container culture classification is preferred. Ornamental plants grown hydroponically are classified based on nutrient culture systems in place (Silber, 2018).

Based on the nutrient culture classification, the hydroponic system is sub-classified based on the solution dispensary or irrigation delivery system (Abd-Elmoniem et al., 2006). The system is therefore grouped as a stagnant, flowing, mist, drip irrigation, or sub-irrigation hydroponic system. Another classification of the hydroponic system is based on the type of substrate used, namely, the organic and inorganic hydroponic system (Pardossi et al., 2011). Lastly, the water culture is based on the drainage of the nutrient solution and is classified as an open (free-drainage) or a closed (recirculation) system. Most commercial leafy vegetable hydroponic classifications are based on the water culture system (Christie, 2014).

The Open versus Closed System

The open hydroponic systems use a nutrient solution supply only once; it flows through and is not recirculated or recycle. The closed hydroponic system reuses nutrient solutions recirculating it throughout the production cycle of the crop (Christie, 2014; Douglas, 1959). The open hydroponic system can significantly reduce the possibility of contaminated, however, this system requires high

amounts of water and nutrients. The open hydroponic system is therefore expensive with regards to water, reagent, and disposal of used nutrient solution (Christie, 2014; Pardossi et al., 2011). The closed hydroponic systems pose a higher risk of contamination relative to the open hydroponic system because the solution is constantly recirculated. However, pathogens may be reduced and disinfection and regular sterilization of the closed system. The closed hydroponic systems require less water and nutrients but also require personnel with technical know-how to manage and control disease and pest incidence (Christie, 2014).

The Nutrient Film Technique (NFT)

The nutrient film technique (NFT) of hydroponic culture was first introduced by Allen Cooper and his team. NFT was a major turnaround point for the acceptance of hydroponic production by commercial growers (Resh, 2013). NFT is mostly employed in closed system hydroponics. Running NFT on a closed system requires addition of topping up solutions to the starting nutrient solution to maintain the composition of the nutrients. In NFT, the plant root system penetrates the through the plugs to assess the nutrient solution before transplanting (Riggio et al, 2019).

FOOD SAFETY

General Overview of Food Safety

Humans and other animals derive their nutrients from food in order to survive. However, food may also serve as a vehicle for transporting foodborne pathogens that threaten human health. Foodborne illness is a major public health issue. In the United States there are approximately 48 million foodborne illnesses annually (Pignata, Angelo, Fea, & Gilli, 2017). Surveillance carried out by the Foodborne Diseases Active Surveillance Network (FoodNet) in 2008 in the United States showed

that children under 5 years had most foodborne infections while people above 65 years old suffered most hospitalizations and deaths. FoodNet revealed that foodborne diseases resulted in 43182 illness, 55961 hospitalizations and 1,351 deaths annually (Scallan et al., 2011). The increase of foodborne illnesses over time is counterintuitive to the advancement of science, medicine, and technology (Wu et al., 2019). Foodborne illnesses are still relevant due to alteration in food production, different food choices, and a favorable environment created by humans for the pathogens to thrive (Painter et al., 2013). Some of these pathogens have developed resistance to chemical treatments and physiological control measures (Schwaiger et al., 2012). Food products have been the host to several antibiotic-resistant pathogens. Several pathogens have been associated with foodborne diseases (Leff & Fierer, 2013), with bacteria contributing to about 60% of reported outbreaks (Whipps et al., 2008; Solomon et al., 2002). Major foodborne pathogens include *Campylobacter*, *Cryptosporidium*, *Cyclospora*, *Listeria*, *Salmonella*, Shiga toxin–producing *Escherichia coli* (STEC) O157, *Shigella*, *Vibrio*, and *Yersinia* (Avila-Vega et al., 2014; Olaimat & Holley, 2012; Berger et al., 2010; CDC, 2010).

In 1875, journalist Lafcadio Hearn reported his findings from the stockyard farms in the US pertaining to poor sanitation. In response to this, government agencies passed acts to improve pork and beef safety. The agencies further broadened their scope to ensure the safety of other food products. According to the Centers for Disease Control and Prevention (CDC) food safety practices can curtail the incidence of foodborne diseases (CDC, 2010). Source identification is an effective measure to control disease outbreak. In foodborne illnesses however, it is challenging to identify the source of infection. The challenges are attributed to three main factors. Firstly, food goes through many links in the food chain for processing before consumption, which increases the likelihood for contamination. Secondly, a wide variety of food serves as host for foodborne

pathogens. Lastly, the incubation period of certain diseases pose a challenge to source identification (Leboffe and Pierce, 2011)

Source of Contamination

All activities geared towards food provision serve as potential sources of foodborne diseases. The environmental safety of food production in all aspects of the food chain is essential in preventing food contamination (Berger et al., 2010a). Food of plant origin, especially fruits and vegetables, have a high risk of contamination. Fruits and vegetables are of high nutritive value and health benefit. They provide the body with vitamins and minerals that help boost the body's immunity to disease (Berger et al., 2010a, 2010b). Fruits and vegetables are added to salad and are mostly eaten raw. Over the years, nutritionists have advocated the significance of incorporating fruits and vegetables in the diet (Hosler & Kammer, 2015c). Marketers of fruits and vegetables stress the fact that they require less preparation and are more convenient for consumer consumption.

Fresh fruits and vegetables rank high in foodborne pathogen transfer and host transmission (Robertson et al., 2016). Increased consumption of fresh and minimally processed fruits and vegetables is directly proportional to food-related microbial diseases (Hosler & Kammer, 2015; Berger et al., 2010a). The World Health Organization (WHO) reported that out of 24 cases of illness caused by foodborne pathogens, 11 originated from fresh produce. In spite of the evidence that fresh fruits and vegetables are major reservoirs of human pathogens, it is extremely difficult to pinpoint the exact source of contamination in the produce-related food supply chain (Orozco, Rico-Romero, & Escartín, 2008). The food industry focuses mainly on consumer handling of food such, as heating to prevent foodborne illnesses. The industry attributes the majority of the foodborne illnesses to poor food handling, and immune deficiencies of consumers. (Berger et al.,

2010a). Though this assertion is valid, it does not address the entirety of foodborne illness transmission and prevention. (Maffei et al., 2016). Human immunity and food handling undoubtedly contribute to foodborne illnesses, however, attention should be focused on the entire food chain to identify possible sources of infection to achieve success in prevention. (Wadamori, Gooneratne, & Hussain, 2016).

The production sector is the first point of entry of pathogens. Practices such as irrigation, manuring, and fertilizer application, among others, make it arduous to localize the exact point of entry this sector (Wadamori, Gooneratne, & Hussain, 2016; Whipps et al., 2008). Vectors of pathogen transfer are either humans, animals/animal products, or environmental resources. Most contaminations in the production sector occur either in the field or during the post-harvest handling of produce (Holvoet et al., 2015; Olaimat & Holley, 2012; Xiao et al., 2015). In the field, possible sources of foodborne pathogen transfer to the produce include the soil, irrigation water, raw or poorly decomposed animal manure, wild animals and insects, and human handling. Post-harvest activities such as handling, washing, and cutting are contributors to foodborne pathogen introduction unto harvested fresh produce (Castro-Ibáñez et al., 2015; Olaimat & Holley, 2012; Yugo & Meng, 2013). Equipment used in harvesting and preparing fresh produce for storage are all important routes for contamination of produce. Adherence to good agricultural practices (GAP) is therefore essential to improve the safety of fresh produce. (Holvoet et al., 2015).

There is an increase in foodborne pathogens resulting from pre-harvest and post-harvest activities. *Salmonella, E. coli* O157:H7, and *L. monocytogenes* are the major organisms associated with fresh produce contamination and cross contamination. Of these, Listeria is the most virulent organism and has caused a high number of lethal cases (Avila-Vega et al., 2014). Listeriosis has a high incidence and severity record for illness and hospitalization in the United States. Among foodborne

pathogen outbreaks, *Salmonella* ranks second to norovirus (Wadamori, Gooneratne, & Hussain, 2016). Incidence of cucumber, alfalfa sprout seeds and raw mung bean sprouts contaminated with *Salmonella* and *S.* Anatum on pre-package lettuce led to 900 and 97 cases of salmonellosis, respectively in United States and Finland. Lettuce, cantaloupe, apple and sprouts have been recorded to be a reservoir of *E. coli* O157:H7 (Wadamori, Gooneratne, & Hussain, 2016)

Risk of Contamination in Soil versus Soilless Production Sector

Soil production has a higher risk of microbial contamination relative to soilless production. The soil is a rich source of microbes including some foodborne pathogens. Contamination can occur at the onset of seed germination to harvest (Christie, 2014; Koseki & Mizuno, 2011). Any portion of the fruit or vegetable in close proximity to the soil is at risk of serving as a host and harboring pathogens. In addition, animals and equipment traversing fields can disperse foodborne pathogens (Holvoet et al., 2015). Soil runoff during heavy stormy days can also spread pathogens (Holvoet et al., 2015).

In soil production, manure application to improve soil fertility may also serve as a source of contamination of produce. Raw or poorly decomposed manure of animal droppings are conducive for the survival of coliform bacteria. There is evidence of long term survival of *S.* Typhimurium and *E. coli* O157:H7 in soil and on leafy vegetables for over 60 days as a result of manure application (Yang, Swem, & Li, 2003).

Water is at the heart of agricultural production and is relevant because it is used in almost all activities ranging from irrigation to washing of the produce. In production, growers normally use wells, surface water, or municipal water for irrigation and fertilizer application (Allende &

Monaghan, 2015). Different techniques of irrigation and fertilizer application can transfer pathogens to the produce. Notably, overhead and sprinkler applications on fresh produce pose a risk of food contamination especially when drawn from surface water (Xiao et al., 2015). In 2006, outbreaks of *E. coli* O157 in bagged spinach and iceberg lettuce in the United States and Sweden, respectively, were attributed to the water used in the production system. The fresh produce industry has taken steps, such as drip irrigation and application of well-composted manure, to reduce cross contamination, but with little success (Allende & Monaghan, 2015; Avila-Vega et al., 2014). In both production systems, contaminated equipment and poor sanitation of post-harvest units can also promote contamination and cross contamination of harvested produce.

Overall there is a significant risk of pathogen contamination in field soil production of raw vegetables. Soilless production carried out in a greenhouse may reduce the risk of human pathogen contamination by eliminating incidental contact with wildlife and use of manure fertilizers. These systems mostly runs on deep well systems or municipal water which further reduces the possibility of human pathogens transfer (Lopez-Galvez et al., 2014; Sikawa & Yakupitiyage, 2010). These, however, do not guarantee total elimination of human pathogens.

Hydroponic production is often done in a controlled environment. This may increase the likelihood of pathogens dispersed through air and recirculating water supply (Riggio et al., 2019). Human pathogens including *Listeria monocytogenes*, *Salmonella enterica* serovars, Shiga toxin-producing *E. coli* (STEC), and human noroviruses have unique characteristics' that enable them to thrive in controlled environment in the hydroponic system (Hirneisen et al., 2012). Foodborne pathogen can survive superficially or internalized in hydroponic produce. In one study, raspberries, strawberries, lettuce, and green onions were found to be contaminated as a result of the water used in the soilless production system (Shaw et al., 2016; Lopez-Galvez et al., 2014). Also, *E. coli*

O157:H7 has been isolated on hydroponically grown lettuce (Riggio et al.,2019; Solomon et al., 2002) and internalization in hydroponic radish sprout and leafy vegetables (Itoh et al., 1998). Water may be a source of contamination in the hydroponic production systems hence pose a greater risk of internalization of pathogen. In the hydroponic system, the use of hydroponic media may impact the rate of absorption of pathogens in produce (Itoh et al., 1998)

Possible Sources of Contamination in the Closed System Hydroponic Lettuce Production

Lettuce (*Lactuca sativa*) belongs to the family Asteraceae, and is one of the most widely commercialized hydroponic leafy vegetables in United States and Canada due to the ease of cultivation in the hydroponic system (Jordan et al., 2018; Christie, 2014; Abd-Elmoniem et al., 2006). Lettuce has a high surface area and is proximal to the growing media and therefore susceptible for microbial transfer from the media. The risk of human transfer of these pathogens is heightened given the fact that it is eaten raw or added to salads. Lettuce creates a suitable environment for many types of bacteria to thrive. The rate of lettuce contamination depends on the prevalence, occurrence, and amount of pathogens in the host (Lopez-Galvez et al., 2014). Hydroponic production runs on nutrient solution, water, and in some cases substrates (Pachepsky et al., 2011; Abd-Elmoniem et al., 2006). All these components are potential sources for human pathogen harboring and transfer unto produce.

Many hydroponic lettuce growers use plugs instead of soil-mix due to the ease of use transplanting and harvesting. The plugs are either from organic or inorganic material including coir and peat. Such plugs may serve as small reservoirs for nutrient storage and serve as potential hosts for microbes including human pathogens. Hydroponic plugs have conductance abilities which aid microbes to affix to their surface. These microbes may help in the conversion of nutrients into

forms readily available for plant use. Sphagnum peat moss and coir are most often used in hydroponic systems due to their high conductance, and relatively high effective cation exchange capacity (Vallance et al., 2011). Substrates used in soilless production may serve as breeding grounds for both bacteria and fungi. Organic substrates tend to be colonized more by fungi while inorganic substrates are colonized more by bacteria (Rastogi et al., 2012). There has been evidence of high microbial count on Rockwool™ and sphagnum peat moss plugs (Riser, Grabowski, & Glenn, 1984). Research dating back to 1984 has shown the presence of coliform bacteria on peat moss plugs (Rastogi et al., 2012).

Water is an important component in the hydroponic system. It is used in irrigation and for preparation of nutrient solution. In the closed system, the nutrient solution is reused through recirculation within unspecified lengths of time (Abd-Elmoniem et al., 2006). The nutrient solution is monitored and changed constantly to ensure optimum function of the system, but microbial counts are not routinely monitored (Resh, 2013). The closed system ensures judicious use of water and nutrient but has a high risk for infection build up. There is, therefore, the need to frequently change the nutrient solution and treat the recycled water in order to minimize microbial contamination (Avila-Vega et al., 2014; Christie, 2014; Lopez-Galvez et al., 2014).

Water is a major pillar in hydroponic production, its microbial quality is importance with regards to food safety. Guidelines for agricultural water is established by the Food and Drug Administration (FDA) through the Food Safety Modernization Act (FSMA) and the Produce Safety Rule (PSR) (21 CFR § 112.42). Specifically, water used during growing activities must meet a geometric mean of ≤126 CFU/100 mL generic *E. coli* and a statistical threshold value of ≤410 CFU/100 mL generic *E. coli* based on a rolling four-year sample dataset (Allende and Monaghan, 2015). According FSMA, water quality is questioned if the water used has direct

contacts with the harvestable part of the crop either during the crops' growth cycle or after harvest (Allende and Monaghan, 2015). This guideline overlooks hydroponic production. Hydroponic leaf greens are not considered to contact the water used for nutrient solution, which allows this water not to comply with the standards above (Allende and Monaghan, 2015; Xiao et al., 2015). This raises the question of whether pre-harvest agricultural water standards should remain the same or be more or less stringent for hydroponic production.

Post-harvest activity is another area for possible pathogen contamination during production. Post-harvest activities have served as mechanisms for human pathogen cross contamination. These activities range from storage and washing to handling and cutting of the produce (Whipps et al., 2008). Handling of harvested produce has resulted in a rise in Hepatitis A and norovirus diseases (Olaimat & Holley, 2012). Post-harvest equipment such as shredders and slicers used for cutting fresh vegetables have contributed to microbial cross-contamination on the surface and inner tissues of the produce (Yang et al., 2003). Hydroponic produce, though generally 'clean' and requiring less washing relative to soil-grown produce, still has a likelihood of microbial transfer onto finished produce irrespective of the number of times the produce is rinsed. Water with unknown microbiological quality can therefore pose important health hazards.

Food Quality/Safety Indicator Test

Microbiological indicator populations help determine the quality and/or hygienic status of food, water and/or the environment. These indicators are categorized into quality and safety indicators (Ray, 2004). Quality indicators assess the microbial presence in food products whereas safety indicators evaluate the conditions associated with the potential risk of exposure to a pathogen. In

the assessment of the microbial quality of food, the sole identification of a species or the quantification of the species may be used (Ray, 2004).

Food spoilage has both microbial and non-microbial etiologies. Microbes cause food spoilage predominantly by increasing in growth and metabolic activity, as well as enzyme secretion (Ray, 2004). Spoilage of food is measured by the change in functional properties of food. Several criteria have been developed as indicators to predict the expected shelf-life of food. These indicators focus on sensory, microbiological, and chemical areas for assessment. Selection of the type of indicator depends on the type of food, the expected shelf life, the storage condition, and the level of microbes in the food (Tortorello, 2003). These indicators can be evaluated individually, however, to increase accuracy, a combination of at least two of these indicators is preferred. Sensory indicators measure and predict food shelf-life using visual characteristics, odor, flavor, and texture. Chemical indicators predict the presence and level of metabolites in food. Microbiological indicators measure the presence of microbes in food (Tortorello, 2003).

With respect to microbiological indicators, enteric microbes serve as a surrogate marker for food safety. The presence of these organisms in food helps to measure the likelihood of fecal contamination (Ray, 2004). Enteric indicators include coliform and fecal coliform (*E. coli*) identification. Selection of either of these depends on the food, water, and environmental conditions. Coliforms are made up of genera such as *Escherichia, Enterobacter, Klebsiella,* and *Citrobacter*. The main sources of food contamination with enteric pathogens are fecal matter from warm blooded animals such as humans, other mammals, and birds (Tortorello, 2003). Assessment of sanitary condition serves as an indirect food safety indicator and is carried out to determine the microbial quality of all sectors in the food supply chain. Aerobic plate count (APC) is used to assess the cleanliness of the production site (Ray, 2004).

Commonly Used Indicator Organisms

Aerobic Plate Count (APC)

APC or Standard Plate Count (SPC) is used to measure the mesophilic microbes in food. APC is not used as a safety indicator for pathogenic microbes however it measures the microbiological load and the cleanliness of production and manufacturing site (Tortorello, 2003). It is an inaccurate measure of quality when used in produce such as sprouts that are known to have high APC (10^8) and in fermented products with a naturally high APC (10^9) due to starter cultures used in the fermentation process (Ray, 2004). It is, however, a good measure in fresh products, and is used to assess the quality of sanitary procedures used during production, and post-harvest activities (Yousef and Carlstrom, 2003). APC has been used as a good quality indicator in drinking water, raw or pasteurized milk and milk products in the United States (Tortorello, 2003).

Coliform Bacteria (CB)

Coliforms consist of several genera which are grouped together based on their characteristic similarities (Tortorello, 2003). Groups found in this genus are facultative anaerobic, rod-shaped, non-spore forming, Gram negative bacteria that ferment lactose to produce gas within 48 hours at 37ºC. The genera include *Escherichia, Enterobacter, Klebsiella*, and *Citrobacter*. Some groups such as *Enterobacter, Klebsiella*, and *Citrobacter* contain species that are from non-fecal origin. Hence, the use of coliforms as an indicator may not necessarily imply food product contamination by fecal matter. Most assessments are specifically done on fecal coliforms as indicators to rule out false implications from non-fecal coliforms (Yousef and Carlstrom, 2003). Coliforms of fecal origin can persist in the soil for a longer time, hence, are mostly present in raw food from plant

and animal origin. High coliform levels may be a result of gross contamination and improper storage conditions (Tortorello, 2003). Food products that are refrigerated can still have increased coliform numbers due to their ability to survive and reproduce under refrigeration (Ray, 2004). Fecal coliform bacteria are hosted in fecal matter of all warm-blooded animals. They consist mainly of *E. coli, Klebsiella* spp.*,* and *Enterobacter* spp. *E. coli is* biochemically differentiated from other coliforms by indole production from tryptone, methyl red reduction due to acid production (red coloration), Voges Proskauer reaction (production of acetyl-methyl carbinol from glucose), and citrate utilization as a carbon source (IMViC) (Ray, 2004).

Listeria spp.

Listeria is a ubiquitous organism that is highly resistant to salt concentrations and environmental stress. It is inactivated by pasteurization but is the most heat-resistant among the common enteric pathogens. *L. monocytogenes* is an important species in the field of public health as it records highly lethal cases (Castro-Ibáñez et al., 2015; Lopez-Galvez et al., 2014; Whipps et al., 2008). It is commonly found in food processing environments. Testing for all *Listeria* species serves as an environmental monitoring mechanism for control of this organism as it is ubiquitous (Tortorello, 2003).

Yeast and Mold (YM)

Yeast and mold are used as quality indicators. Their prevalence and occurrence are indeterminate as they can survive and thrive in almost all environmental conditions. They have the ability to survive in a wide range of pH (2-9), temperature (5 - 35ºC), and water activity (<0.85) (Tortorello, 2003). *Zygosaccharomyces* spp. contains osmophilic yeast that can thrive in < 0.65 water activity

and are used as indicators in low water activity foods such as jam and syrups (Ray, 2004). Their diverse and fast-growing characteristics are concerning for food contamination in the manufacturing site and they are known to be major food spoilage organisms. Other useful indicators include assessment of ingredient acceptability, organoleptic characteristics, stability, and shelf-life of products (Tortorello, 2003).

PREVENTION AND CONTROL OF FOODBORNE PATHOGENS IN CLOSED SYSTEM HYDROPONIC GROWN LETTUCE

Preproduction Strategies

A production system with soilless media free from pathogens will help reduce the rate of pathogen transfer onto produce. In a hydroponic production system, little is known about the effect of preproduction disinfection or sterilization techniques (Tanaka et al., 2011). Disinfection is the use of chemicals, while sterilization uses non-chemical techniques such as heat to control pathogens and pests.

Most growers utilize a non-chemical method for sterilizing their growing media. Heat treatment is the underlying technique for most non-chemical treatments. The selection of the intensity of heat for sterilization of growing media depends on the purpose of the sterilization. Temperature selection must be done with care to prevent killing of beneficial microorganisms (Kelsey, Slizovskiy, Peters, & Melnick, 2010). The temperature used ranges from 120°F for inactivation of oomycetes to 212°F for inactivation of viruses and weeds. For bacterial control, a temperature range between 145°F and 180°F has proven to be efficacious (Castro-Ibáñez et al., 2015; Tanaka et al., 2011). In soil production, heat treatment of the soil has been very beneficial in controlling certain plant disease pathogens such as *Verticillium dahiae*, *Pythium* spp., *Rhizotonia* spp.,

Phytophthora spp., root-knot (*Meloidogyne* spp.), and sting nematodes (*Belonolaimus* spp.) (Samtani et al., 2012). It has been recorded that autoclaving perlite substrates suppresses soil-borne pathogens such as *Pythium* spp. and *Fusarium* spp. (Pardossi et al., 2011). However, there is less focus on how heat treatment can reduce foodborne microbial transfer from substrates to produce.

Solarization is a non-chemical method of sterilizing growing media. Growing media are sun-dried for a specified time period. The timing depends on the intensity and amount of heat produced by the sun (Samtani et al., 2012). However, this method is disadvantageous since the intensity and temperature easily fluctuates. To curtail this problem, some growers use translucent plastic bags to cover their growing media to retain heat which aids in killing harmful microbes. Solarization is mostly used in the tropics and subtropical regions. It is relatively cheap, however its success in killing pathogens is unreliable (Kelsey et al., 2010).

Steam sterilization is another technique that is mostly used in the nursery and in greenhouse production. Steam sterilization is the treatment of media using moist heat. Regulation of temperature and pressure is a key component (Samtani et al., 2012; Tanaka et al., 2011; Gay et al., 2010). It has been successful in eliminating soil-borne pathogens, however, it is relatively costly and more labor intensive than other non-chemical sterilization techniques (Egli et al., 2006).

Oven sterilization is the most widely adopted technique used in laboratory and on commercial scale production. It operates on dry heat (Gay et al., 2010). Its high efficacy in controlling bacteria, bacterial spores, and fungi makes it a good sterilization technique to control human pathogens. A temperature range of 150 -180°F for 30 minutes has been shown to be adequate. A reduced temperature would require longer periods for sterilization (Samtani et al., 2012).

Decontamination Strategies

Consumer preference for fresh vegetables has led producers to use mild preservation strategies to meet the market's expectation. Mild preservation strategies such as modified atmosphere or vacuum packaging and refrigeration provides quality produce with longer shelf life (Weller et al., 2013). A high diversity of microbes has been recorded on whole and cut fresh processed vegetables. Pathogenic microbes have been found on this mildly preserved produce. Mild preservation creates a new environment for microbes to thrive (Chaidez et al., 2018; Fraisse et al., 2011). Inefficient washing and sanitization processing of harvested produce will allow surface microbes to survive and persist in mildly preserved produce. In the case of hydroponic lettuce, harvested lettuces are packaged and sold with intact bulk of growing media. This may transfer pathogens from the root or plugs unto the leaves irrespective of the use of mild preservation techniques. One way to mitigate this is by using decontamination strategies such as sanitizer application (Olaimat & Holley, 2012; Fraisse et al., 2011; López-Gálvez et al., 2010). This will possibly reduce or control the microbial load on the plugs or roots to reduce contamination of produce.

Chlorine-releasing chemicals such as bleach have mostly been used as sanitizers in vegetable production due to the low cost, strong oxidizing ability, antibacterial effect, and safe status (Chaidez et al., 2018; Fraisse et al., 2011; López-Gálvez et al., 2010). Fresh and cut vegetables use chlorine-releasing agents to reduce microbial load and create an unfavorable environment that prevents pathogens from surviving or being transferred (Weller et al., 2013). Dosage ranges from 50-200 mg/L and a contact time of 1-2 minutes have been recorded to be efficient for microbial load reduction (Chaidez et al., 2018; Fraisse et al., 2011). Research on honeydew and cantaloupe exposed to chlorination resulted in significant reduction ($p \leq 0.05$) relative to unwashed and water-washed produce (Weller et al., 2013). Chlorine-releasing agents are bactericidal and impair

bacterial enzymes and protein through irreversible binding of sulfhydryl groups. Its efficacy is variable due to deactivation in the presence of organic matter. A concentration of 200 ppm has been found to reduce a microbial load of *Yersinia enterolitica* on tomato by 4.77 log units while a microbial load of *E. coli* on cilantro was reduced by only 1 log CFU. (Weller et al., 2013). Sodium hypochlorite (NaOCl) is the most commonly used chlorine-releasing agent in small scale fruit and vegetable production (López-Gálvez et al., 2010; Weller et al., 2013).

Peroxyacetic acid (PAA), is a solution made from the reaction of hydrogen peroxide and acetic acid (González-Aguilar et al., 2012). PAA is an approved sanitizer for fruits and vegetables. The US Food and Drug Administration (FDA) approved a concentration ≤ 80 ppm in wash water for produce (Weller et al., 2013). It acts as a good antimicrobial agent against several pathogens. It is effective against bacteria, viruses, bacterial spores, and protozoan cysts. PAA has shown a slower reactivity to organic matter relative to chlorinated compounds. It has a larger oxidation potential than many other sanitizers (González-Aguilar et al., 2012). Its antimicrobial ability is based on the release of active oxygen molecules. It oxidizes essential enzymes that block vital biochemical pathways leading to impairment of active transport across membranes. The measure of its efficacy depends on its exposure time and the concentration used. PAA significantly reduces microbial growth on the surface of cut produce and maintains a low microbial count for up to 21 days. PAA (80 ppm) has been found to reduce *Salmonella* on tomatoes by 5.5 log units (González-Aguilar et al., 2012).

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CHAPTER 2

IDENTIFICATION OF POSSIBLE SOURCE(S) OF CONTAMINATION IN CLOSED HYDROPONIC LETTUCE PRODUCTION SYSTEM

ABSTRACT

The aim of this study was to assess the safety and potential sources of contamination in a closed hydroponic system. Lettuce was used as the test crop. Leafy greens including lettuce are known to be a vehicle for foodborne pathogen transfer. Lettuce is mostly used in salad and eaten raw, increasing its risk of transmitting foodborne diseases. The cleanliness and safety attributed to the hydroponic system needs a thorough investigation due to identified foodborne pathogens on hydroponic produce.

In this study water, peat moss plugs, and lettuce samples were collected from a functioning hydroponic facility. Water was sampled from the facility tap, 'water reservoir', 'water outlet' and 'water inlet.' Peat samples consisted of fresh (unused) peat moss plugs and actively 'growing peat plugs' and the lettuce samples included 'onsite lettuce leaves' (preharvest), roots, and harvested (packaged) lettuce leaves. Samples were enumerated for aerobic plate count (APC), coliform bacteria (CB), and yeast and mold (YM). *Listeria* spp. detection was performed on all samples.

Presumptive positive *Listeria* spp. isolates were found in samples from water reservoir, water outlet, peat plugs (fresh and growing) and plant roots. However, none of the presumptive positive colonies confirmed positive for *Listeria* after the agglutination test.

Growing peat moss plug had the highest count for APC, CB and YM. Root counts for all enumerations were nearly as high as those obtained from peat plugs partly due to being embedded in the growing peat moss plug. Counts in tap water were the lowest of all samples. Among the water samples, the water reservoir yielded the highest count for APC and CB while water outlet

had the highest count for YM, likely the result of the use of a *Trichoderma* biocontrol product used for seeding. Onsite lettuce leaves demonstrated lower APC, CB, and YM compared to harvested leaves. This finding is significant because most harvested hydroponic lettuces are packaged and sold with 'root ball', i.e. intact roots and plugs used in cultivation. With a high microbial load found on the plugs there might be a possible transfer from root and/or plugs unto the harvested lettuce leaves.

INTRODUCTION

Advocacy for healthy eating has led to an increased dietary proportion of fruits and vegetables in the United States (Oluwaseun, Singleton, & Sant, 2018; Uyttendaele et al., 2015; Berger et al., 2010) and the fresh produce industry has expanded to meet the high demand for these products. The increased production of fresh produce, as well as increased handling, wider distribution, mechanization and awareness have contributed to an increase in foodborne infections attributed to contaminated produce (Uyttendaele et al., 2015). In the United States, an increased fresh produce consumption between 1998 and 2007 was positively correlated with foodborne diseases, accounting for 14.8% of foodborne disease outbreaks and 22.8% of cases of illness during this time period (Gould, 2019; Uyttendaele et al., 2015; Sivapalasingam, Friedman, & Cohen, 2004). According to the FDA, Foods of Non-Animal Origin (FoNAO), including fresh produce including salads, vegetables, fruits, and juice are the main vehicles for foodborne diseases (Olaimat & Holley, 2012; Berger et al., 2010). This has been attributed, among other causes, to direct contact of the edible portion the plant with the soil or growing medium (Johnston et al., 2005). Hydroponic production may reduce this risk, as the growing media has little to no contact with the edible portion (Settanni et al., 2013).

Hydroponic production systems have been adopted by many commercial leafy green and fruit growers (Lopez-Galvez et al., 2014). It is a fast-growing sector as it provides assurance of an all-year round production and relatively higher productivity with decreased land requirements. Closed hydroponic systems run on recycled and reused "spent" nutrient solution. Most growers utilize the closed system as it saves time, labor, and money. In spite of its safety, however, foodborne pathogens of public health concern have been found in hydroponic production systems (Lopez-Galvez et al., 2016; Lopez-Galvez et al., 2014). Source identification of the point of infection, as well as the activities that promote pathogen survival and persistence is challenging. The closed hydroponic system of production creates a potential breeding ground for foodborne pathogens to thrive as it recycles spent nutrient solution (Christie, 2014). Moreover, the source of water and the type of media used could potentially introduce microbes to the hydroponic system. Several agronomic practices carried out also serve as a potential source of contamination (Lopez-Galvez et al., 2014; Riser, Grabowski, & Glenn, 1984).

Globally, water has been a major vehicle for foodborne pathogen transfer to food (Castro-Ibáñez et al., 2015). Irrigation with contaminated water sources has yielded *Salmonella* Newport on tomatoes, *Escherichia coli* O157 and *Cyclospora* on iceberg lettuce, and *Salmonella* Saint Paul in peppers, among others (Allende, 2016; Steele & Odumeru, 2004; Solomon, Yaron, & Matthews, 2002). In hydroponic production, water is utilized in the nutrient solution preparation and for irrigation. Evidence of *Salmonella* Typhimurium, *E. coli,* and *Staphylococcus aureus* on hydroponic produce makes water source an area to be investigated as a possible source of contamination (Lopez-Galvez et al., 2014). The choice of water usage is influenced by its availability and proximity to the production site (Uyttendaele et al., 2015). Water contamination depends on several factors such as exposure to animals and their fecal matter, runoff and proximity

to sewage or waste disposal (Lopez-Galvez et al., 2016; Fung, 2007; Duffy et al., 2005). In the hydroponic system, sources of water for irrigation and nutrient solution mix range from surface water and reclaimed water to groundwater and municipal water. Municipal water possesses the best microbiological quality while surface water is the most likely to be contaminated (Uyttendaele et al., 2015).

Although the hydroponic system uses no soil, the substrates used in the production have properties similar to soil with the potential of harboring pathogens. Most organic hydroponic substrates have high absorptive and cation exchange capacity that enable them to retain nutrients for plant growth and development (Sikawa & Yakupitiyage, 2010), and have conductance ability to help fix microbes on their surface. These microbes may help in the conversion of nutrients into forms readily available for plant use (Pardossi et al., 2011). Among the substrates used, sphagnum peat moss and coir have the highest conductance and effective cation exchange capacity. Their high water-holding capacity, slightly acidic pH (~4-5), and high nutrient retention ability makes them good substrates for most hydroponic crops. Their ability to host microbes, however, may be detrimental to humans. Organic substrates preferentially host more fungi while inorganic substrates host bacteria (Rastogi et al., 2012). Evidence suggests a high microbial count on Rockwool™ and sphagnum peat moss substrates used in hydroponic production of lettuce (Riser et al., 1984).

Understanding and identifying possible sources of contamination in the hydroponic system will require screening of the entire system. In this study, samples (water, growing media-peat, leaf, and root) were obtained from a working hydroponic production site. Tests were run using microbiological quality parameters aimed at determining the most likely source of contamination

in the system. These parameters include aerobic plate count, coliform count, and fungi comprising of yeast and mold.

MATERIALS AND METHODS

Sample Collection and Preparation

Water, peat moss and lettuce samples were obtained from a hydroponic site. The hydroponic site runs on the closed hydroponic system of operation and utilizes peat moss substrate for lettuce production. Before planting, substrates are routinely 'seeded' in water with a commercial biocontrol product (*Trichoderma* spp.). The International Commission on Microbiological Specifications for Foods (ICMSF) 1978 sampling technique was used (ICMSF, 1978). For water samples, two 50 ml aliquots were aseptically collected into sterile conical tubes. Water samples were obtained from the water reservoir, water inlet, water outlet, and tap. Peat moss plugs were aseptically removed from the trough and separated from the root into sterile stomacher bags. Fresh peat moss plug samples were also obtained. For the lettuce samples, two samples of roots, preharvest leaves, and harvested leaves were aseptically sampled into stomacher bags on each individual sampling day. Samples were placed in an insulated cooler, transported, and refrigerated until analysis (within 24 h). The surface rinse technique was used for analyses; approximately 10 g of lettuce, water, or peat moss samples were weighed aseptically into sterile stomacher bags. The weight of the root varied, mostly ranging from 5.2 to 10 g. A 1/10 dilution (w/v) of the commodity was made using 0.1% Buffered Peptone Water (BPW) (Alpha Biosciences, Baltimore, MD). Samples were homogenized for 2 minutes. Serial dilutions were made by pipetting 1 ml of content from the stomacher bag into 9 ml of BPW tubes.
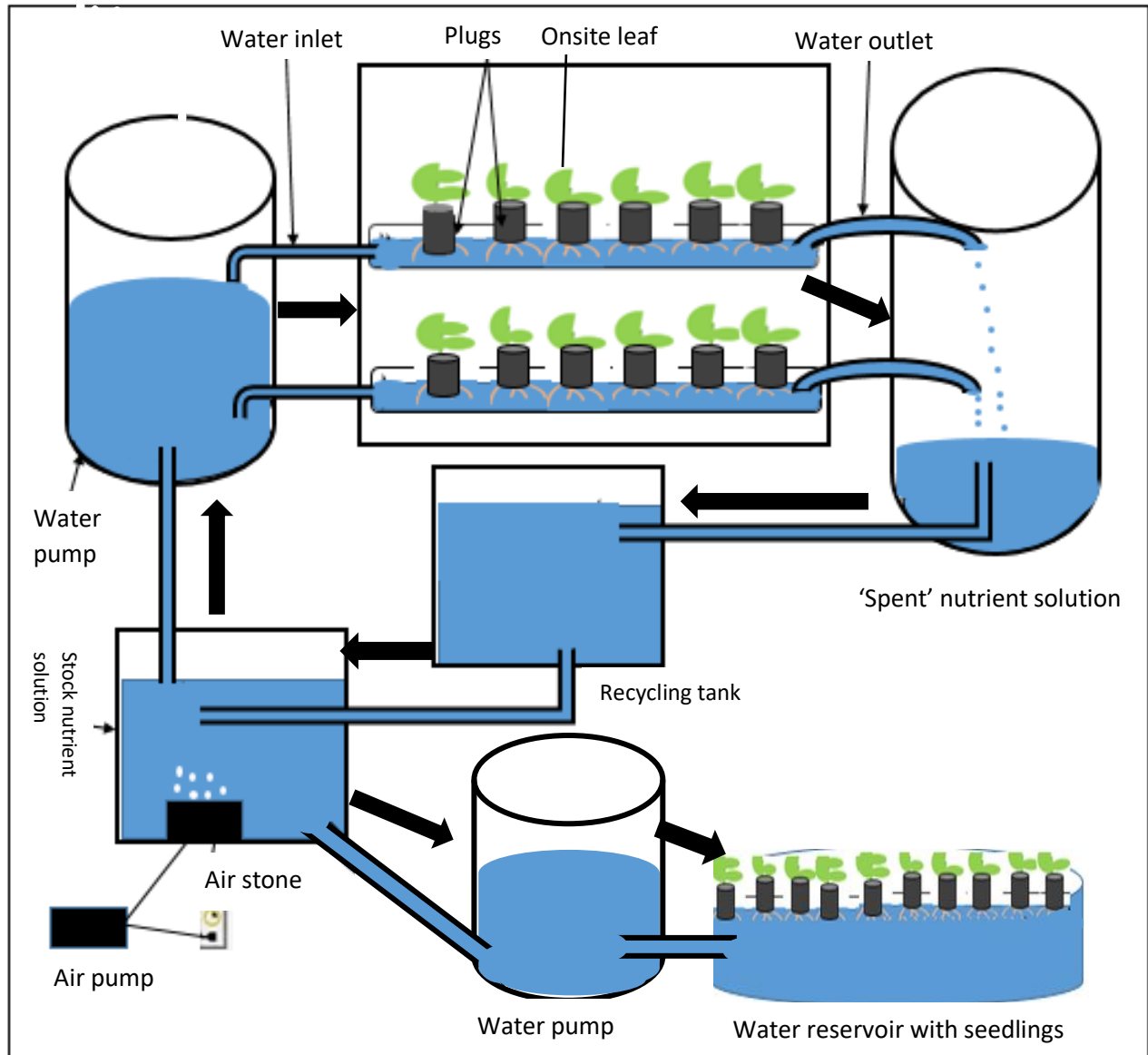
Figure 2. 1 Schematic representation of hydroponic closed system from which samples were collected

Water reservoir- stagnant nutrient solution containing germinated seedling at 3 leaf stage; conditioned plants before transplanting.
Water inlet- point of entry of nutrient from stock solution into transplanted seedlings
Water outlet- use to expel used up nutrient solution into spent nutrient solution tank for recycle.
Plugs- substrate use in place of soil as growing media
Onsite leaf- leaves actively growing in the system

Enumeration of Aerobic Plate Count (APC), Yeast and Mold (YM)

ICMSF, 1978 method was used with slight modification (ICMSF, 1978). From the results of preliminary testing, a countable range of 10-fold serial dilutions were prepared using 0.1% peptone water blanks. Each sample was plated in duplicate on tryptic soy agar (TSA) (Alpha Biosciences, Baltimore, MD) and acidified potato dextrose agar (APDA) (Alpha Biosciences, Baltimore, MD) plates inverted, and incubated at 37°C for 48h or 25°C for 5 days in the dark, respectively. Dilutions within a countable range (20-200 colonies/15-150 colonies, respectively) were counted using a standard counting rule. Counts were averaged and recorded as colony forming unit per gram (CFU/g).

Enumeration of Coliform Bacteria (CB)

The 3 Tube Most Probable Number (MPN) technique was used for CB enumeration (BAM, 2010). One ml of appropriately diluted sample was transferred in triplicate into 9 ml lactose broth (LB) (Alpha Biosciences, Baltimore, MD) tubes fitted with inverted Durham tubes. Three dilutions per sample were used for a total of 9 tubes per sample. Tubes were incubated at 37°C for 24h. Incubated LB tubes were observed for gas production in the Durham tubes and record using the profile '+/-' as presumptive positive. Confirmation of presumptive coliform bacteria was carried out using a subsequent 3 Tube MPN in *E. coli* broth (EB) (Alpha Biosciences, Baltimore, MD) fitted with inverted Durham tubes and incubated at 37°C for 24h. EB tubes were observed for turbidity and gas formation in the Durham tubes to confirm positive coliform bacteria. Positive EB tubes were recorded using the profile '+/ -' and converted to MPN/g using standard MPN tables (Appendix A) (Feng et al., 2001). MPN/g adjustments were made according to sample mass inoculated in presumptive tubes.

Detection of *Listeria* spp.

Twenty-five (25) grams of each of the leaf and water samples, as well as maximum available weight of plug and root samples (average of 20 and 5 g respectively) were aseptically weighed into sterile stomacher bags. A 1/10 dilution (w/v) of the sample was made using Listeria enrichment broth (LEB) (Alpha Biosciences, Baltimore, MD), homogenized for 2 minutes, and incubated at 30°C for 24h. Enriched LEB were streaked onto both modified Oxford agar (MOX) (Alpha Biosciences, Baltimore, MD) and PALCAM agar (BD Diagnostic, Franklin Lakes, NJ). Plates were incubated at 32°C for 24 - 48h and examined for colonies with morphology typical of *Listeria* spp. Presumptive colonies from PALCAM agar and MOX were re-streaked on a non-selective medium, TSA, and incubated at 32°C for 24h. Colonies were confirmed by an initial oxidase test. Agglutination test for oxidase-negative isolates were further carried out using the Listeria latex kit (Microgen Bioproducts Ltd, Camberley, UK).

Statistical analysis

Results of APC, YM and CB were analyzed using R statistical package (R Foundation for Statistical Computing, Vienna, Austria). Counts were log transformed to conform to normality assumption. The analysis of variance (one-way ANOVA) was performed to compare the means of each sample and was followed by Tukey HSD mean separation test at $\alpha = 0.05$. A contrast post ANOVA was run to determine the test trend of significant samples.

Aerobic Plate Count (APC)

Enumeration of APC for water and lettuce samples are displayed in Figure 2.2 and 2.3, respectively. APC in the water reservoir was significantly higher ($p < 0.05$) compared to all the other water samples, suggesting that the stagnant water had a role to play in the APC. Municipal water sample directly obtained from the tap was below the detection limit for APC enumeration. There was no statistically significant difference ($p < 0.05$) between water inlet and water outlet, but APC levels in both samples were lower than that observed in the reservoir.
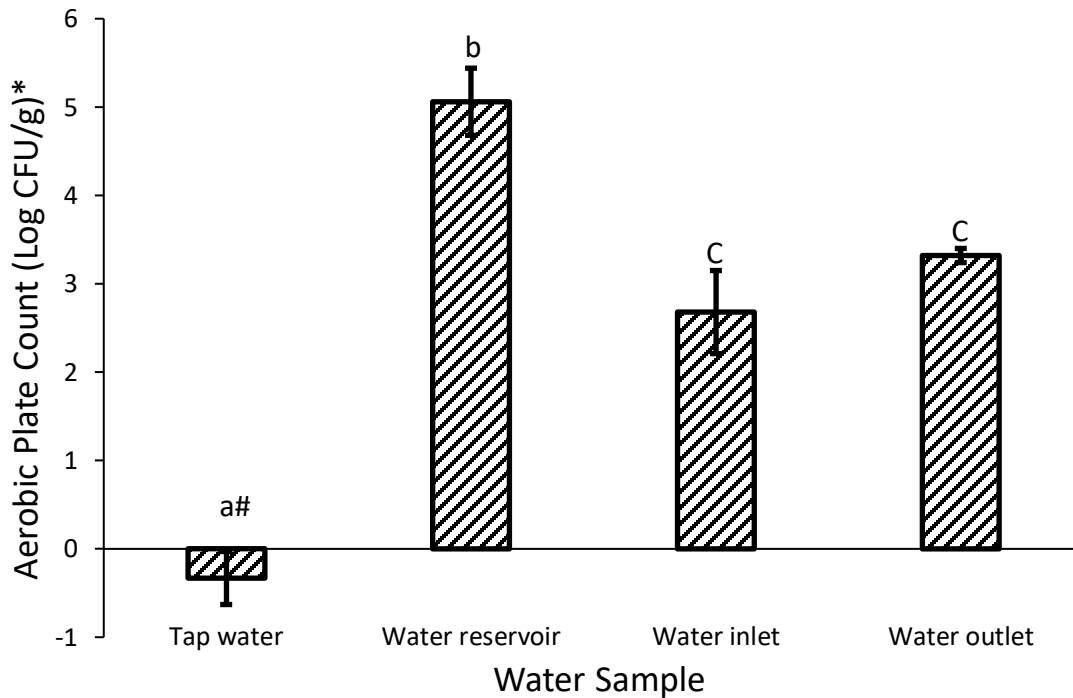
Figure 2. 2 Enumeration of aerobic plate count in water samples from closed hydroponic production system (n=8).

* colony forming unit/gram
# estimated count < 1 CFU/g
The mean APC is shown for each group. The bars represent the standard deviation.
Different letters indicate significant difference (p<0.05) among groups based on a one-way ANOVA followed by a Tukey's post hoc test
Error bars represent standard deviation

APC on the fresh peat plugs was 5.67 log CFU/g, indicating an initial high APC on the peat moss plug samples. This slightly, but significantly, increased to 6.75 log CFU/g when used in cultivation of lettuce. Lettuce leaf onsite (preharvest) had a lower APC (1.67 log CFU/g) relative to harvested lettuce leaf (4.14 log CFU/g), indicating a likelihood of possible transfer during harvest and/or packaging.
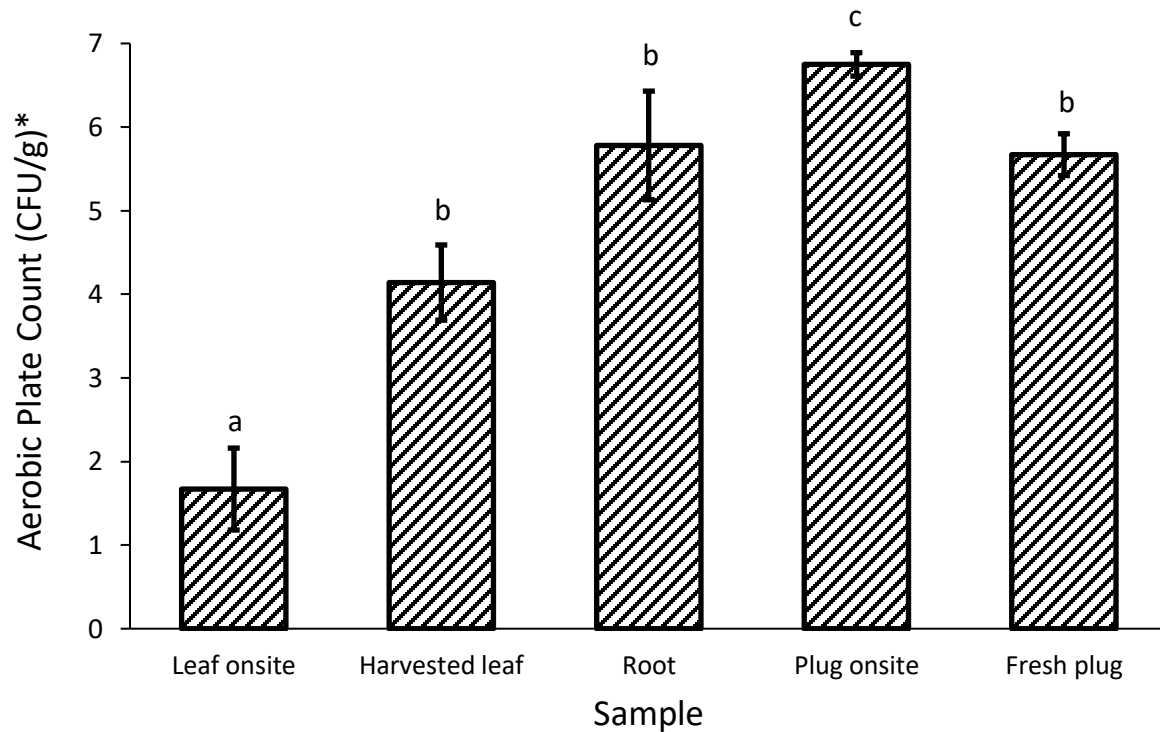
Figure 2. 3 Enumeration of aerobic plate count for hydroponic lettuce and peat samples (n=8).
*colony forming unit/gram
Different letters indicate significant difference (p<0.05) among groups based on a one-way ANOVA followed by a Tukey's post hoc test
Error bars represent standard deviation

Coliform Bacteria (CB)

Results of coliform count for water and lettuce samples are shown in Figure 2.4 and 2.5, respectively. Data shown are from analysis of confirmed CB testing only. Results of both samples followed a similar trend as the APC. Water reservior had the highest CB count with municipal tap water recording the lowest count (not detected, or < 3 MPN/ml). CB count of water outlet was slightly higher than water inlet count.

CB count on fresh peat moss plug was 2.57 log MPN/g which increased to 4.48 log MPN/g in the cultivated peat moss plug. Lettuce leaves from both onsite and harvested had no detectable CB count.
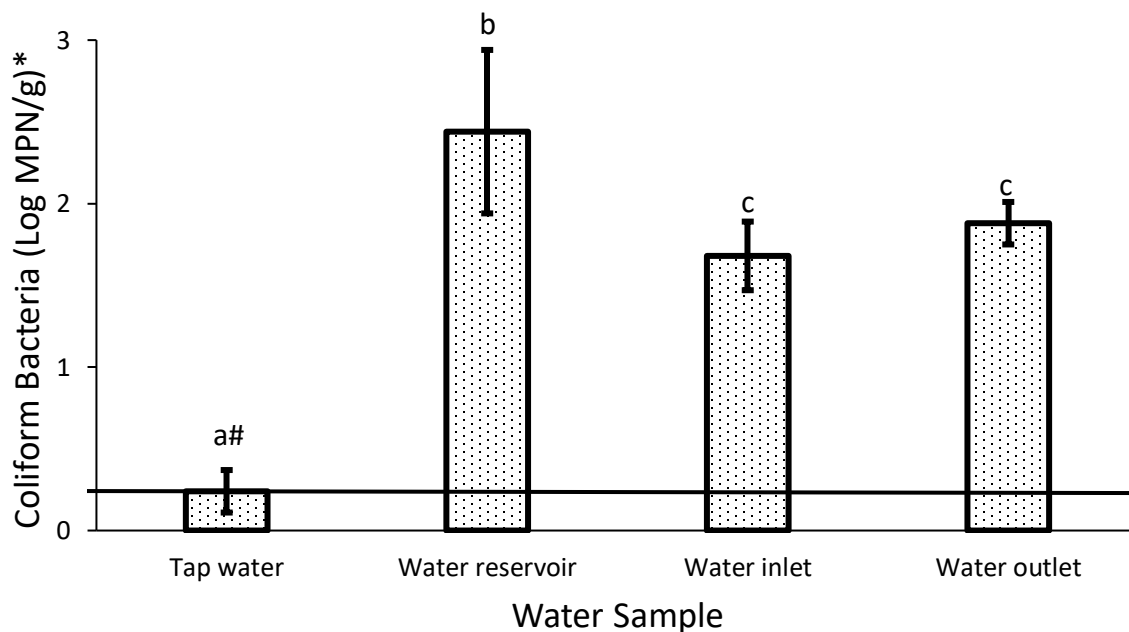
Figure 2. 4: Enumeration of coliform bacteria for water samples using most probable number technique (n = 8).
*Most probable number per gram
#estimated MPN/ml count with no positive confirmed tube(s) recorded as < 30 MPN/ml
Different letters indicate significant difference (p<0.05) among groups based on a one-way ANOVA followed by a Tukey's post hoc test
Error bars represent standard deviation, line represents minimum detection limit (30 MPN/ml)
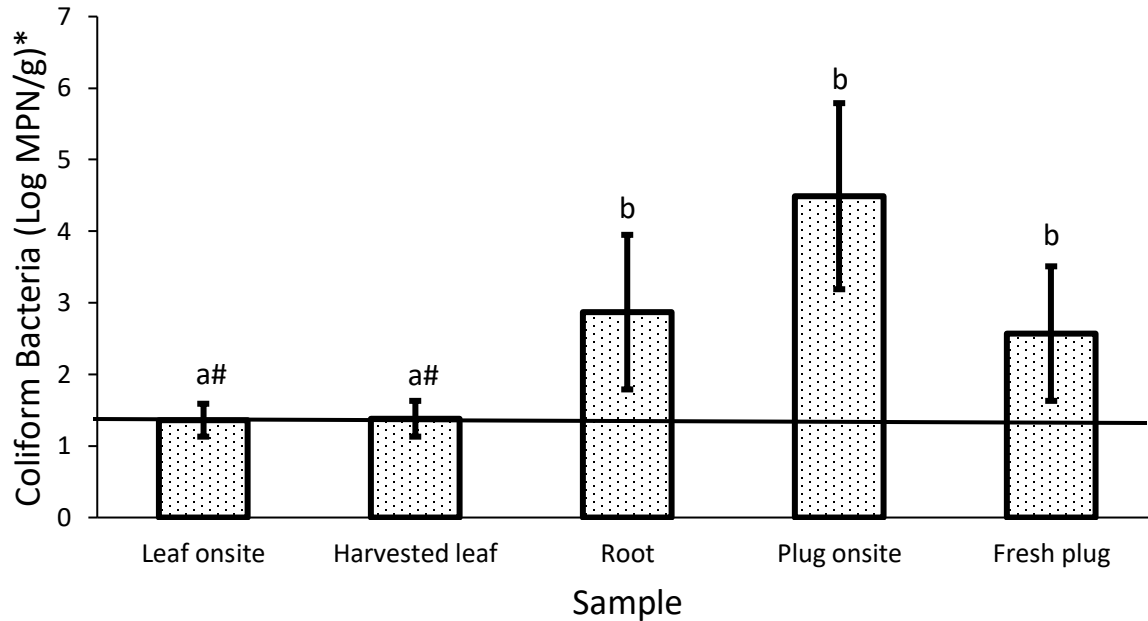
Figure 2. 5: Enumeration of coliform bacteria for lettuce and peat samples using most probable number technique (n = 8)

* Most-probable number per gram

Estimated MPN/g count with no positive confirmed tube(s) recorded as < 3 MPN/g

 Different letters indicate significant difference (p<0.05) among groups based on a one-way ANOVA followed by a Tukey's post hoc test

Error bars represent standard deviation, line represents minimum detection limit (3 MPN/ml)

Yeast and Mold (YM)

YM count from water samples is displayed in Figure 2.6. Overall YM trend of water samples was slightly different from the water samples' APC and CB count. Samples from water outlet and water reservoir had the highest count for yeast. For the mold counts, there were no significant differences among the water samples except the water from the tap, which yielded significantly lower counts.
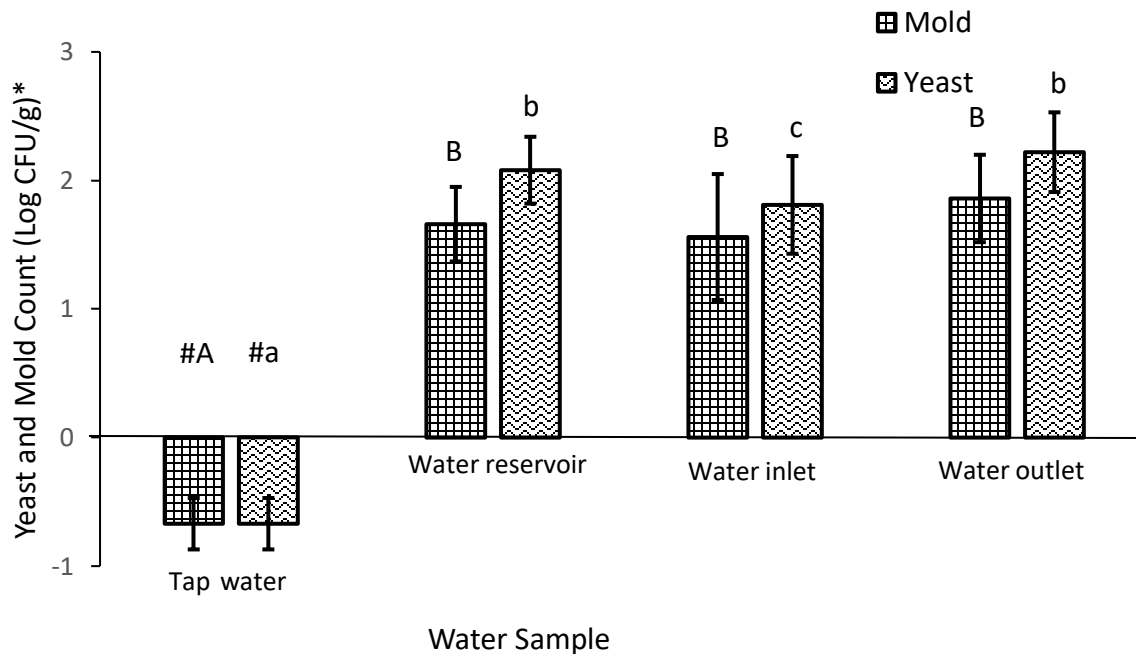
Figure 2. 6: Yeast and mold count on water samples (n = 8)
*colony forming unit per gram
#Estimated yeast and mold count recorded as < 1 CFU/g
Error bars represent standard deviation
Different letters indicate significant difference (p<0.05) among groups based on a one-way ANOVA followed by a Tukey's post hoc test within population count

Figure 2.7 shows the YM count on peat and lettuce samples. YM count was below detectable limit (10 CFU/g) in the leaf onsite samples. Yeast was always higher than mold count except in the fresh peat moss plug. Initial YM count on fresh peat moss plugs was significantly lower than that on the growing peat moss plug used in cultivation, suggesting a favorable condition in the system increased their survival and growth rate. Like the APC and CB result, growing peat moss plug samples had the highest count for both yeast and mold similar to the APC and CB result which indicates that its organic nature aided in a high fungal count and growth. Onsite leaves had the lowest YM count, implying less support for fungi growth. However, harvested leaves were significantly higher in YM count than onsite leaves.
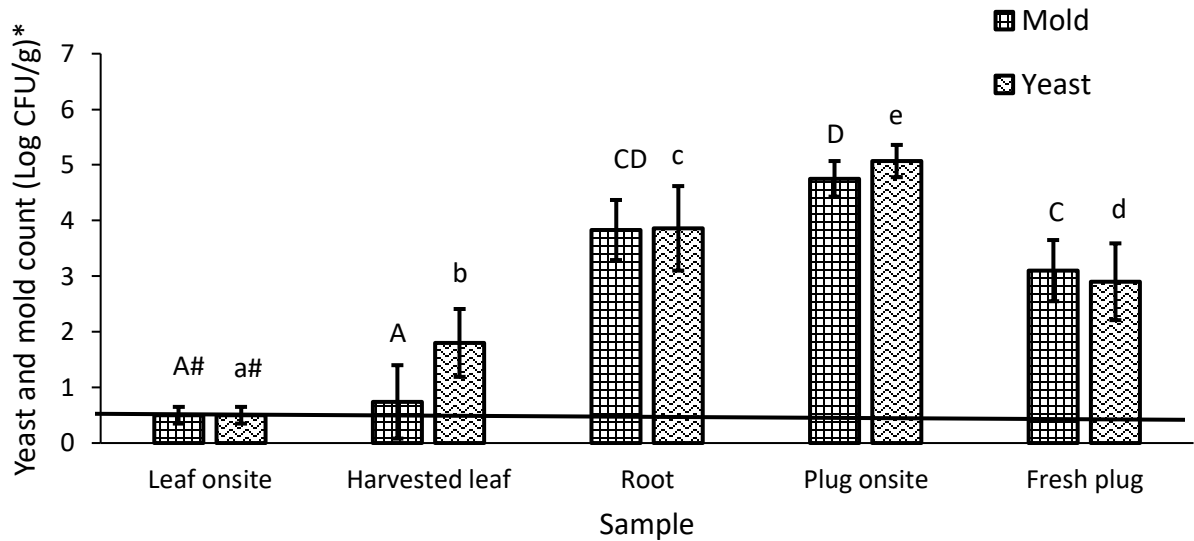
Figure 2. 7: Yeast and mold count on lettuce and peat moss samples (n = 8)
*colony forming unit per gram
#Estimated yeast and mold count recorded as < 1 CFU/g
The mean YM count is shown for each group. The error bars represent the standard deviation
Different letters indicate significant difference ($p<0.05$) among groups based on a one-way ANOVA followed by a Tukey's post hoc test (upper case indicate significant difference in mold count and lower case indicates significant difference in yeast count

Listeria Detection

Positive presumptive *Listeria* spp. result was obtained from root, peat moss plugs, water reservoir, and water outlet samples (Table 2.1). However, none of the presumptive colonies confirmed positive for *Listeria* spp.

Table 2. 1: Listeria detection on water, peat moss and lettuce samples (n=8)

Sample	*Listeria* Detected [a]	
	Presumptive test	Confirmed test
Tap water	-	-
Water reservoir	+	-
Water inlet	-	-
Water outlet	+	-
Onsite leaves	-	-
Harvested leaves	-	-
Roots	+	-
Fresh peat moss plug	+	-
Growing peat moss plug	+	-

[a] *Listeria* detection code + implies present; - implies absent.

DISCUSSION

The results from this study show that peat moss plugs may be a potential source of contamination in the closed hydroponic system. Since the municipal tap water samples were below the detectable limit for all populations, water may be ruled out as a potential source in the system investigated. The acceptable coliform count <2.2 CFU/100 ml or 1000 coliforms/100 ml for municipal and agricultural water, respectively (Allende and Monaghan, 2015). Generally municipal water and portable water are the lowest risk source of microbial contamination. They are known to be regularly tested and held to legal standards of hygiene, hence most hydroponic growers have adapted to its use to provide healthier and safer produce (Uyttendaele et al., 2015). Peat moss, an organic substrate, has an inherent ability to host and harbor microbes. Its organic origin contributed to support of a high YM count (Lopez-Galvez et al., 2016; Rastogi et al., 2012). Its high effective cation exchange capacity and absorptive ability enable it to reserve nutrients for plant use (Pardossi et al., 2011). This also contributes to making it a good host for microbes to thrive and survive.

Onsite lettuce leaves were relatively low in all counts as there was no contact between the leaves and either water or the peat moss substrate. The embedment of the root in the peat-moss substrate contributed to an overall high microbial load on the root.

A high count in all the enumerated microbes from the water reservoir and the water outlet may be due to length of contact time with the peat moss used. Water reservoir was relatively higher in APC and CB than the water outlet. This may be due to the fact that stagnant water was in close proximity to the peat moss plug (Riser et al., 1984) used for seeding the lettuce. Lack of replenishment allows microbes to reproduce in the water reservoir while the water outlet allows water to be expelled after its nutrient are utilized.

Onsite leaves were low in APC, CB, and YM. This is likely explained by the absent contact of the leaf portion to the growing media. The hydroponic system is believed to be safe due to little or no contact of the growing substrate to the edible portion (Allende, 2016). In soil production microbial contamination is may be as a result of the contact of the edible portion to the growing media and the frequent splash of water from the soil unto edible surfaces (Fung, 2007; Gagliardi et al., 2003). A rise in APC and YM on the harvested lettuce leaves may be a result of the harvesting procedure and packaging method used. Postharvest methods have been shown to increase APC and YM on hydroponic harvested produce. This has been attributed to harvesting equipment, personnel, and water used in washing and rinsing the equipment (Holvoet et al., 2015). In this system, none of these factors was applicable. However, the marketable size lettuce was harvested and packaged with intact root and peat moss plugs to help prolong its shelf life. Although counts for all populations on both onsite and harvested lettuce leaves were within expectations for edible produce, packaging was associated with significantly higher counts. Packaging processes are

believed to contribute to transfer of microbes from plugs to leaves. Also, buildup of moisture content in the packaged lettuce may have created a favorable environment for microbes to thrive.

Coliform count (done by MPN) in the water samples ranged from less than <1 to 2.4 log MPN/ml. The legal level of generic E. coli in agricultural water is ≤126 Most Probable Number (MPN)/100 mL (rolling geometric mean n=5) and ≤235 MPN/100ml for any single sample (Allende and Monaghan, 2015). With exception of tap water (CB below detectable limit) the CB levels in the water samples were high, hence the proliferation of this bacteria in the system is a concern. In lettuce, the counts were higher and ranged from <1.5 to 4.48 log MPN/g. Coliforms are suggested to be common microbes in most raw vegetables and leafy greens, but are considered to be indicators of potential contamination (Castro-Ibáñez et al., 2015; Rastogi et al., 2012). CB count on fresh peat moss plug increased from 2.57 to 4.48 log MPN/g in growing media. This may be partially due to the favorable conditions and constant supply of nutrients and moisture to the plant. Coliform introduction into agricultural systems are often a result of fecal contamination from the use of raw animal manure and water from untreated sewage. Coliform levels documented in this work are of some concern for the safety of hydroponic produce as they indicate the potential for survival and growth of enteric pathogens, if pathogenic species were introduced into the environment, hence pre and post treatment of the system will be key to ensure reduction or elimination of harmful human potential on produce.

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CHAPTER 3

EVALUATION OF THE EFFICACY OF BEHAVIOR MODIFICATION

AND/OR   SANITIZATION IN THE REDUCTION OF

MICROBIAL COUNT ON HARVESTED

PRODUCE THROUGHOUT

EXPECTED SHELF

LIFE

ABSTRACT

This study aims at evaluating the effect of behavior modification and/or sanitization to the reduction of microbial counts on harvested produce throughout the expected shelf life. Research dating back to 1984, corroborated by recent studies, suggest that peat moss substrate used in the hydroponic cultivation of lettuce is a possible source of microbial contamination in the system. Currently, most hydroponic lettuce growers harvest and package their marketable size lettuces with intact root ball and flip the lettuce over during packaging. With a high microbial load on the peat moss substrate, there is a risk of microbial transfer unto the edible portion. Since the system is believed to be clean, no sanitizer wash is performed before storage.

In this study, we sanitized the root ball and modified the packaging to evaluate the effect on microbial load and shelf life of the lettuce. Treatment consisted of 3 factors: factor 1 - packaging ('flipped over' and 'no flipped over', referring to manual inversion of the lettuce to wrap roots around peat plug), factor 2 - sanitizers (chlorine [Cl-200 ppm], peroxyacetic acid [PAA-80 ppm], sterile distilled water [SDW], applied as a manual dip, and no treatment) and factor 3 - shelf life (day 1 and 14 of refrigerated storage). Treatments were grouped using factor 2 (sanitizers) and sub-grouped using factors 1 and 3 (package/shelf life). Each subgroup was dipped in a sanitizer/

SDW 3 times and packaged for storage. Leaves, roots, and peat moss were aseptically removed and enumerated for aerobic plate count (APC), coliform bacteria (CB), yeast and mold (YM), and *Listeria* detection. Colorimetric analysis was done on leaf samples.

Presumptive positive *Listeria* spp. was found on root and plugs but not harvested leaf. However, none of the presumptive positive colonies confirmed positive for *Listeria* after the agglutination test. Over a storage time of 14 days the samples still looked appealing in all treatment groups, however control treatment exhibited a lower Hunter *b value (less yellow color) and color intensity (chromo).

The APC results suggested that the reduction in counts was influenced by both sanitizer and storage time. These factors significantly influenced the effect of microbial reduction on lettuce portions used in the analysis. Packaging did not significantly reduce APC except in samples treated with PAA. PAA significantly reduced APC count on all portions, with 1.8 log CFU/g reduction on the leaf. Storage effect on portions indicated that APC increased with time. The highest APC increase was seen in roots over time. This study therefore suggests that the efficacy of PAA as a sanitizer wash in reducing APC is dependent on the initial microbial load and the time of storage.

There was no significant interaction effect between factors in reduction of CB. Leaves had the lowest CB with chlorine being the most effective in reducing CB. Package method did not have any significant effect in reducing CB. Unlike APC, CB levels decreased during storage on the plug and root samples.

Overall, yeast count increased over time on the water treated portions. PAA significantly reduced yeast count. For mold count, PAA and no flip packaged reduced mold counts on the lettuce root and leaf samples while chlorine and no flip packaged reduced counts on the plug.

The findings in this study are significant because they demonstrate that application of sanitizer to the plugs of harvested hydroponic lettuce can reduce microbial load, improving microbial quality without affecting its sensory quality.

INTRODUCTION

Hydroponic production is an expanding sector for vegetable production. Lettuce is one of the most popular crops in this type of system. In hydroponic production most commercial growers utilize substrates for anchorage and support (Pardossi et al., 2011). The substrates are grouped as organic and inorganic. The organic substrates have high conductance and absorptive properties that enable them to absorb microbes and nutrients unto their surface for plant use (Lopez-Galvez et al., 2014). Organic substrates are known to harbor more fungi while inorganic substrates can harbor more bacteria (Pardossi et al., 2011). This enables the substrates to serve as nutrient reservoirs for plants use. In hydroponic lettuce production marketable size lettuces are harvested and packaged with the 'root ball' (root and substrate). It is believed that this provides a longer shelf life for the plant. However, some studies suggest that the substrates host and harbor many microbes with a risk of microbial transfer to the edible portions (Riser, Grabowski, & Glenn, 1984). In most soil production operations, washing and sanitizer dips are carried out to remove and detach impurities, foreign materials, and microbes to ensure product safety (Banach et al., 2015; Yu et al., 2013; Yang, Swem, & Li, 2003). However, in hydroponic lettuce production, the system is believed to be clean due to lack of contact of the edible portion with the growing substrate. As a result, little to no post-harvest management practice like washing and sanitizer dips are used to reduce the microbial load (Lopez-Galvez et al., 2014). Since most hydroponic lettuces are packaged with intact root ball and are eaten raw there is a need to explore strategies to reduce microbial load on harvested produce.

Fresh fruits and vegetables are known to have high microbial diversity and are potential hosts for most food borne pathogens (Robertson et al., 2016; Berger et al., 2010a, 2010b). Most soil growers incorporate sanitization methods to reduce microbial populations on the surface of fresh produce (Yoon & Lee, 2018; Alexandre, Brandão, & Silvaa, 2011; Fraisse et al., 2011). The extensive use of sanitizer wash on produce reduces microbial load in some instances and promotes the shelf life of produce, but is primarily employed to reduce the likelihood of cross contamination (Neal et al., 2012). Sanitizer efficacy may vary depending on the type of produce, the microbial presence and their behavior, and the concentration of application and processing time (Yoon & Lee, 2018).

Chlorine-based sanitizers are commonly used by fresh produce growers. They is preferred by growers due to the ease of use and low cost (Yoon & Lee, 2018; Petri, Rodríguez, & García, 2015). Concentrations ranging from 50-200ppm are known to be effective. Chlorine-based sanitizers are effective at reducing *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* on fresh produce such as spinach, lettuce, and bell pepper (Chaidez et al., 2018; Trinetta, Linton, & Morgan, 2013; Yu et al., 2013; López-Gálvez et al., 2010). They act by changing the permeability of the cytoplasmic membranes of microbes, damaging DNA with the chlorine product called chloramine, and inhibiting enzymes involved in cell wall component synthesis (Yoon & Lee, 2018).

The efficacy of chlorine-based sanitizers on fresh produce is reportedly declining due to microbial biofilm formation, and pathogen internalization in produce. The efficacy is further decreased in the presence of pH fluctuations and organic matter components. The application of some chlorine-based sanitizers is reported to produce toxic residues on produce. Indiscriminate application could result in the production of trihalomethanes and haloacetic acids, which are potential carcinogenic in humans (Olaimat & Holley, 2012; López-Gálvez et al., 2010).

Peroxyacetic acid (PAA) is an alternative sanitizer that is an effective oxidizing agent used against microbes on fresh produce (Baert et al., 2009; Vandekinderen et al, 2009) and has superior antimicrobial activity relative to chlorine-based sanitizers. It possesses unique characteristics such as robustness against suspended organic matter, switches in pH and temperature, and non-toxic by-products (mainly water and acetic acid). These properties make PAA a preferred alternative to chlorine (Yu et al., 2013). PAA functions by targeting thiol groups in enzymes and proteins, disrupting cell membrane permeability, and inhibiting protein synthesis. The FDA recommended concentration for use as a disinfectant on food produce is ≤ 80 ppm (Yoon & Lee, 2018).

The use of sanitizer dip to treat lettuce root ball before packaging is likely to reduce the microbial population, minimizing the rate of microbial transfer unto the edible portion. Sanitizer application may significantly affect the shelf life and sensory quality of the produce (Alwi & Ali, 2014). There is therefore the need to evaluate the effect of behavior modification and/or sanitization in the microbial count on harvested produce throughout the expected shelf life. In this study, three (3) factors, namely sanitizers, shelf life, and method of packaging were evaluated. Marketable size lettuce was harvested and treated with sanitizers before packaging and storage. Lettuce leaves, root and plug samples were tested to assess the reduction of microbial load and sensory quality on hydroponic lettuce. The microbiological quality parameters assessed were aerobic plate count, coliform count, and fungi comprising of yeast and mold. The sensory parameters used were appearance and color change.

MATERIALS AND METHODS

Experimental Design and Preparation

The experiment was a 2x4x2 factorial arranged in randomized complete block design. Factor one (1) consisted of modified packaging. The packaging used mimicked the system packaging method termed 'flipped-over packaged' and a modified packaging termed 'no flipped-over packaged'. This consisted of manual removal of marketable size lettuces from hydroponic growing system and placement into commercial package. Factor two (2) comprised sanitizers used in the experiment. Commercial chlorine (Cl) and peroxyacetic acid (PAA) were used as sanitizers for this experiment. Sterile distilled water (SDW) and no treatment (control) were used as controls. Concentration used for Cl and PAA were 200 ppm and 80 ppm respectively, representing maximum allowable concentrations for produce wash water. Two (2) L of sanitizer wash was prepared in tap water and changed 2 times for each group of packaging. Factor three (3) was storage time. Two storage times were used; day 1 and day 14 storage which implies storage of harvested and treated lettuce for 1 day and 14 days respectively before analysis with either sensory or microbial parameters.

Sampling

Marketable size lettuce ranging between 98 -128 g were harvested from a functioning commercial hydroponic facility. Harvested lettuce were grouped using sanitizer-package-day. In all, there were 4 main groups (based on sanitizer grouping) with each group consisting of 2 subgroupings (based on package-day). Two liters each of PAA and Cl sanitizers and SDW were prepared twice into a container. Each subgroup lettuce was dipped 3 times into the sanitizer/SDW treatment before packaging. A 'no dipped' before packaging sample was used as a control. Samples were placed in

an insulated cooler, transported, and refrigerated until analysis (within 24 h or 14 days). The experiment was repeated 4 times.

Preparation, Enumeration of APC, YM, and CB, and *Listeria* detection.

Sample preparation technique used for enumeration of APC, YM, CB, and *Listeria* detection was same as t has been previously described in chapter 2.

Appearance and Colorimetric Analysis

Analysis was conducted for each storage group prior to microbiological quality test. Samples were enclosed in a chamber and pictures were taken. Samples were then used for colorimetric analysis. Colorimetric value of the leaves was determined by Hunter L, a, and b analysis. Leaves were cut into a disc-like shape of approximately 5 cm in diameter. Cut leaf was then analyzed using a Hunter L, a, b Model II color difference meter with a 4 cm optical diameter. L, a, and b values were recorded once, and the sample was then rotated 1/3 of a turn two times. Value were recorded again after each turn to give a total of three L, a, and b values for each group. The three values were averaged by the computer to give one overall value for L, a, and b per sample. Chroma, which indicated the intensity of the color, was calculated using the formula $(a^2 + b^2)^{1/2}$. Each treatment combination was replicated twice and repeated four times throughout the experiment.

Statistical analysis

Results of APC, YM, CB and color change were analyzed using R statistical package (R Foundation for Statistical Computing, Vienna, Austria). Counts for APC, YM and CB were log transformed to conform to normality assumption. Color change data were averaged before

analyzing. The analysis of variance (one-way ANOVA and two-way ANOVA) was performed to compare the means of each sample and the interaction effect. Data was analyzed using analysis of variance (one-way ANOVA) with Tukey HSD mean separation test at $\alpha = 0.05$. A contrast post ANOVA was run to determine the test trend of significant samples.

RESULTS

Aerobic Plate Count (APC)

ANOVA analysis on sample portion (leaf, root and plugs) is shown in Table 3.1, which shows that factor 2 (sanitizers), factor 3 (storage condition), and portion used in the analysis significantly affected APC (p <0.001 and p <0.05). An interaction effect was seen among sanitizer/storage time/package, and between storage time/ portion and sanitizer/portion. This suggests that the main factors affecting APC reduction on the lettuce were storage time, sanitizer, and package. However, since the levels that make up the portion (plug, root and leaf) are of greatest interest in this study, further analysis was done by splitting each level in the portion to assess how effective the factors used in this study were able to reduce APC on them.

Table 3. 1: Extrapolated Anova table showing significant factor and interaction effects for APC enumeration~ (n=16).

Source	Df	SS	MS	F value	Pr(>F)
Storage time	1	103.70	103.70	102.806	<2e-16*
Sanitizer	3	52.10	17.40	17.22	1.33e-09*
Portion	2	1229.00	641.50	609.23	<2e-16*
Storage time: Portion	2	23.70	11.90	11.77	1.85e-05*

Sanitizer: Portion	6	48.30	8.10	7.99	1.94e-07*
Storage time: Sanitizer: Package	3	8.50	2.80	2.81	0.04*

~Df=degree of freedom; SS= Sum of squares; MS= Mean sum of square; F= F-statistics;
Significant difference (p<0.05) among groups based on ANOVA analysis

Figure 3.1 shows effects of interaction of sanitizer, storage time and package on the reduction of APC on lettuce plug (A), root (B) and leaf (C). On the plug samples, the effect of all the factors in reducing APC was low. Between PAA and the control treatment no significant reduction of APC was observed. Water treated plugs significantly (p<0.05) increased on storage day 14 and no flipped packaged samples. The sanitizers effectively reduced APC on the root and the leaf samples. On the root samples PAA significantly (p<0.05) reduced APC at storage day 1 and 14. All PAA treated root samples with the no flipped packaged on day 14 of storage had a reduction in APC. For the leaf samples, count was significantly reduced from 4.7 log on the control to 2.1 log on the PAA treated samples. PAA and chlorine significantly (p<0.05) reduced APC on samples stored for one day with no flipped packaging. On day 14 of storage, PAA reduced APC on the leaf sample. This result suggests that the efficacy of the sanitizers decrease over the storage time. Further analysis was run on the storage time and sanitizers to determine its effect on reducing APC.
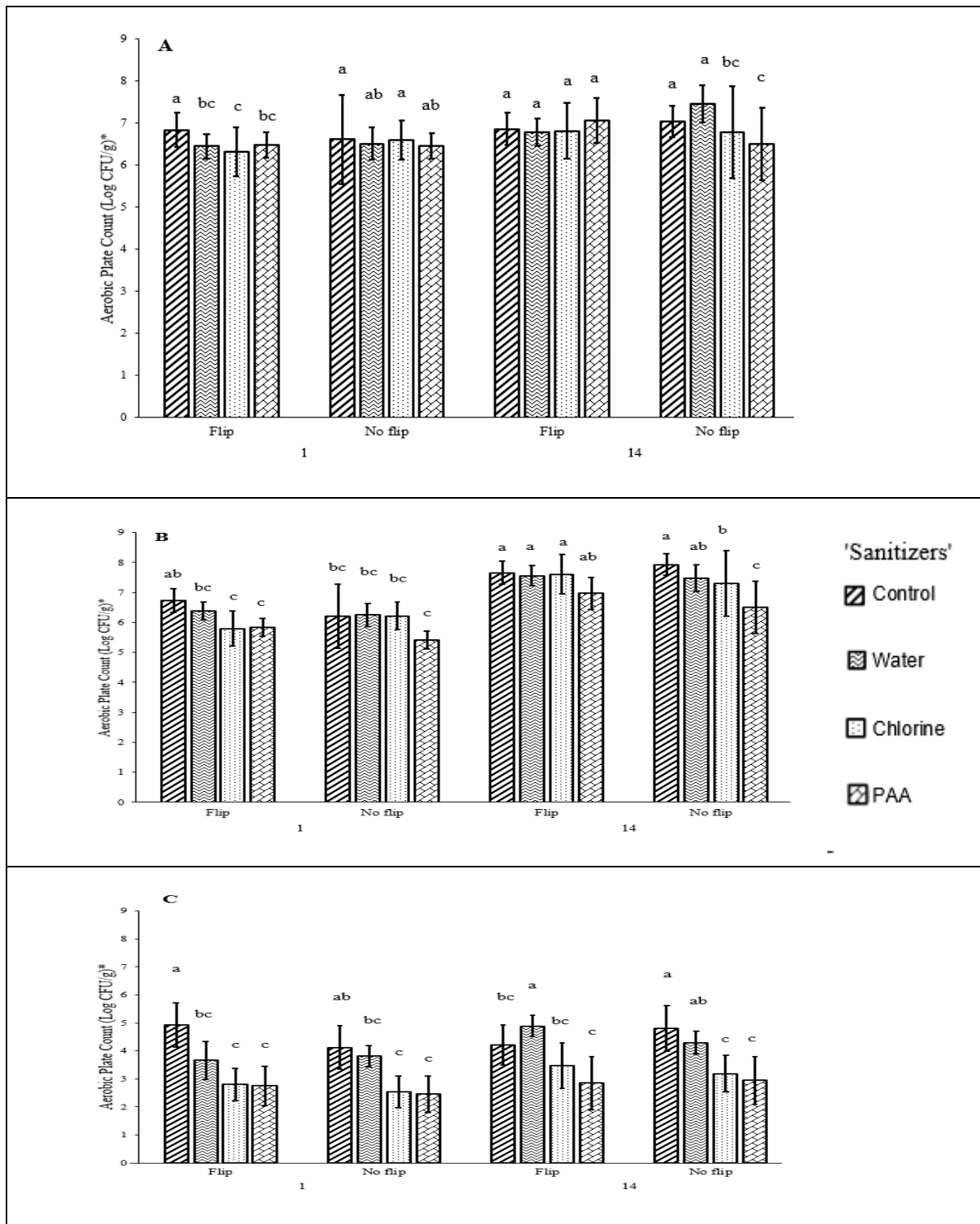
Figure 3. 1 Effects of interaction of sanitizer, storage time and package on the reduction of APC on lettuce plug (A), root (B) and leaf (C) (n=16).

*colony forming unit/gram

Error bars represent the standard deviation of means

Letters indicate significant difference (p<0.05) among groups based on ANOVA followed by a Tukey's post hoc test

Coliform Bacteria Count (CB)

Table 3.2 shows the ANOVA table with significant factor effects for CB enumeration. Overall, there was no significant interaction effect. Each factor used in this study significantly affected coliform counts. Further analysis was run on the factors to understand how each contributed to the overall CB level.

Table 3. 2: Extrapolated Anova table showing significant factor effects for CB enumeration~ (n=16).

Source	Df	SS	MS	F value	Pr(>F)
Storage time	1	1.10	1.06	4.65	0.03*
Sanitizer	3	3.30	1.10	4.83	<0.00*
Portion	2	336.40	168.19	739.15	<2e-16*
Package	1	1.10	1.06	4.65	0.03*

~Df=degree of freedom; SS= Sum of squares; MS= Mean sum of square; F= F-statistics;
Significant difference (p<0.05) among groups based on ANOVA analysis

Figure 3. 2 shows effect of sanitizer, packaging and storage time in the reduction of coliform bacteria on lettuce plug (A), root (B) and leaf (C). The coliform bacterial count on the leaf samples was below the detection limit (3 MPN/g). Storage time different have any significant effect on Day 1 of storage of treated plug and root samples with flipped packaging had high CB count on control treatment. Overall, chlorine and PAA were less effective in reducing CB levels on the plug samples. Roots samples decreases over time were observed only in samples treated with chlorine or PAA, regardless of flipping. Coliform levels increased from day 1 to 14 on the roots of samples that were untreated or dipped in water.
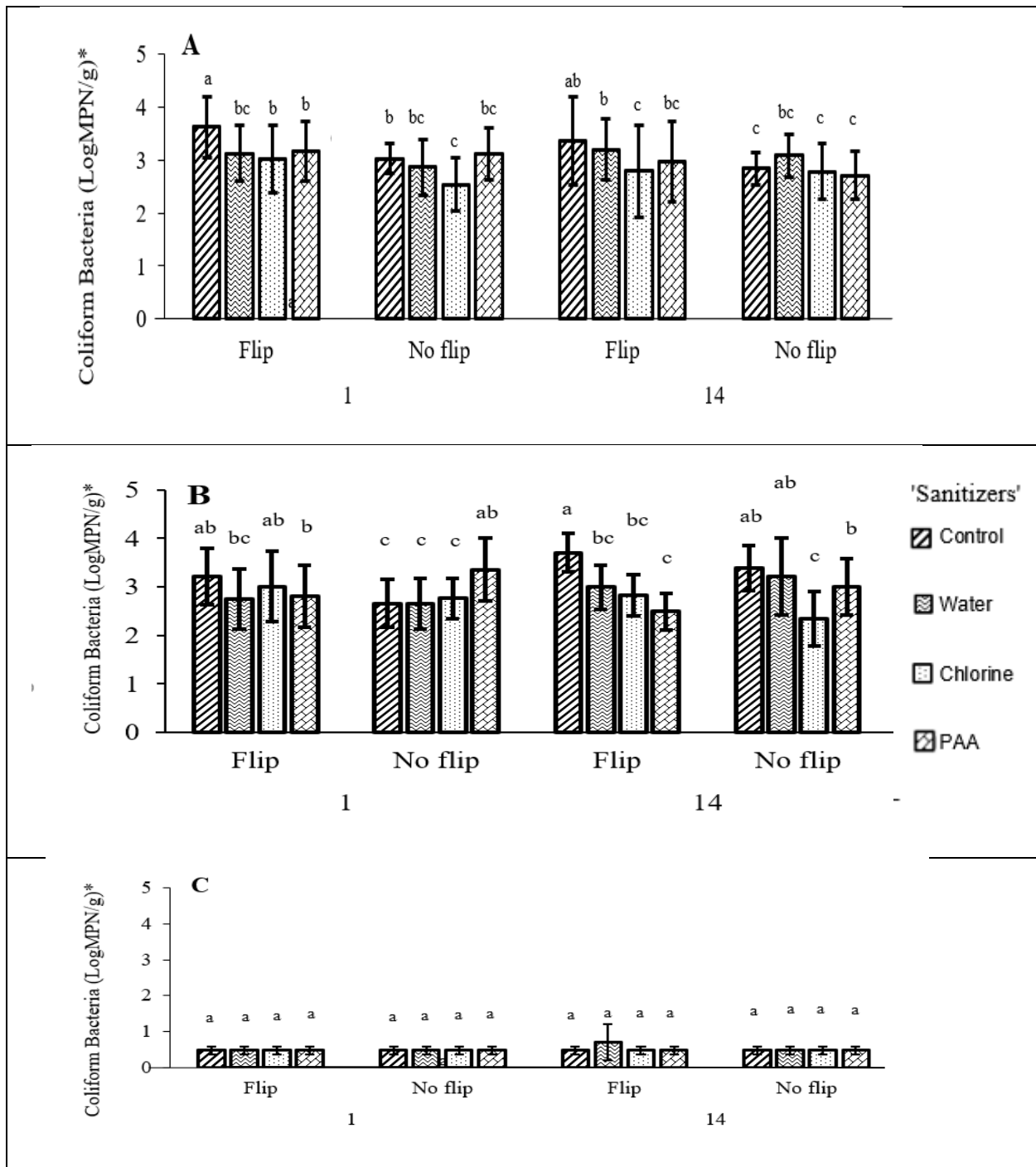
Figure 3. 2 Effect of sanitizer, packaging and storage time in the reduction of coliform bacteria on lettuce plug (A), root (B) and leaf (C) (n=16).

*Most probable number/gram

Error bars represent the standard deviation of means

Letters indicate significant difference (p<0.05) among groups based on ANOVA followed by a Tukey's post hoc test

Yeast and Mold Count (YM)

From the Anova analysis, some factors used in the experiment were different in yeast (table 3.3) and mold (table 3.4) counts. In yeast count interaction, effects were seen in sanitizer and portion while in mold count, effects were seen in sanitizer and package. In both yeast and mold a three-factor interaction was found in sanitizer, package, and portion. Package as a main effect played an insignificant role in reducing YM. Hence, storage time and sanitizer were the dominating factors influencing YM. Analysis was further carried out to determine how each of these factors significantly contributed.

Table 3. 3: Extrapolated Anova table showing significant factor and interaction effects for yeast enumeration~ (n=16).

Source	Df	SS	MS	F value	Pr(>F)
Storage time	1	4.68	4.68	0.00	<0.00*
Sanitizer	3	1.70	0.57	0.02	0.02*
Portion	2	153.70	307.421	0.00	<2e-16*
Sanitizer: Portion	6	2.19	0.36	0.04	0.04*
Storage time: Portion: Package	6	2.50	0.42	0.022	0.02*

~Df=degree of freedom; SS= Sum of squares; MS= Mean sum of square; F= F-statistics;
*Significant difference (p<0.05) among groups based on ANOVA analysis

Table 3. 4: Extrapolated Anova table showing significant factor and interaction effects for mold enumeration~ (n=16).

	Df	SS	MS	F value	Pr(>F)
Storage time	1	1.73	1.73	0.00	<0.00*
Sanitizer	3	1.70	0.57	0.02	0.02*
Portion	2	223.82	111.91	0.00	<2e-16*
Sanitizer: Pack	3	1.34	0.45	0.26	0.01*
Sanitizer: Portion: Package	6	3.27	0.54	0.022	<0.00*

~Df=degree of freedom; SS= Sum of squares; MS= Mean sum of square; F= F-statistics;
Significant difference (p<0.05) among groups based on ANOVA analysis

Efficacy of sanitizer and storage time in the reduction of mold and yeast count of lettuce plug, root, and leaf samples are shown in figures 3.4 and 3.5 respectively. Storage time only influenced the yeast count on water treated leaf samples stored for 14 days. This indicates that the reduction in YM could solely be due to sanitizer/water treatment effect. On leaf samples chlorine and PAA led to 1log reduction on day 1. Neither factors had any effect on mold on the leaf samples.
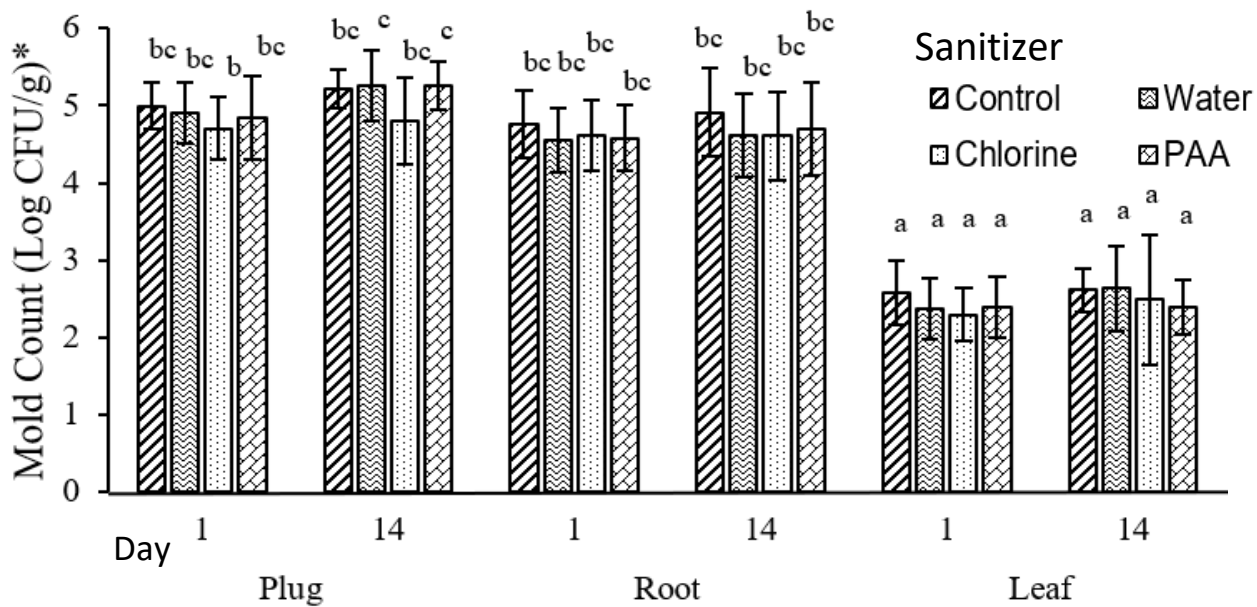
Figure 3. 3 Efficacy of sanitizer, and storage time in the reduction of mold count on lettuce plug, root, and leaf (n=16).

[*]colony forming unit/gram

Error bars represent the standard deviation of means

Letters indicate significant difference (p<0.05) among groups based on ANOVA followed by a Tukey's post hoc test
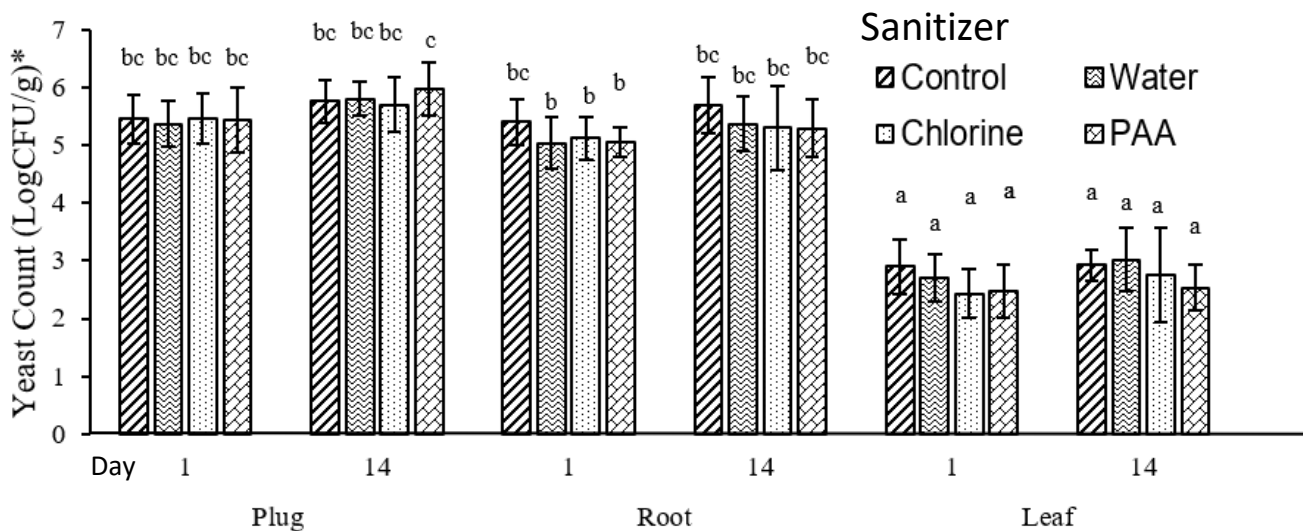
Figure 3. 4 Efficacy of sanitizer, and storage time in the reduction of yeast count on lettuce plug, root, and leaf (n=16).

[*]colony forming unit/gram

Error bars represent the standard deviation of means

Letters indicate significant difference (p<0.05) among groups based on ANOVA followed by a Tukey's post hoc test

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Listeria Detection

Positive presumptive *Listeria* result was found on root and plug samples (Table 3.5). However, none of the presumptive colonies confirmed positive for *Listeria* spp.

Table 3. 5: Listeria detection on harvested lettuce leaf, plug, and root (n=16)

Sample	*Listeria* Detected [a]	
	Presumptive test	Confirmed test
Plug	+	-
Root	+	-
Leaf	-	-

[a] *Listeria* detection code + implies present; - implies absent.

Appearance and Colorimetric Analysis

Lettuce retained its appealing appearance over time (Appendix B). No phytotoxic effect was seen on the lettuce leaves treated with either Cl or PAA, suggesting that the concentration applied for each did not have any harmful effect on the sensory parameter (color change). The water and no treatment (control) samples lost their texture over time.

Table 3.6 shows the colorimetric analysis on the lettuce leaves. Overall, storage time did not significantly affect the color change. The L (lightness) was around the midpoint on a 0-100 scale for all samples and the a (redness) was in the negative predicting the greenish coloration of the

leaves. Both the L and a were not significantly different among each of the factors. The b (yellowish) and chromo (color intensity) were significantly affected in the control flip samples indicating a lower color intensity and a less yellowish leaf color.

Table 3. 6: Colorimetric analysis on lettuce leaves (n=16).

Leaf sample treatment (sanitizer and Package)	L	A	b	Chromo
Chlorine flip	52.81 a*	-15.13 a	41.50 a	44.18 a
Chlorine no flip	51.35 a	-15.08 a	41.26 a	43.93 a
PAA flip	50.98 a	-14.56 a	38.33 a	41.00 a
PAA no flip	50.71 a	-15.13 a	39.43 a	42.24 a
Water flip	52.81 a	-14.90 a	40.37 a	43.03 a
Water no flip	51.45 a	-15.01 a	40.19 a	42. 90 a
Control flip	51.28 a	-14.51 a	25.67 b	30.00 b
Control no flip	52.23 a	-14.71 a	40.87 a	43.44 a

***Different letters within each column indicate a significant difference ($p<0.05$) among groups based on a one-way ANOVA followed by a Tukey's post hoc test.

DISCUSSION

The overall CB levels were low on the leaves, and not problematic. However, the relatively high levels of coliform documented in plugs and on roots highlights the potential for harborage of gastrointestinal pathogens. Because these portions are packaged with the edible leaves, transfer of such contaminants is a realistic concern. Counts were highest in the peat moss plugs samples and treatments applied significantly influenced the level of APC on leaves sampled and CB for root samples. PAA and chlorine were most effective when the initial microbial loads were low.

The results from this study show that sensory parameters of the harvested lettuce are maintained as a result of dipping of the substrate. The sensory parameter analysis from this study suggests that dipping the peat substrate in any solution promotes plant metabolic activity after harvesting which enables the lettuce to retain its freshness and appealing look over 14 days. All metabolic activity occurred in the produce after harvest. However, depending on the rate, these activities may either result in produce deterioration or extend the shelf life (Tanaka et al., 2011). A crop that is uprightly taken from its parent source of nutrient supply can easily lose its expected shelf life due to stress shock. This may have been the case of the no treated (control) sample resulting in the low color intensity and yellowness over time.

Application of any solution can physiologically keep the plants alive and gradually reduce its metabolic activity in order to store up energy hence extending its shelf life, however, the moist environment serves as breeding grounds for most pathogenic microbes (Banach et al., 2015). The sterile distilled water application used in this study had an increased count for all microbial enumerations even though its sample was still fresh and appealing. The lack of antimicrobial agents in the SDW made the samples treated with SDW a preferred host the microbes relative to the chlorine (Cl) and peroxyacetic acid (PAA) (Petri et al., 2015). Though washing is known to reduce superficial microbes on produce it is mostly focused on the sensory quality of removing dirt to create an appealing and acceptable produce for consumer acceptability (Fraisse et al., 2011).

Results from this study also suggest that reduction of microbial population on the lettuce is influenced by several factors. Microbial population increase during storage time may be attributed to loss of efficacy of the sanitizers and adaptation of microbes to storage conditions. Package modification reduced APC with no pattern seen on the YM. Microbial transfer is mostly increased during post-harvest activities which involve more handling. YM have diverse propagules such as

spore and mycelium for dispersal, hence with less handling of the produce or packaging method used, it will have a less significant effect in reducing their growth and reproduction (López-Gálvez et al., 2010). Microbial count on the harvested leaves was very low in cases where microbial population was reduced on the peat (the portion with the highest microbial load). This suggests that the sanitizers were efficacious when microbial concentration was relatively low. No *Listeria* spp. was found in the system. CB was relatively low in the harvested leaves. This suggests that the system ensures good sanitation measures as CB is of fecal matter origin and *Listeria* spp. are ubiquitous (Schwaiger et al., 2012; Whipps et al., 2008). PAA was the most effective sanitizer in APC and YM. Chlorine and PAA were most effective against CB on the root samples. In APC, PAA and no flipped packaging method were effective in reducing microbial count. PAA is an effective oxidizing agent whose efficacy is not influenced by organic matter, pH, and temperature changes (Weller et al., 2013; González-Aguilar et al., 2012). Chlorine was effective in against CB control. However, with a reduction in CB count as storage time progressed, chlorine is not assured of retaining its efficacy should microbial load increase. Also, the formation of biofilm by CB may significantly reduce chlorine's efficacy over time (Lianou & Koutsoumanis, 2013; Strayer, 1994). From this study we can deduce that, storage time and sanitizers can help reduce the microbial load on the produce however the efficacy of the sanitizers are lost over time.

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CHAPTER 4

EVALUATION OF MICROBIAL LOAD ON COMMERCIAL PEAT MOSS SUBSTRATES AND EFFECT OF HEAT TREATMENT

ABSTRACT

This study builds on previous work to assess the effect of heat treatment on peat moss plugs and survey microbial load in different brands of peat plug. Substrates are added to hydroponic production to provide anchorage for plant roots. They are grouped as either organic or inorganic. Organic substrates are more often used due to their inherent ability to support plant growth and their ecologically friendly nature. The ability of peat to balance the water-to-oxygen ratio and to retain water and nutrients makes it preferable to other substrates in most commercial production systems. The rate of decomposition and drying of the peat moss results in varying chemical and physical properties in the final product. Some peat mosses are grouped as "white" or "light" due to the color resulting from low decomposition of the layers. Others are grouped as "black" if the peat moss is well decomposed. It is not known if the time for decomposition and/or heat treatment (drying) will help to reduce the microbial population on peat moss substrates.

In this study, peat moss plugs were obtained from five separate manufacturers. One group of substrates was obtained from the actively growing hydroponic system from which samples were taken for analysis as described in chapters 2 and 3. These plugs were subdivided into three treatment groups (coded H1, H2, and H3), of which two were heat treated. The H1 group received heat treat at 180ºF for 30 min, H2 at 150ºF for 30 min and H3 had no heat treatment (control). Substrate analysis for greenhouse parameters were carried out on H1, H2, and H3. The remaining peat moss samples consisted of three spongy, moist plugs coded Com1, Com2, and Com3 and a compact dry plug coded D1. Microbiological quality assessment was carried out on all the peat

moss samples using aerobic plate count (APC), coliform bacteria (CB), and yeast and mold (YM) as indicators.

The results show that APC and YM can be heat controlled. Overall, microbial populations were significantly lower on the compact dry plug (D1). Among the spongy plugs, Com2 was significantly lower in all counts compared to Com1 and Com3, despite similar water activity and pH. This study indicates heat treatment can reduce the microbial load in peat plugs, however the amount of reduction is dependent on the temperature and time of treatment applied. Also, this study predicts that the different mode of manufacturing of peat moss plugs can confer its ability to support microbial populations. Mostly, the drier, compacted, black peat moss had low microbial counts, but these are likely to increase during active use for growing.

INTRODUCTION

Plant root zone environment is an important pillar for plant survival which requires an optimum balance of oxygen and water (Bar-tal et al., 2008). A deficit in the water-to-oxygen balance results in impairment of photosynthesis and respiration leading to low nutrient uptake, poor growth, and reduced yield. In soil production, the soil serves as a medium to provide plants with nutrients for growth (Tanaka et al., 2011). However, a deficit in the use of soil as a medium is that its pores either hold water or air pockets at a given time. Soil imbalance in water-to-oxygen ratio takes a longer time to be corrected compared to soilless growing environments (Xiao et al., 2015). In severe cases this results in a highly aerated or waterlogged soil (Settanni et al., 2013). In hydroponic production this is overcome by using substrate to provide a better water-to-oxygen balance needed by the plant. Hydroponic production utilizes substrates to provide support for plant roots, enhance aeration, and retain moisture for plant use (Jordan et al., 2018; Abd-Elmoniem et

al., 2006). The substrate's larger particle size enables it to create room for absorption of water and oxygen at the same time. The selection of the type of substrate depends on availability and the type of crop (Carlile, Cattivello, & Zaccheo, 2015). Substrates are classified as organic or inorganic based on the material used, and this influences the capacity and properties of the substrate (Lind, 2016).

In most hydroponic lettuce production, peat moss substrates are utilized. Peat moss is an organic layer of decomposed, fibrous material. Its organic material composition makes it sustainable and easily disposed of after use. It is the largest available organic material that is produced with mire. The incomplete decomposition of bryophyte mosses from the genus *Sphagnum* is utilized in the production of peat moss as a substrate for the hydroponic system and other agricultural purposes such as soil amendment and potting mix (Carlile, Cattivello, & Zaccheo, 2015). It is highly utilized for commercial production due to its water holding capacity, cation exchange capacity (560-15800) and adsorptive potential. Its characteristics differ depending on the mode of production and rate of decomposition (Bar-tal et al., 2008). Peat moss is produced as either milled or sod type. In milled peat moss, milling machinery are used to remove the surface peat layer from peatland which is then further dried and aggregated into windrows or piles for marketing. Sod peat are traditionally cut into large pieces and dried. Sod peat has larger particle size, hence higher air content than milled peat. The peat moss is further classified using the 'practical von Post scale'. On this scale peats are grouped as H1-H3 (undecomposed of low humification), H4-H6 (partly decomposed, and H7-H10 (highly decomposed) (Carlile, Cattivello, & Zaccheo, 2015). Decomposition of peat confers its final coloration and property for cultivation. H1-H3 indicates white peat while H7 and above indicates black peat coloration. Natural peat moss is acidic, and white peat moss has a pH of 3-4 while a black peat has a pH of 5.5-7.3 (Bar-tal et al., 2008).

Peat moss is known to retain nutrients unto its surface due to its high cation exchange capacity. This reservoir ability also makes the peat substrate a good host for microbes. Research, as well as our previous studies, have suggested that peat moss is a potential source of contamination in hydroponic lettuce production (Riser, Grabowski, & Glenn, 1984). Further drying of peat moss by dry heat application confers the final chemical and physical properties of the substrate. However, not much is known about the effect of the dry heat on the microbial population on the peat substrate. Hence, further evaluation of different kinds of peat moss and heat sterilization will provide insight into their microbial host potential abilities.

MATERIALS AND METHODS

Sample Size and Preparation

Five different peat moss substrates were used for this study. This included substrates from the active hydroponic lettuce grower from whom the samples for chapters 2 and 3 were obtained. These substrates were divided into three groups. One group was heat treated at 180°F for 30 mins (coded as H1), another was heat treated at 150°F (coded as H2) for 30 mins, and the other was not heat treated (control- coded as H3). Details on the other four peat mosses used can be found in Appendix C. Briefly, peat moss Com1, Com2, and Com3 had a soft, spongy texture with brownish black coloration and were slightly soaked. Peat moss D1 had a hard, dry, compacted texture with black coloration. Peat moss was aseptically removed from the package and about 10 g each of peat moss sample was weighed aseptically into sterile stomacher bags. The weight of the peat moss coded 'D1' was about 5.3 g per plug. A 1/10 dilution (w/v) of the commodity was made using 0.1% buffered peptone water (BPW). Samples were homogenized for 2 minutes. Serial dilutions

were made by pipetting 1 ml of content from the stomacher bag into 9 ml of BPW tubes. This study was repeated three times.

Enumeration of Aerobic Plate Count (APC), CB, YM.

All procedures used are similar to the previously described methods in chapter 2.

Determination of pH and Water Activity

Peat moss plug samples were chopped into pieces and approximately 2 g were weighed into plastic Falcon tubes (VWR Brand; Boston, MA). Samples where choked in 1 ml sterile distilled water. The pH of the samples was determined with an Orion Model 320 PerpHecT LogR meter (Beverly, MA). For water activity analysis, approximately 1 g of each chopped samples was taken. Analysis was done using an Aqua Lab CX-2 water activity meter (Decagon Devices Inc., Pullman WA)

Statistical Analysis

Results of APC, YM and CB were analyzed using R statistical package (R Foundation for Statistical Computing, Vienna, Austria). Counts for all enumeration were log transformed to conform to the normality assumption. The analysis of variance (one-way ANOVA) was done to compare the means of each sample. Data was analyzed using analysis of variance (one-way ANOVA) with Tukey's HSD mean separation test at $\alpha = 0.05$. A contrast post ANOVA was run to determine the test trend of significant samples.

RESULTS

Aerobic Plate Count (APC)

Figure 4.1 shows result of enumeration of aerobic plate count. Peat moss Com2 and D1 were significantly lower in count compared to all other samples. Heat treatments of the plugs (H1, and H2) slightly reduced the count compared to H3 (no heat treatment) but this reduction was not significant. The other alternative spongy wet peat moss substrate had APC levels comparable to those in the plugs used for prior research (H).

Figure 4. 1 Enumeration of aerobic plate count for peat moss samples (n=6).
*colony forming unit/gram
~ H1=180°F heat treatment, H2=150°F heat treatment, H3= no heat treatment, Com1=Rapid rooter, Com2=Root riot, Com3=Viagrow, and D1 = Junlinto
Error bars represent the standard deviation, line indicates minimum detection limit (10 CFU/g)
Different lowercase letters indicate significant difference (p<0.05) among groups based on a one-way ANOVA followed by a Tukey's post hoc test

Coliform Bacteria (CB)

Figure 4.2 shows the result of enumeration of coliform bacteria (confirmed counts only). Peat mosses Com2 and D1 were relatively low in count. Heat treatments of the plugs H1 (180°F) significantly reduced the CB compared to H2 (150°F), and H3 (no heat treatment). This indicates that the application of higher temperatures of heat to the plugs reduced the coliform load. The other plug had a similar trend as the APC enumeration. The other alternative spongy wet peat moss substrate had a high APC except for Com2.
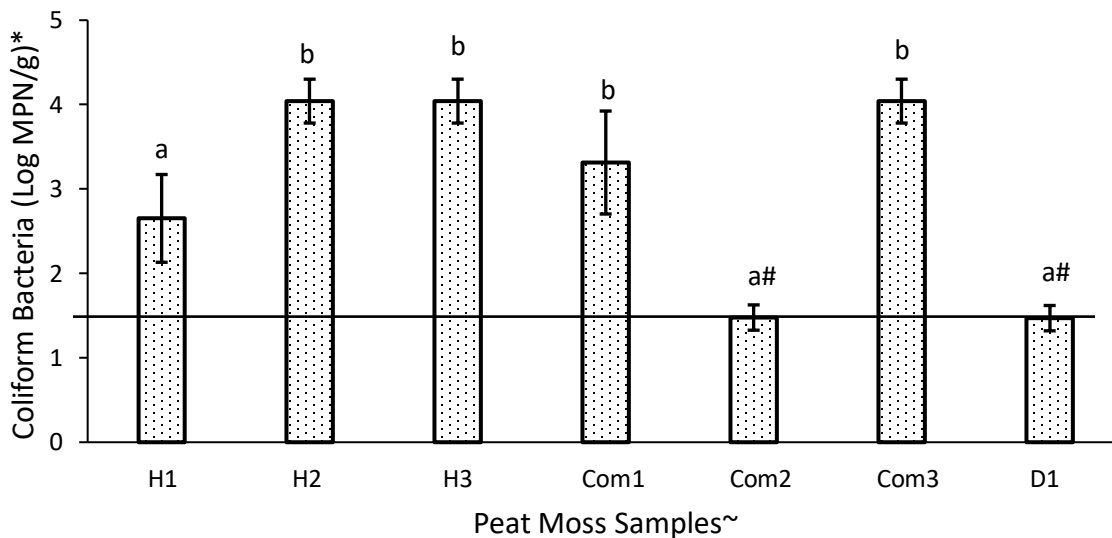
Figure 4. 2 Enumeration of coliform bacteria count for peat moss samples (n=6).
* Most probable number/gram
~ H1=180°F heat treatment, H2=150°F heat treatment, H3= no heat treatment, Com1=Rapid rooter, Com2=Root riot, Com3=Viagrow, and D1 = Junlinto
Error bars represent the standard deviation, line indicates minimum detection limit (<30 MPN/g)
Different lowercase letters indicate significant difference (p<0.05) among groups based on a one-way ANOVA followed by a Tukey's post hoc test
estimated count < 30 MPN/g.

Yeast and Mold Count (YM)

Figure 4.3 shows the result of enumeration of yeast and mold. Heat treatment of the plugs H1 had no significantly, effect in reducing yeast and mold count. The other 3 spongy like plugs (Com1,

Com2, and Com3) were between log 2.5-2.8 CFU/g. The dry compact plug (D1) had the lowest

counts for both yeast and mold, suggesting its composition does not support YM.
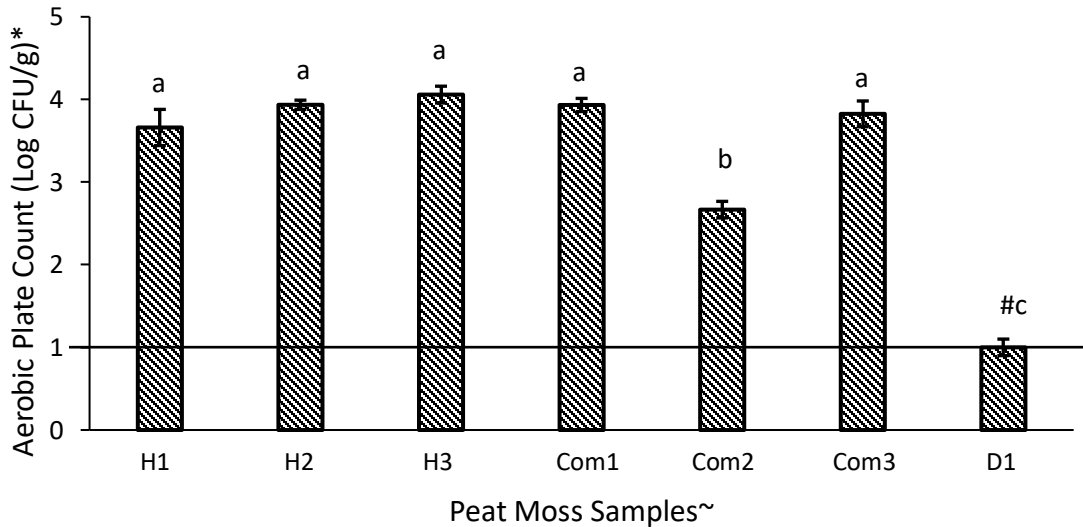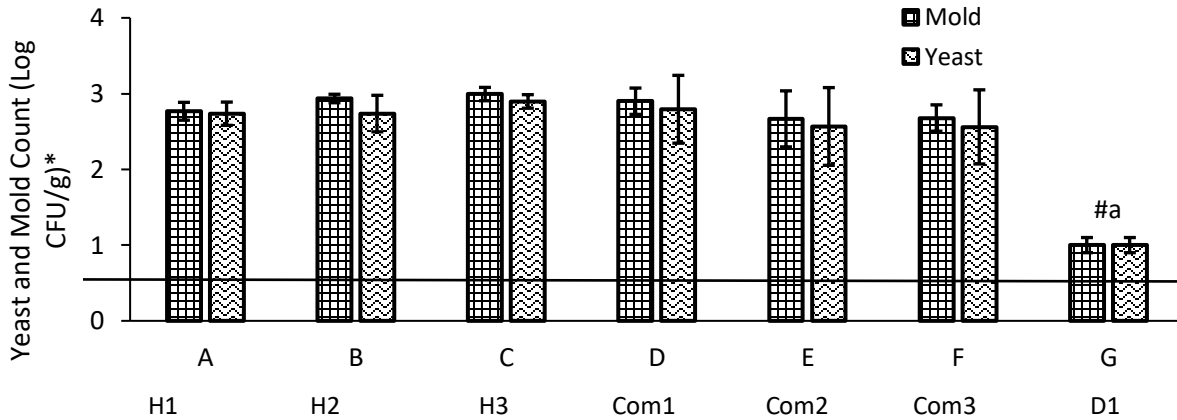

Figure 4. 3 Enumeration of yeast and mold count for peat moss samples (n=6).
*colony forming unit/gram
~ H1=180°F heat treatment, H2=150°F heat treatment, H3= no heat treatment, Com1=Rapid rooter,
Com2=Root roit, Com3=Viagrow, and D1 = Junlinto
Error bars represent standard deviation, line indicates minimum detection limit (10 CFU/g)
[a] indicates significant change ($p < 0.05$) among groups based on a one-way ANOVA followed by a
Tukey's post hoc test
estimated count < 1CFU/g

pH and Water Activity

Table 4.1 shows pH and water activity of the various peat moss samples. Overall all the peat

moss plug samples were slightly acidic with a high-water activity, expect D1. D1 was

significantly different from all the other samples.

Table 4. 2 pH and water activity of the various peat moss samples

Peat Plugs~	Characteristics	pH	Water Activity
H1	180 F @30 min	5.4 a	0.85 a
H2	150 F @ 30 min	5.3 a	0.98 a
H3	No heat treatment	5.4 a	0.95 a
Com1	Moist and spongy	5.8 a	1.00 a
Com2	Moist and spongy	5.6 a	1.00 a
Com3	Moist and Spongy	5.9 a	1.00 a
D1	Dry and compact	2.5 b	0.34 b

Different lowercase letters indicate significant difference (p<0.05) among groups based on a one-way ANOVA followed by a Tukey's post hoc test
~ H1=180°F heat treatment, H2=150°F heat treatment, H3= no heat treatment, Com1=Rapid rooter, Com2=Root riot, Com3=Viagrow, and D1 = Junlinto

DISCUSSION

The results from this study show that different temperatures used in heat treatment of peat plugs have relatively varying effects on the rate of reduction of microbial populations. The180^{o}F treatment of plugs for 30 mins had a greater reduction effect on microbial load than 150°F treatment for 30 mins, as expected. However, this treatment led to a significant reduction of only coliform bacteria. The exact ideal temperature ranges for heat treatment of peat plugs is not known, however, soil sterilization to control pathogens has shown to be effective between 120°F for inactivation of oomycetes and 212°F for inactivation of viruses and weeds. Spore forming fungi

and dominant vegetative propagules such as sclerotium, require a temperature range higher than a 160°F. Studies on bacterial control have demonstrated efficacy between a temperature range of 145°F and 180°F (Castro-Ibáñez et al., 2015; Tanaka et al., 2011). This work suggests that an equivalent range is not sufficient for decontamination of peat plugs.

This study also suggested that different composition, pH, water activity, texture, and rate of decomposition significantly influence the ability of microbes to grow and survive in peat plugs. Results from commercial peat moss plugs suggested that the drier, compacted black plugs had less ability to support the growth/survival of all microbial populations enumerated relative to the spongy, wet black plugs. This can be attributed to high conductance abilities of the spongy wet brownish black plugs which aids microbes to affix to their surface and particularly to the presence of water required for microbial metabolism. The high fungi levels in the spongy, wet black plugs are in line with Riser's (1984) finding which suggested that peat moss aided fungi growth due to their organic matter composition.

Overall, heat treatment of the plugs did not significantly reduce the microbial populations enumerated except CB. High temperatures above 150°F will be more effective, however, the issue of killing beneficial microbes may put heat treatment at disadvantage. Other microbe-reducing methods should therefore be evaluated. One of these methods is the mode of manufacturing of the peat moss. Since the dry compacted black peat had a relatively low microbial load, adaption of techniques that reduce the water holding potential while retaining the required characteristics to support the plant growth will be an area worth evaluating.

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APPENDIX A: MOST PROBABLE NUMBER TABLE

Table A. 1 Most Probable Number (MPN) table by FDA Bacteriological Analytical Manual (Feng et al., 2001).

For 3 tubes each at 0.1, 0.01, and 0.001 g inocula, the MPNs per gram and 95 percent confidence intervals.

Pos. tubes 0.10	0.01	0.001	MPN/g	Conf. lim. Low	High	Pos. tubes 0.10	0.01	0.001	MPN/g	Conf. lim. Low	High
0	0	0	<3.0	--	9.5	2	2	0	21	4.5	42
0	0	1	3.0	0.15	9.6	2	2	1	28	8.7	94
0	1	0	3.0	0.15	11	2	2	2	35	8.7	94
0	1	1	6.1	1.2	18	2	3	0	29	8.7	94
0	2	0	6.2	1.2	18	2	3	1	36	8.7	94
0	3	0	9.4	3.6	38	3	0	0	23	4.6	94
1	0	0	3.6	0.17	18	3	0	1	38	8.7	110
1	0	1	7.2	1.3	18	3	0	2	64	17	180
1	0	2	11	3.6	38	3	1	0	43	9	180
1	1	0	7.4	1.3	20	3	1	1	75	17	200
1	1	1	11	3.6	38	3	1	2	120	37	420
1	2	0	11	3.6	42	3	1	3	160	40	420
1	2	1	15	4.5	42	3	2	0	93	18	420
1	3	0	16	4.5	42	3	2	1	150	37	420
2	0	0	9.2	1.4	38	3	2	2	210	40	430
2	0	1	14	3.6	42	3	2	3	290	90	1,000
2	0	2	20	4.5	42	3	3	0	240	42	1,000
2	1	0	15	3.7	42	3	3	1	460	90	2,000
2	1	1	20	4.5	42	3	3	2	1100	180	4,100
2	1	2	27	8.7	94	3	3	3	>1100	420	--

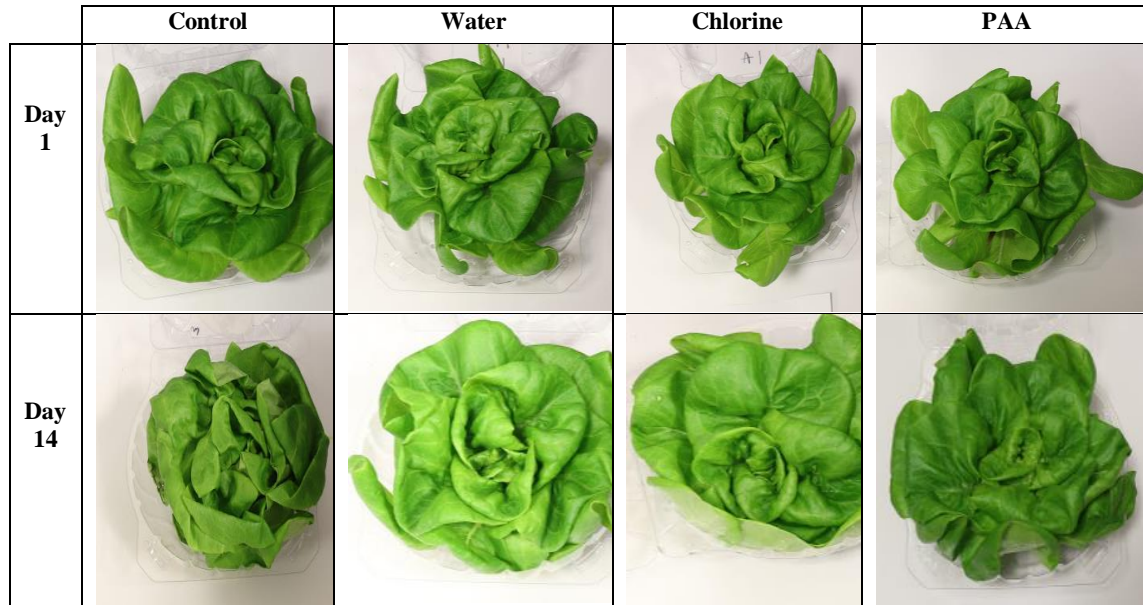**APPENDIX B: PHOTOGRAPHS OF EFFECT OF TREATMENT ON LETTUCE**

	Control	Water	Chlorine	PAA
Day 1				
Day 14				

Figure B. 1 Photographs of lettuces treated with chlorine, peroxyacetic acid (PAA), sterile distilled water, and no treatment (control)

APPENDIX C: DETAILED INFORMATION OF PEAT PLUGS USED

Table C. 1 Peat moss start plugs details

Peat Plugs	Commercialized Name/Manufacture Details.
H1	Peat Plugs, Grow Tech, South Portland, ME
H2	Peat Plugs, Grow Tech, South Portland, ME
H3	Peat Plugs, Grow Tech, South Portland, ME
Com1	Rapid root (General hydroponic Inc, Santa Rosa, CA)
Com2	Root riot (Hydro Dynamics International, Lansing, MI)
Com3	Viagrow Super Plugs, 25 Organic Seed Starter Plugs (Viagrow, Atlanta, GA)
D1	Junlinto,5Pcs Peat Pellets Seed Nursery Starting Plugs Pallet Seedling Soil Block

BIOGRAPHY OF THE AUTHOR

Adwoa Safoa Dankwa was born in New Tafo in the Eastern region of Ghana. She attended high school at the Cocoa Research Institute of Ghana (CRIG) and graduated with a First-Class Honors from the Kwame Nkrumah University of Science and Technology (KNUST) with a Bachelor of Science in Agriculture, and a major in Plant Pathology and subsequently served as a Teaching and Research Assistant at the Department of Crop and Soil Science in KNUST. Her professional experiences include serving as a Teaching/Graduate Assistant at the University of Maine, Field Researcher at the Presque Isle Research farm, Extension Personnel Assistant at the Ministry of Food and Agriculture in Ghana, and a Research Assistant at CRIG. She has five publications to her credit as well as several others in press and is a member of the International Students Union at the University of Maine. She is a candidate for the Master of Science degree in Plant, Soil, and Environmental Science from the University of Maine in May 2019.