

ABSTRACT

RIVERO PENA, WENDY CHIQUINQUIRA. Effect of Plasma-activated Liquids on the Growth, Quality, and Microbiological Safety of Fresh Produce. (Under the direction of Dr. Deepti Salvi).

Plasma, the fourth state of matter, generates reactive oxygen and nitrogen species when exposed to water that is referred to as plasma-activated water (PAW). PAW was reported to reduce microbial contamination, including foodborne pathogens, and to enhance plant growth. Hydroponic plants are susceptible to food safety risks in the pre-harvest or post-harvest stage. Hydroponic farming uses nutrient solution (NS) instead of water. The literature on plasma-activated nutrient solution (PANS) is limited in terms of plant growth and microbial inactivation. Plasma technology could be adapted to agriculture as irrigation water or NS, to enhance plant growth while maintaining microbial sanitation, and in microbial inactivation as post-harvest wash. Current sanitation techniques face challenges: chemical residue, limited effectiveness due to organic matter, or cost. In contrast, PAW and PANS could provide effective decontamination without chemical residue. The objectives of this study were: 1-Evaluate the effect of PANS on sweet basil growth in an ebb-and-flow hydroponic system in terms of yield, morphology, and quality; and 2-Investigate the application of PAW in the microbial inactivation of *E. coli* DH5 α and quality on alfalfa sprouts, broccoli sprouts, and clover sprouts.

The effect of PANS on the growth of hydroponic sweet basil was measured in terms of yield (fresh weight, dry weight, and moisture content), morphology (nodes, branches, plant height, width, node appearance rate, and leaf index) and quality (color, texture, aromatic oils, and leaf tissue mineral composition). Control sweet basil plants were grown in NS. Although the concentrations of total nitrogen, nitrate-nitrogen, zinc, and copper were higher in PANS, there was no significant difference in terms of yield or quality ($p < 0.05$) in the plants grown in NS or PANS. However, plants grown in PANS had a significant increase in number of branches and

nodes, and node appearance rate. These results suggest that PANS can be incorporated into the hydroponic farming of sweet basil plants without affecting product quality, with the additional benefit of inducing plant growth.

PAW was studied in terms of chemical composition, as well as its effect to microbial inactivation (*E. coli* DH5 α and aerobic mesophilic microorganisms), and quality (total color difference (ΔE) and electrolyte leakage (EL)) of alfalfa sprouts, broccoli sprouts, and clover sprouts. Chlorine (Cl) (200 ppm) and deionized water (DI water) were the controls. PAW significantly reduced *E. coli* DH5 α on alfalfa sprouts ($3.5 \log \text{CFU g}^{-1} \pm 0.9 \log \text{CFU g}^{-1}$), broccoli sprouts ($1.8 \log \text{CFU g}^{-1} \pm 0.5 \log \text{CFU g}^{-1}$), and clover sprouts ($1.4 \log \text{CFU g}^{-1} \pm 0.4 \log \text{CFU g}^{-1}$). On alfalfa sprouts and broccoli sprouts, each sanitizing solution (DI water, Cl, or PAW) significantly reduced between $\sim 1 \log \text{CFU g}^{-1}$ to $2 \log \text{CFU g}^{-1}$ in aerobic mesophilic organisms. On clover sprouts, DI water was ineffective, while PAW and Cl were effective. No significant change in color or EL, an indicator of tissue damage, were observed between PAW and Cl. The results suggest that PAW and Cl could increase the inactivation of aerobic mesophilic microorganisms and inoculated *E. coli* DH5 α on sprouts in comparison to DI water wash, while achieving a similar effect on the quality of the sprouts. PAW is a promising alternative to chlorine-based sanitizers for washing sprouts.

In conclusion, plasma treatment of NS or water could be adapted to agriculture in the pre-harvest stage to improve plant growth in hydroponic farming, or in the post-harvest stage for improving food safety of sprouts without compromising the food quality.

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Effect of Plasma-activated Liquids on the Growth, Quality, and Microbiological Safety of Fresh Produce

by
Wendy Chiquinquirá Rivero Peña

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APPROVED BY:

Dr. Deepti Salvi
Committee Chair

Dr. Josip Simunovic

Dr. Ricardo Hernandez

DEDICATION

To the women elders from the families Pena Nava and Rivero Sanchez: *abuela* Mercedes, *bisabuela* Rosa, *bisabuela* Maria Hortensia, *abuela* Nora, and *abuela* Ramona. And to *mi familia*: *papi* Wilfredo, *mami* Diana, Wildi Jhoan, *los sobrinos* Weyner and Weyler, and *campeon*.

BIOGRAPHY

Wen was born in the city of Maracaibo, State of Zulia, in the country of Venezuela. Wen came to the USA to learn English, and they continued to study an undergraduate degree in Chemical Engineering at Montana State University. Afterward, Wen came to North Carolina State University for a master's degree in Food Science, and interned at LC America. Wen was Dr. Salvi's first graduate student and Dr. Wang's first mentee as a post-doctoral associate. During their master's degree, Wen was key in starting up the *Salvi's lab*, which they hope will develop into a large food engineering research powerhouse.

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CHAPTER 1: Introduction

The Food and Agriculture Organization of the United Nations (UN) has estimated that world population will reach 9.8 billion by 2050 (Le Mouél, 2017). In the year 2017 about 124 million people experienced acute food insecurity; it was at the stage that called for immediate emergency intervention, a 13% increase in acute food insecurity from the previous year (Food and Agriculture Organization, 2018). Almost 36% of countries experiencing increased undernourishment were facing severe agricultural draught (Food and Agriculture Organization, 2018). Furthermore, climate variability or extreme climate are one of the leading non-conflict causes for severe food crisis. From 1990 to 2016, there have been an average of 213 climate-based events in the world, including extreme heat, drought, floods, and storms (Food and Agriculture Organization, 2018). Thus, climate variability and extreme climate events are a threat to conventionally grown agricultural foods. The consequences can not only jeopardize the food supply for current global population, but also make direr the challenge of producing 70% more food by 2050 required to feed an increasing population (Food and Agriculture Organization, 2018).

According to Clark (2017), global agriculture involves multiple tradeoffs despite its capability to feed current population levels. Among the hard tradeoffs are the production of greenhouse gases (25-33%), land usage (40% of the Earth's surface), and water usage (more than 70% of freshwater use). For less tangible effects, is the acidification of water and land ecosystems by agrochemicals (pesticides, herbicides, fertilizer, etc.), deforestation, and habitat fragmentation. Therefore, traditional agricultural practices face a severe sustainability challenge.

In addition to environmental drawbacks, there are microbial safety challenges associated with the food supply. Since 1998, there have been 9.6 million total cases of foodborne outbreaks

in the United States, where leafy greens accounted for 22% of illnesses, 14% of hospitalizations and 6% of deaths (Dewey-Mattia, Manikonda, Hall, Wise, & Crowe, 2018). The Food and Drug Administration released the Final Rule on Produce Safety in 2018; this guidance aids farmers and food manufacturers in decreasing the risk of contamination, and thereby decreasing the possibility of foodborne outbreaks. Produce contamination occurs in the form of bacteria, viruses, and parasites. The contamination can happen either in the field, or during the processing steps used to make produce ready for consumption: washing, conveying, handling, cutting, packaging or transportation (Salvi, 2017). By optimizing these steps, it is possible to improve produce safety in addition to matching the necessary food production targets. Produce is susceptible for contamination during various steps involved in its life cycle when it is grown in conventional farming. Controlled environment agriculture is an option where improved intervention techniques can be used to reduce occurrence of pathogen contamination.

Controlled environment agriculture (CEA) is a farming method that involves reducing plant stresses and optimizing growing conditions (Lieth, 2017), with the goal of improving food freshness and safety. Vegetable crops commonly grown in controlled environment agriculture are basil, microgreens, tomatoes, melons and lettuce (Nemali, 2017). Systems currently used include greenhouse, tunnels, in-door and nurseries, which currently may rely on soil media, hydroponics, aeroponics, or aquaponics (Duston, 2017). De Anda (2017) defines hydroponic as growing plants without soil by using a water solution enriched with nutrients. Hydroponic farming may be more efficient than conventional farming for certain crops. For instance, according to Barbosa et al. (2015), hydroponic lettuce farming produces higher yields and consumes significantly less water than conventionally grown lettuce. Despite high energy consumption, the authors recommended the use of hydroponic for water-scarce locations

(Barbosa et al., 2015); an instance of this would a draught prone region such as Arizona, or a location with limited water supply, such as California.

Despite hydroponics usage of optimized growing parameters, the produce is still susceptible to food-borne illnesses. There exists a plethora of alternatives to increase produce safety post-harvest. Among them are the use of chlorine dioxide, hydrogen peroxide, organic acids, quaternary ammonium compounds, irradiation, ozone, and chlorine (Huang, Tian, Salvi, Karwe, & Nitin, 2018). These methods are effective in reducing the microbial load in the water used for the washing step, but do not perform as well in decreasing the microbial load on the produce surface itself (Huang et al., 2018), and chemical residue levels must be carefully monitored to avoid harmful concentrations at the time of consumption (Chen, X. & Hung, 2018). Furthermore, if the wash water is maintained at sub-optimal condition, it can be a vulnerability for spreading contamination since these systems often use recirculated water.

Plasma is the fourth state of matter, achieved by adding energy to the gas phase. During the phase transition, reactive species are produced. Plasma from air when exposed to water generates plasma-activated water (PAW), which is rich in reactive nitrogen and reactive oxygen species. The effect of plasma-activated water in vegetables had been studied in: seed germination (Judée, Simon, Bailly, & Dufour, 2018; Zhou et al., 2016), length of plants grown using PAW (Kim, Je-Wook, Puligundla, & Mok, 2017), yield of plants grown using PAW (Brar et al., 2016), and microbial inactivation (Chen, T., Liang, & Su, 2018; Guo, J. et al., 2017; Kamgang-Youbi et al., 2009; Ma et al., 2015; Shen et al., 2016; Xiang et al., 2019). Consequently, it is hypothesized that the application of plasma-activated water (PAW) in soil-less production and post-harvest processing of vegetables may lead to an improvement in yield, safety or quality of the produce, while providing a chemical residue free alternative to current farming practices.

This study focuses on the optimization of soil-less farming of sweet basil with plasma-activated nutrient solution, with the intention of reducing the risk of produce contamination before harvest, lessening the sustainability impacts of conventional agriculture, and increasing yield under conditions less vulnerable to climate variability. In addition, *this study focuses on the optimization of post-harvest treatment of sprouts (alfalfa, broccoli, and clover) with PAW*, in order to improve the sanitation of produce after harvest with chemical free technologies. These markers will be assessed through the following objectives.

Objectives

1. Evaluate the effect of plasma-activated nutrient solution (PANS) on sweet basil growth in an ebb-and-flow hydroponic system in terms of yield, morphology, and quality.
2. Investigate the application of plasma-activated water (PAW) in the microbial inactivation of *E. coli* DH5 α on alfalfa sprouts, broccoli sprouts and clover sprouts, and its effect on the quality of these sprouts.

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CHAPTER 2: Literature Review

2.1. Hydroponic farming

Hydroponics is an agricultural growing method which relies on a nutrient solution instead of soil to achieve plant growth. In 2014, hydroponically grown crops accounted for 63% of the greenhouse food sales, which amounted almost \$800 million (Gilmour, Bazzani, Nayga Jr., & Snell, 2019). Hydroponics alone was estimated to be a \$8.1 billion market in 2019, and it is predicted to grow to \$16 billion by 2025 (Concepcion, 2019). There are several benefits to hydroponic farming. Reduction of water usage compared to soil-based farming, up to 95% (Gilmour et al., 2019). Since crops grown hydroponically do not depend on the properties of the soil or the weather, there is flexibility in location of the farm and year round growing cycles (Lee & Lee, 2015). A cleaner product may be obtained, since exposure to soil-borne plant pathogens is reduced, and soil particles do not need to be removed from the surface of the food product (Gilmour et al., 2019). Finally, hydroponics rely heavily on automation for irrigation, lighting, among other tasks, which reduces labor costs (Lee & Lee, 2015).

Crops commonly grown in commercial hydroponic systems include fresh vegetables and flowers. In the vegetable category, a variety of plants are grown, including tomatoes (*Lycopersicon esculentum*), beans (*Phaseolus vulgaris*), spinach (*Spinacia oleracea*), strawberries (*Fragaria ananassa*), cucumbers (*Cucumis sativus*), sweet pepper (*Capsicum annuum*), lettuce (*Lactuca sativa*) (Gómez et al., 2019; Lee & Lee, 2015). Another market involved in hydroponics is the production of pharmaceutical and medicinal crops, such as cannabis (*Cannabis sativa*) (Gómez et al., 2019).

2.1.1. Types of hydroponic production systems

Hydroponic production systems vary depending on how the nutrient solution is provided to the plants. Common setups are: drip, aeroponics, wick, nutrient film technique (NFT), deep flow technique, and ebb and flow (Controlled Environment Agriculture Center, The University of Arizona, 2004; Lee & Lee, 2015; Mohammed, 2018; Phibunwatthanawong & Riddech, 2019). Drip system consists of nutrient solution fed individually to each plant through spaghetti drip lines (Mohammed, 2018). In aeroponics, plant roots are suspended in air and the nutrient solution is fed by a misting system (Controlled Environment Agriculture Center, The University of Arizona, 2004). In the wick system nutrient solution is obtained from wicks connecting to the plant instead of misting (Lee & Lee, 2015). Nutrient film technique takes advantage of gravity by continuously flowing the nutrient solution on a tilted channel as a thin film, and recirculating the solution back to the system (Lee & Lee, 2015). Deep flow technique employs rectangular tanks lined with plastic, where plant roots are submerged in the nutrient solution (Phibunwatthanawong & Riddech, 2019). As seen on figure 2.1, in an ebb and flow system the plants are anchored to an inert substrate, such as rockwool or perlite, and they receive intermittent nutrient solution flow (Lee & Lee, 2015; Phibunwatthanawong & Riddech, 2019). Hydroponics can be employed in different production systems depending on the preference of the grower or the constraints of the farming operation.

2.1.1.1. Environment control

Hydroponic production systems are less vulnerable to external factors, such as the weather, since they are often located inside greenhouses. Environmental conditions within the greenhouse are controlled depending on the requirements of the crops being produced (Gómez et al., 2019; Lee & Lee, 2015). The environmental conditions of interest may include concentration

of carbon dioxide (CO₂), relative humidity (RH%), temperature, quality of light (daily light integral, DLI) and lighting time (photoperiod). In addition, plants may be anchored to an inert substrate, and the properties of the substrate may affect nutrient solution retention (Lee & Lee, 2015). The carbon dioxide concentration requirement varies from ambient concentration (about 400 $\mu\text{mol mol}^{-1}$) to supplemental CO₂ injection in order to optimize plant growth (Lee & Lee, 2015). Aerial humidity, absolute and relative, is affected by irrigation, ventilation, and plant transpiration; it should be maintained between 80% to 85% (Gómez et al., 2019). Temperatures are measured as average daily temperature (ADT), which is the average temperature over a 24-hour period. ADT influences the rate of plant development, where plant growth may be limited or enhanced by optimized temperatures. Daily light integral is the photosynthetically active radiation (PAR) experienced by the plant as the moles of light, i.e. photons, received in a square meter over the duration of a day. It is measured in $\text{mol m}^{-2} \text{day}^{-1}$. The daily light integral (DLI) requirement of food crops vary from 12 $\text{mol m}^{-2} \text{d}^{-1}$ to 30 $\text{mol m}^{-2} \text{d}^{-1}$, and the photoperiod may depend on the specific crop and the market the grower is targeting (Lee & Lee, 2015). By maintaining optimum environmental conditions, crop efficiency can be optimized.

2.1.1.2. Nutrient solution

The nutrient solution is one of the most important aspects of hydroponic farming (Controlled Environment Agriculture Center, The University of Arizona, 2004; Gómez et al., 2019; Lee & Lee, 2015; Mohammed, 2018). The nutrient solution is water enriched with a concentrated solution of plant nutrients (Chekli et al., 2017). It contains the nutrients that are conventionally provided to plants in the field by the combination of soil and fertilization. There are 17 essential elements for plant growth, among them carbon, oxygen, nitrogen, phosphorus, calcium, magnesium, sulphur, and trace elements such as iron, manganese, boron, copper, zinc,

molybdenum, sodium, chlorine, aluminum (Chang, Hong, & Fu, 2018). Hydroponic systems take advantage of osmosis, pH of the nutrient solution and controlled environment to promote photosynthesis (Mohammed, 2018). Osmosis involves the transport of water across a semi-permeable membrane, from high water concentration to low water concentration; plants employ their root systems as a semi-permeable membrane to uptake the nutrient solution (Mohammed, 2018). At the nutrient solution level, the parameters commonly monitored include pH, electrical conductivity (EC), dissolved oxygen (DO), and solution temperature (Chang et al., 2018). Plants use a mechanism of ion exchange to feed the roots from the nutrients present in the water, so pH and EC are measured and maintained as an indication of the plant nutrients (Ding et al., 2018). Specific levels of pH and EC are required depending on the plant and its growth stage.

2.1.2. Hydroponic farming of sweet basil

Sweet basil is an annual herb from the family of *Lamiaceae*, and the genus *Ocimum* (Simon, 1985). Commercially, the most common uses are as a spice, as essential oils for flavoring, and as an ornament (Walters, Kellie J. & Currey, 2015). Key quality markers for sweet basil in the fresh market are color, flavor and texture. Customers expect to see dark green leaves, to taste a sweet and spicy flavor, and a texture ranging from “smooth and shiny to curled and hairy” depending on the species (Simon, 1985). The chemical composition of the essential oils in basil includes methyl chavicol, eugenol, and linalool (Hiltunen, 1999). These three components also are responsible for the sweet, floral and clove-like notes that characterize basil flavor and aroma (Hiltunen, 1999). In addition, its nutritional profile includes vitamin A, calcium, potassium, vitamin C, flavonoids and antioxidants (Hiltunen, 1999).

Basil is best suited for soilless propagation. Sweet basil can be grown in the field or indoors in controlled environments (Walters, Kellie J. & Currey, 2015). The optimization of the

production of hydroponic sweet basil based on hydroponic production system and controlled environment parameters was studied by Walters (2015). The researchers looked at the effects of temperature, electrical conductivity (EC), nutrient solution concentration, and daily light integral (DLI). EC did not have a significant effect in shoot mass, but increasing the concentration of nutrients in the nutrient solution correlated to a significant increase on the nutrients on the tissue of the basil leaves. DLI and temperature increases achieved a significant increase in fresh weight, dry weight, height, and node number. Rates of fresh mass and dry mass gain increased with increasing average daily temperature until the temperature of 29 °C (M.P. Kaczperski, Carlson, & M.G. Karlsson, 1991; Walters, Kellie Jean, 2015). Basil plants grown under optimized environmental conditions may see the highest increases in yield and morphological traits.

2.1.3. Hydroponic farming of sprouts

The fresh sprouts market was predicted to grow to \$250 million by 2018 (Cooper & Reed, 2018), and for the period of 2019 to 2023, it is expected to offer a compound annual growth rate of 11.52% (MarketWatch, 2019). Hydroponic sprouts farming is popular as sprouts are grown within controlled environment conditions in short output cycles, and have reduced cropland costs using any viable seed (Kim, Joo, Lee, Kim, Park, & Jeong, 2017). Any viable seed could be used to grow fresh sprouts (Cooper & Reed, 2018). Examples of fresh sprouts products include sprouts of alfalfa, broccoli, mung bean, clover, wheat grass, or radish (Baker, 2016). Fresh sprouts have antioxidant and anticancer properties, in addition to a high nutritional content of vitamins (A and C), protein, dietary fiber compounds, iron, and calcium (Davis, 2016; Kim, Joo et al., 2017). In the western diet, sprouts are commonly consumed raw or lightly cooked to add a crunchy texture to dishes such as stir-fry and sandwiches (Baker, 2016).

Hydroponic farming of sprouts uses epigeal germination, or stem germination, which is the preferred method of growing sprouts for human consumption (Davis, 2016). Epigeal sprouts grow into a white thin stem, which tangles among the stems of the sprouts around it, and proliferates two dark green leaves (Davis, 2016). During growth the stem shoots upwards, and the seed becomes the “head” of the plant as the shoot extends (Davis, 2016). The seed is shed when the sprout reaches maturity, after 3 to 8 days (Davis, 2016). Since the seed are grown in a warm and humid controlled environment, hydroponic sprouts are farmed under conditions that are vulnerable to food-borne illness (Baker, 2016).

2.1.4. Microbial contamination in hydroponic farming

Pathogens, including bacteria and fungus, affect hydroponic farming and cause diseases in plants (Stewart-Wade, 2011). Waterborne pathogens can rapidly spread through the entire systems since nutrient solutions are recirculated and the plants are connected through water tubing systems (Takahashi et al., 2018). These infectious agents may multiply and accumulate in the tubing or the nutrient solution, potentially causing a severe disease outbreak in the system (Lee & Lee, 2015). Preemptive hydroponic management to avoid microbial contamination can involve improving drainage, usage of sterilized medium, and aeration (Hiltunen, 1999). Treatments to improve microbial safety of nutrient solution during continuous operation of a hydroponic system include ultraviolet (UV) irradiation, chemical compounds (i.e. alkylating and oxidizing agents, sodium hypochlorite), ozone, steam, and heating (Evans, 1994). Drawbacks for the current pre-harvest treatment methods for nutrient solutions include high running cost, maintenance, and limited efficacy due to the presence of organic matter (Takahashi et al., 2018; Zhang & Tu, 2000).

Also, the treatments of the nutrient solution may not focus on decreasing the microbial load of the final product for human consumption. Microbial safety is a not a major concern in hydroponic basil, but it is a great challenge in hydroponic sprouts. During the period of 1996 to 2015, fresh basil was associated with four outbreaks which resulted in 1187 food-borne illnesses (U.S. Food and Drug Administration, 2020). The microorganisms of concern were *Cyclospora cayetanensis*, *E. coli* O157:H7, and *Shigella sonnei* (U.S. Food and Drug Administration, 2020). Sprouts have been related to more than 1800 food-borne illnesses since 1996 (Oregon Public Health Division, Belabre, Dekevich, & DeMent, 2018). To address the food safety concern in the farming industry, in 2015 the FDA developed a standard rule for produce safety and a separate guidance for sprouts safety, through the Food Safety and Modernization Act (U. S. Food and Drug Administration, 2017). In this guidance draft, the FDA covers best practices for microbiological safety, such as to avoid contamination from water, soil, production practices, pests, workers, or transport (Davis, 2016). Testing for *E. coli* is encouraged in the case of sprouts, since they are most vulnerable to this microorganism (Davis, 2016).

2.2. Plasma

Plasma is the fourth state of matter which results from ionizing a neutral gas. To create plasma, an input of energy is necessary. Microwave, radio frequency, thermal and electromagnetic sources can be employed, among others, as energy input to generate plasma (Misra, Schlüter, & Cullen, 2016). By ionizing a neutral gas feed, reactive species, electrons, UV, photons, ions, free radicals, atoms and molecular species are created. These exist only for a short period of time due to the inherent instability of the plasma phase (Roth, 2001). However, these reactive species are “more numerous, different in kind, and-or more energetic” when compared to chemical reactors (Roth, 2001). Thus, plasma can be useful in applications where

chemical treatment is not efficient in changing the surface or chemical properties of the product, or where it would be economically challenging to get that efficiency through chemical reactors.

Depending on the properties of the plasma generated, it can be classified into two general types: thermal plasma and nonthermal plasma. Thermal plasma occurs when the ionized products are in thermodynamic equilibrium. Nonthermal plasma exists in non-equilibrium; therefore, the ions and neutrons remain at a lower temperature than electrons, which still maintain high temperatures (104 K to 105 K) (Zainal, Redzuan, & Misnal, 2015).

The phase transition to the plasma state generates a variety of chemical species: electrons, ions, free radicals, atoms, UV irradiation, reactive molecules, among others. These chemical species can bombard the surface of a material such as textiles, causing the splitting of carbon-carbon bonds or carbon-hydrogen bonds, and ejecting protons out of said surface (Cornelius, 2009). As the scission process develops throughout the surface of the material, free radicals start chain reactions to form oxy, peroxy, carbonyl, carboxyl, or hydroxyl radicals (Cornelius, 2009), depending on the feed gas and equipment configuration. Other possible reactions are cross-linking or double-bonding among these free radicals and molecular chains nearby (Cornelius, 2009). To induce a specific set of reactive species or combination thereof, a knowledge of the plasma operating parameters and kinetics is needed.

Another option to optimize the composition of the reactive species or the plasma chemistry is the feed gas. The composition of the gas used to generate plasma can be pure gas or a mixture of gases, based on the purpose of plasma generation. In general, noble gases are used such as Argon or Neon, while in the food applications use of compressed air is common. It is also possible to use other gases such as ambient air, or a combination of gases like an Argon/molecular Oxygen blend (Misra et al., 2016). The gas composition will affect the

reactants available for plasma chemistry, which will influence the reactive species and the byproducts produced (Hertwig et al., 2015; Nikiforov, Leys, Li, Nemcova, & Krcma, 2011). In the case of producing plasma for water treatment, air has been a more effective feed gas in generating reactive nitrogen species (NO_x, HNO_x) and solution acidification compared to helium, nitrogen, or oxygen (Zhou et al., 2016).

2.2.1. Cold Atmospheric Pressure Plasma

Cold atmospheric pressure plasma (CAPP) is a sub-classification of non-thermal plasma where three conditions must be met within the neutral gas: neutrons and ions remain at near room temperatures, electrons remain at high temperature, and the overall gas pressure must be atmospheric (Niemira, 2012). Therefore, the application of cold atmospheric pressure plasma onto food does not alter or detriment its bulk properties.

2.2.1.1. Equipment configurations to generate CAPP

In order to induce a phase change in gas, high amounts of energy must be added to the system. Technologies to produce plasma vary on the energy method applied to the feed gas, such as microwave or radio frequencies (Pankaj, Shashi K. & Keener, 2017). The plasma discharge may also vary depending on the internal configuration of the plasma generator, which may be a corona discharge, dielectric barrier discharge, plasma jet (Pankaj, Shashi K. & Keener, 2017), and gliding arc, among others.

Gliding arc plasma generators take advantage of a vortex plasma flow (Kim, Hyoung S. et al., 2013). The feed gas is added tangentially into the gap between the electrodes (Kim, Hyoung S. et al., 2013; Lewis et al., 2020). The plasma discharge moves along the circumference of two circular electrodes, hence it is termed a gliding arc plasma (Kim, Hyoung S. et al., 2013).

This configuration allows for optimizing the residence time of the plasma discharge (Chernets, Nirenberg, Rabinovich, & Fridman, 2012; Kim, Hyoung S. et al., 2013).

Another configuration to generate plasma are plasma jets, which use two electrodes arranged at a set distance, usually in the range of millimeters. The electrodes direct a stream of plasma to an object at the exposure distance. Power sources can be used to power plasma jets are direct current (DC), radio frequencies (13.56 MHz or 27.12 MHz), microwaves, and pulsed power; and the voltage obtained may vary from hundreds to thousands of volts (Ziuzina, 2015). The electrodes can be designed parallel to each other or in a special ring (Ziuzina, 2015). To obtain cold atmospheric pressure plasma, the temperature of the feed gas and the power consumption must be fine-tuned. Although the design of plasma jets varies on the literature, previous equipment configurations include: “coplanar-electrodes, hollow-electrode, twin injection-needle, jet from a flexible tube, and pencil-type electrode” devices (Ziuzina, 2015).

2.2.1.2. Applications of CAPP

CAPP has been researched in medical applications, textiles, automobile, food processing, air pollution, and degradation of pesticides (Bai, Chen, Mu, Zhang, & Li, 2009; Phan, Phan, Brennan, & Phimolsiripol, 2017; Preis, Klauson, & Gregor, 2013; Ziuzina, 2015). Surface treatment is the most relevant application to food technologies, where CAPP has been researched in terms of food decontamination (Bhide, Salvi, Schaffner, & Karwe, 2017; Butscher, Van Loon, Waskow, von Rohr, & Schuppler, 2016; Niemira, 2012), functional packaging (Pankaj, S. K. et al., 2014), in-package decontamination (Min et al., 2017; Misra et al., 2014), seed germination (Brisset & Pawlat, 2016; Shapira et al., 2017; Sivachandiran & Khacef, 2017).

In biological systems, CAPP has been applied to microbial inactivation in a variety of ways (in-package, or directly onto foods), and CAPP has also shown effectiveness in promoting

plant growth. Seeds are pretreated using fungicides or insecticides, and these pretreatments may hinder the germination rate of seeds by consequence of the protective layer (Puligundla, Kim, & Mok, 2017a). However, CAPP can provide antimicrobial protection for seeds and it has also been applied onto a variety of seeds to enhance their germination rate (Brisset & Pawlat, 2016; Gerard J J B de Groot, Hundt, Murphy, Bange, & Mai-Prochnow, 2018; Kim, Je-Wook et al., 2017; Meiqiang, Mingjing, Buzhou, & Tengcai, 2005; Puligundla et al., 2017a; Puligundla, Kim, & Mok, 2017b; Shapira et al., 2017; Sivachandiran & Khacef, 2017; Zahoranová et al., 2018; Zhou et al., 2016). Reactive species disrupt the protective layer on the seed and allow for increased water availability (Gerard J J B de Groot et al., 2018; Shapira et al., 2017; Zahoranová et al., 2018). Reactive species also affect the metabolic processes of plant growth at the seedling stage (Sivachandiran & Khacef, 2017).

2.2.2. Plasma-activated Water

The transition of a given gas to the plasma state incurs in the generation of a variety of chemical species as explained earlier. When the plasma interacts with a liquid medium, it is believed that the reactive species may be carried into the medium or that other reactions take place at the plasma-liquid interface. When the liquid medium is water, the treatment of water by plasma changes the properties of the medium and its chemistry (Park et al., 2013). The water treated with plasma is referred to as plasma-activated water (PAW). The activation of water can be accomplished through different plasma discharge configurations: discharging gas plasma onto the liquid surface, or infusing plasma gas into the liquid through bubbling (Maniruzzaman, Sinclair, Cahill, Wang, & Dai, 2017). In addition to the plasma generation configuration, the efficacy of PAW may depend on excitation voltage, working gas, and treatment time (Joshi, 2017).

According to Joshi (2017), there are four reactions taking place at the water-plasma interface: acid-base reactions, oxidation reactions, reduction reactions, and photochemical reactions. The two most relevant group of species generated in PAW are reactive nitrogen and oxygen species. Diverse chemical reactions occur in plasma due to the ions and neutral chemical species which, excited from the energy from the plasma state, collide and exchange charges. The product of the collisions are reactive species, which are free radicals and highly reactive molecules. Oxidation reactions occur through reactive nitrogen species; these species have been identified as nitric oxide radical (NO^*), peroxyxynitrite (ONOO^-), nitrogen dioxide radical (NO_2^*), and it is thought that there may be more oxides to be identified. The most relevant reactive oxygen species have been characterized thus far as atomic oxygen (O), hydroxyl radical (OH^*), and ozone (O_3) (Joshi, 2017), and they are responsible for the reduction reactions attributed to plasma-activated water.

The chemistry of PAW can be employed as an indirect method to plasma treat food products. Although CAPP and PAW could effectively inactivate microorganisms (Guo, L. et al., 2018), CAPP has been reported to reduce the quality of a food product while PAW had no detrimental effect (Frías et al., 2020). Therefore, PAW has been explored as a pre-harvest and post-harvest treatment in food products. PAW has been researched in the areas of plant growth (Brar et al., 2016; Judée et al., 2018; Lindsay et al., 2014; Sivachandiran & Khacef, 2017; Zhou et al., 2018), and microbial inactivation (Chen, T. et al., 2018; Guo, J. et al., 2017; Joshi, Salvi, Schaffner, & Karwe, 2018; Kamgang-Youbi et al., 2009; Ma et al., 2015; Ma et al., 2016; Naïtali, Kamgang Youbi, Herry, Bellon-Fontaine, & Brisset, 2010; Oehmigen et al., 2010; Shen et al., 2016; Traylor et al., 2011).

2.2.2.1. Effect of PAW on plant growth

Enhancement in plant growth has been observed in plants irrigated with PAW. The enhancement in plant growth has been attributed to the presence of reactive oxygen and nitrogen species. During plasma treatment, the solution becomes acidified from the presence of reactive nitrogen species that react into the acids HNO_2 and HNO_3 . Reactive nitrogen species (RNS) in PAW are often attributed to the increase in plant growth (Brar et al., 2016; Eun-Jung, Muhammad Saiful Islam Khan, Shim, & Yun-Ji, 2018; Zhou et al., 2016), since they participate in cell metabolic processes or as signaling molecules. Reactive oxygen species (ROS) and NO_x , a form of reactive nitrogen species (RNS), may be responsible for inducing defense mechanisms in the plant (Eun-Jung et al., 2018). PAW may also lead to enzymatic and non-enzymatic oxygen scavenging (Zhou et al., 2016). In the literature, an increase in the enzymes catalase (Zhou et al., 2016) and nitrate reductase (Brar et al., 2016) has been observed. Other compounds that have significantly increased in plants treated with PAW would be ascorbate, γ -aminobutyric acid, and asparagine (Eun-Jung et al., 2018). An increase in these enzymatic activity or nutritional compounds may result from the activity of the defense mechanism of the plant (Eun-Jung et al., 2018). Although PAW has achieved an enhancement in plant growth, it may need to be optimized for each type of crop (Sivachandiran & Khacef, 2017).

2.2.2.1.1. Effect of PAW on seed germination

The ability of PAW to improve seed germination has been researched in mung bean seeds (Zhou et al., 2016), lentil seeds (Judée et al., 2018), radish seeds, tomato seeds, and sweet pepper seeds (Sivachandiran & Khacef, 2017). Germination rate of the lentil seeds was similar between the treatment and control group (Judée et al., 2018). Zhou et al. (2016) observed that mung bean seeds irrigated with PAW achieved a higher germination index (35% higher), and germination

percentage (approximately 65% higher) than the control group. The combination of CAPP treatment and PAW irrigation may be the most effective treatment to improve seed germination, but the length of treatment needs to be optimized to avoid damaging the seeds (Sivachandiran & Khacef, 2017).

2.2.2.1.2. Effect of PAW on plant length

The terms height (Brar et al., 2016; Lindsay et al., 2014) or length (Judée et al., 2018; Sivachandiran & Khacef, 2017; Zhou et al., 2016) has been used interchangeably in the literature of PAW applications to refer to how tall a plant grew after treatment. In this thesis, it will be referred to as height. The height of plants irrigated with PAW has been researched with mixed results. The height of tomato, radish and marigolds plants was higher for plants irrigated with PAW, but the increase was not significantly different (Lindsay et al., 2014). In the case of mung bean seeds, the height index was 3 times higher than control) and the seedling height was 3 mm to 5 mm longer than control (Zhou et al., 2016). Lentil seedlings treated with PAW showed an increase in height of 128.4% after 6 days of treatment at the germination stage, compared with tap water controls (Judée et al., 2018). The height of *A. thaliana* plants was significantly higher for PAW treated plants compared to control from tap water, about 5 cm (Brar et al., 2016). When tomato and sweet pepper seeds were irrigated initially with PAW and finished with tap water, the plant height increase by 60% compared to controls (Sivachandiran & Khacef, 2017). Therefore, the effect of PAW in plant height may depend on performing the PAW treatment in the early phases of plant growth (Judée et al., 2018; Sivachandiran & Khacef, 2017), and it may be affected by the type of seed used (Brar et al., 2016; Lindsay et al., 2014; Zhou et al., 2016).

2.2.2.1.3. Effect of PAW on plant yield

A significant increase in shoot dry mass was observed in radishes, tomatoes, and marigolds irrigated with PAW (Lindsay et al., 2014). Soybean sprouts grown with PAW in a continuous system displayed a significant increase in fresh weight after harvest (Eun-Jung et al., 2018). *A. thaliana* plants irrigated with PAW have shown a significant increase in the number of flowers and seeds after harvest (Brar et al., 2016).

2.2.2.2. Effect of PAW on microbial inactivation

Studies have explored the ability of PAW to inactivate microorganisms in planktonic solution (Chen, T. et al., 2018; Oehmigen et al., 2010; Shen et al., 2016; Traylor et al., 2011), on food contact surfaces (Chen, T. et al., 2018; Kamgang-Youbi et al., 2009), and on food products: mung bean sprouts (Xiang et al., 2019), strawberries (Ma et al., 2015), Chinese bayberries (Ma et al., 2016), grapes (Guo, J. et al., 2017), and grape tomatoes, limes, and spiny gourds (Joshi et al., 2018).

Researchers have observed that increased time for PAW treatment or plasma generation leads to the most effective microbial inactivation (Ma et al. (2015), Xiang et al. (2019), Chen et al. (2018)). Kamgang-Youbi et al. (2009), and Xiang et al. (2019) attributed the antimicrobial effect to the acidic pH, as well as the concentration of nitrites, nitrates and H₂O₂. Chen et al. (2018) also suggested that the presence of metal ions may contribute to the antimicrobial capability of PAW. Also, peroxyxynitrite (ONOO⁻) plays a critical role in terms of microbial inactivation. Peroxyxynitrite can degrade the cell membrane at the lipid bilayer, as well as induce peroxidation and nitration of lipids and proteins within the cell (Zhou et al., 2018). Hence, PAW can aid in microbial inactivation through a complex chemistry that can be utilized in conjunction, compared to other sanitizers that may be based on a much simpler chemistry.

PAW treatment at levels that accomplish microbial inactivation may not decrease the quality of food products. Guo et al. (2017), Ma et al. (2015) and Xiang et al. (2019) have reported no detrimental effect on food quality immediately after PAW treatment. Ma et al. (2016) observed an improvement in the quality of Chinese bayberries on day 8 after treatment, in terms of firmness, redness color attribute, and total soluble solids.

2.2.2.2.1. Microbial inactivation in planktonic solution

Shen et al. (2016) studied the effect of plasma-activated water on *S. aureus* in planktonic solution. PAW was stored at four different temperatures (25 °C, 4 °C, -20 °C, and -80 °C), and for five time periods (1 days, 3 days, 7 days, 15 days and 30 days). A reduction of 3 log CFU mL⁻¹ to 4 log CFU mL⁻¹ of *S. aureus* was achieved by the storage temperature at -80 °C after 30 days of storage. The researchers saw that the microbial inactivation decreased over the storage time of PAW for storage temperatures higher than -80 °C, which correlated with decreasing concentration of reactive species (H₂O₂, NO₂⁻, and NO₃). Therefore, the effectiveness of PAW could be correlated to the presence of reactive oxygen and nitrogen species.

Chen et al. (2018), Traylor et al. (2011), and Oehmigen et al. (2010) investigated the use of plasma-activated water on *E. coli* in planktonic solution. Chen et al. (2018) used a nonthermal micro-hollow cathode discharge plasma to generate PAW, and they observed an inactivation below detection level after incubating the bacterium for 30 min in PAW. In another study, an indirect dielectric barrier discharge plasma was employed to create PAW, which achieved a reduction in cell viability by ~5 log CFU mL⁻¹ (Traylor et al., 2011). Oehmigen et al. (2010) used a surface dielectric barrier discharge plasma to treat the planktonic solution directly, and they observed an inactivation below detection level after 5 min to 15 min of plasma treatment. In

general terms, PAW has been reported to be effective in completely inactivating *E. coli* in planktonic solution at optimized plasma generation times and optimized treatment times.

2.2.2.2.2. Microbial inactivation in food contact surfaces

Kamgang-Youbi et al. (2009) looked at the microbial inactivation achieved by plasma-activated water in food contact surfaces (stainless steel or high-density polyethylene, HDPE) contaminated with bacteria or yeast. Plasma was generated through a gliding arc configuration at atmospheric pressure. The treatment times were 10 minutes, 20 minutes and 30 minutes.

Kamgang-Youbi et al. (2009) saw that the microbial reduction obtained was greater than 5 decimal logarithmic units after a treatment of 30 min on all solid substrates, except for *L. mesenteroides* in HDPE and *S. cerevisiae* on all solid substrates. Also, Chen et al. (2018) investigated the use of plasma-activated water on biofilm of *S. aureus* in food contact surfaces. Plasma was generated in two different feed gases: pure oxygen or air. Biofilm reduction was most effective after treatment of PAW for 3 hours, which inactivated 99% of biofilm in the case of oxygen, and 99.9% in the case of air. Therefore, PAW is more effective in inactivating bacteria on food contact surfaces compared to biofilms.

2.2.2.2.3. Microbial inactivation in food products

Xiang et al. (2019) evaluated plasma-activated water application on mung bean sprouts, specifically its effect on microbial and physicochemical quality. The generation of plasma-activated water (PAW) was achieved by an atmospheric pressure plasma jet with an internal gliding arc configuration. After plasma generation, mung beans were immersed in PAW for three separate times: 10 min, 20 min, and 30 min. The results showed that the highest reduction was 2.3 log CFU g⁻¹ for aerobic bacteria, and 2.8 log CFU g⁻¹ for yeasts and molds after 30 min of immersion. In another study, Guo et al. (2017) researched the capabilities of plasma-activated

water in inactivating yeast (*S. cerevisiae*) in grapes. Plasma was generated using a plasma jet, which treated water for either 30 minutes or 60 minutes. Inoculated grapes were treated by PAW for 30 min. Guo et al. (2017) saw a reduction of yeast by 0.4 log CFU g⁻¹ to 0.5 log CFU g⁻¹. Ma et al. (2015; 2016) studied the effect of applying plasma-activated water on the natural microflora (aerobic mesophilic bacteria, yeasts and molds) of strawberries and Chinese bayberries. For strawberries, a reduction of 2 log CFU gr⁻¹ was achieved immediately after treatment, and for bayberries, a 1.1 log CFU gr⁻¹ was achieved after 8 days of storage. Joshi et al. (2018) observed a significant reduction in *E. aerogenes* in grape tomatoes (4.7 log CFU per surface ± 1.3 log CFU per surface), spiny gourds (1.7 log CFU per surface ± 0.2 log CFU per surface) and limes (3.2 log CFU per surface ± 1.4 log CFU per surface) after treatment with PAW generated by a plasma jet. In comparison to bacteria in planktonic solution, the inactivation of bacteria on food products was less consistent and less effective. The type of food product (surface characteristic, bacterial attachment, and chemical composition), the plasma generation time, and the PAW treatment time may have an impact on the effectiveness of PAW.

2.2.2.3. Effect of Plasma-activated Nutrient Solution on hydroponic farming

Due to the promise of plasma-activated water in aiding in plant growth, and microbial inactivation, its extension into hydroponic farming has been researched. Plasma-activated nutrient solution (PANS) results from the plasma treatment of hydroponic nutrient solution. Takahashi et al. (2018) researched the application of a recirculating plasma water treatment unit for the greenhouse production of hydroponic tomatoes; Takano et al. (2016) looked at the effect of plasma-activated nutrient solution in the plant growth of hydroponic Japanese mustard spinach (*Brassica para var. perviridis*); and Takahashi et al. (2018) has investigated PANS as an effective decontamination method for background microflora and *R. solanacearum* in tomato.

Takahashi et al. (2018) used two corona plasma reactors in continuous operation to generate plasma-activated nutrient solution, which was used to grow greenhouse tomatoes. Total plate counts and plant pathogen counts of *Ralstonia solanacearum* in the nutrient solution were assessed throughout the growth cycle. The authors saw a 2 log CFU mL⁻¹ reduction of standard microflora after 2 days of continuous treatment, which further decreased to non-detectable after 18 days. *R. solanacearum* was reduced to undetectable levels after 8 days of continuous treatment. The authors attributed the reduction effect to ozone generation during plasma treatment.

Takano et al. (2016) employed a plasma reactor with two electrodes to discharge plasma directly into the nutrient solution reservoir, which was recirculated to a hydroponic growth chamber for Japanese mustard spinach (*Brassica para var. perviridis*). Although the increase in dry weight was not significant, there was a significant increase in both height and leaf nitrogen content. It was also observed that plants in the control group experienced a more significant depletion in nitrate concentration in the nutrient solution throughout the growth period, compared to the PANS group. The result suggested that plasma treatment aided in supplementing nitrogen, which lead to significant increases in plant growth.

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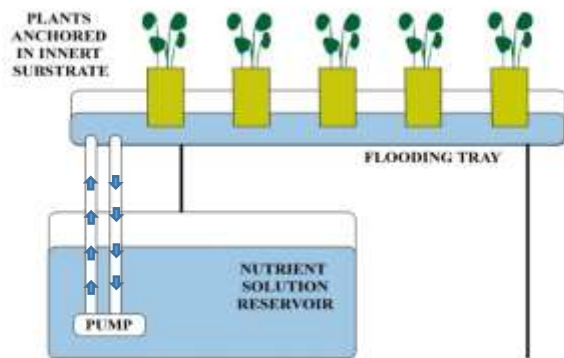


Figure 2.1: Schematic of ebb-and-flow system. Adapted from D'Anna (2018).

CHAPTER 3: Effect of Plasma-activated Nutrient Solution (PANS) on Microbiological Inactivation of *E. coli* DH5 α , and on the Growth and Quality of Hydroponic Sweet Basil

3.1. Abstract

Hydroponic farming utilizes a water solution enriched with nutrients (NS), instead of soil, to optimize yield. Plasma, the fourth state of matter, generates reactive oxygen and nitrogen species when exposed to water, which is known as plasma-activated water (PAW). Similar to PAW, application of plasma-activated nutrient solution (PANS) has shown to increase plant growth or microbial inactivation in hydroponic systems, but its effects on food quality are unexplored. The objectives of this study were to understand the chemical characteristics and microbiological inactivation efficacy of PAW and PANS, and to compare the yield, morphology, and quality of sweet basil plants grown hydroponically in an ebb-and-flow system with either PANS or NS. PAW and PANS were prepared from tap water, and the solutions were treated by the gliding arc plasma discharge until pH dropped to 4.0 ± 0.5 . Chemical characterization of PANS and PAW involved quantification of oxidation-reduction potential (ORP), electrical conductivity (EC), pH, the concentration of reactive species: nitrate-nitrogen and nitrite, and plant nutrient concentration. Microbial inactivation on planktonic *E. coli* DH5 α at stationary phase with PAW and PANS for a treatment time of 5 min. Yield (fresh weight, dry weight, and moisture content), morphology (nodes, branches, plant height, plant width, node appearance rate, and leaf index) and quality (color, texture, aromatic oils, and leaf tissue mineral composition) on plants grown in NS and PANS were analyzed. Despite no significant difference in EC, ORP rose significantly from 46.0 ± 1.46 mV to 152.0 ± 19.9 mV, suggesting that reactive species were generated from the acidification process. Nitrate-nitrogen concentration in PANS was not significantly different from NS, while nitrite increased significantly from negligible levels to

33.9 ± 2.6 ppm. There was no significant difference ($p < 0.05$) on the inactivation efficacy of PAW and PANS, which was up to approximately 5 log CFU mL⁻¹ reduction. Although the composition of PANS showed higher concentrations of total nitrogen, nitrate-nitrogen, zinc, and copper, there was no significant difference in terms of yield, width, height, chlorophyll fluorescence, or quality ($p < 0.05$). However, PANS had a significantly effect in plant growth based on an increase in number of branches and nodes, and node appearance rate. These results suggest that PANS could affect plant morphology without affecting product quality. Plasma is a promising treatment technology for nutrient solutions. Further research is needed on the interaction between plasma and plant growth, along with the effect of multiple PANS treatments during a single growing cycle.

3.2. Introduction

Plasma is the fourth state of matter generated from a neutral gas. To create plasma, an input of energy is necessary. Microwave, radio frequency, thermal and electromagnetic sources can be employed, among others, as energy input to generate plasma (Misra et al., 2016). By ionizing a neutral gas feed, reactive species, electrons, UV, photons, ions, free radicals, atoms and molecular species are created (Zainal et al., 2015). Diverse chemical reactions occur in plasma from the ions and neutral chemical species which, excited from the energy input needed for the plasma state, collide and exchange charges (Zainal et al., 2015). The product of these collision is referred to as reactive species, and these compounds exist only for a short period of time due to the inherent instability of the plasma phase (Roth, 2001). Cold atmospheric pressure plasma (CAPP) is a sub-classification of non-thermal plasma where the plasma is generated at atmospheric pressure, and the temperature of ions and neutrons remains near room temperature while the electrons remain at high temperature (Misra et al., 2016). The effect of CAPP has been studied in seed germination and plant growth (Kim, Je-Wook et al., 2017; Meiqiang et al., 2005; Puligundla et al., 2017a; Puligundla et al., 2017b), and microbial inactivation on the surface of food products (Bhide et al., 2017; Ziuzina, 2015).

When CAPP encounters a liquid medium, the reactive species may be carried into the medium or other reactions may take place from the plasma-liquid interaction. The treatment of water by plasma changes the properties of water and its chemistry (Park et al., 2013). Water treated by plasma has been referred as plasma-activated water (PAW) hereafter. According to Joshi (2017), there are four reactions taking place at the water-plasma interface: acid-base reactions, oxidation reactions, reduction reactions, and photochemical reactions. The two most relevant species generated in plasma-activated water (PAW) are reactive nitrogen and oxygen

species. Oxidation reactions occur through reactive nitrogen species; these species have been identified as “nitric oxide radical (NO^*), peroxyxynitrite (ONOO^*), nitrogen dioxide radical (NO_2^*)” (Joshi, 2017), but it is thought that there may be more oxides of nitrogen. Plasma treatment of water has been researched for translating this technology into agriculture in terms of plant growth (Brar et al., 2016; Judée et al., 2018; Lindsay et al., 2014; Sivachandiran & Khacef, 2017), microbial inactivation in planktonic solutions (Chen, T. et al., 2018; Kamgang-Youbi et al., 2009; Naïtali et al., 2010; Shen et al., 2016), and post-harvest decontamination of produce (Guo, J. et al., 2017; Joshi, 2017; Ma et al., 2015; Xiang et al., 2019).

PAW has shown success in enhancing plant growth. Sivachandiran et al. (2017) applied a non-thermal dielectric barrier plasma discharge (DBD) to create PAW for the irrigation of radishes, tomatoes, and sweet red peppers. The researchers observed that by growing the plants first in PAW followed by tap water resulted in a significant increase (60%) of stem height compared to control. In addition, Lindsay et al. (2014) used a plasma reactor at atmospheric pressure in a glow discharge configuration to generate PAW to grow radishes, tomatoes, and marigolds sown in pots with soil for 4 weeks. Their results showed a significant increase ($p < 0.05$) in shoot dry mass for tomatoes and radishes, which were 1.7 to 2.2 larger for plasma-activated water than control, and no significant difference for marigolds, compared to tap water controls. Also, Brar et al. (2016) employed a non-equilibrium gliding arc plasma in air to generate PAW and grow *A. thaliana* plants for 4 weeks in soil. Brar et al. (2016) observed that height, leaf area, number of seeds and number of flowers of *A. thaliana* was significantly higher for plants watered with PAW than control. The researchers attributed the growth increase to the presence of reactive nitrogen species and the enhanced activity of nitrate reductase. Judée et al. (2018) utilized a dielectric barrier discharge plasma to treat tap water used to irrigate hydroponic

lentils for 6 days. In comparison with control lentils, seeds watered with PAW showed an increase in length by day 2 of 34.0% (1.4 ± 0.7 cm in control versus 2.0 ± 0.8 cm in PAW) and by day 5 an increase of 128.4% (1.9 ± 1.0 cm in control vs 4.3 ± 2.2 cm in PAW). The researchers noted that plasma-activation did not affect the height increase uniformly on all lentil seeds, so it was pointed out that plasma-activation could elicit discrepant results that depend on other factors, such as seed internal mechanisms. Hence, reactive species may be involved in the mechanism by which PAW enhances plant growth.

Although PAW has been effective in improving growth characteristics in plants, hydroponic growers commonly use a water solution enriched with nutrients, i.e. nutrient solution (NS), to grow their crops instead of water. One of the main challenge faced by hydroponic systems is contamination of nutrient solution by plant pathogens or food-borne pathogens (Evans, 1994). Growers use recirculated hydroponic nutrient solution in a closed loop to reduce cost, which allows for the spread of the contamination throughout the system (Takahashi et al., 2018). Therefore, treatment technologies should be added to the system as hurdles to maintain a clean environment. Treatment strategies to reduce microbial contamination in NS include chemical disinfection, ozone, UV irradiation, ultrafiltration, ultrasonics, and heating (Evans, 1994). The drawbacks of these traditional methods include low oxidation power, high running cost, or periodically cleaning maintenance (Takahashi, Saito et al. 2018). Traditional methods could cause improper chemical application, which may hinder plant growth and lead to monetary losses for the grower. Alternative methods such as UV are limited by the organic matter presence in the NS, and they may incur in a reduction of non-target bacterial populations such as beneficial microflora on the rhizosphere of the root zone (Zhang & Tu, 2000). Therefore,

research using new technologies such as cold atmospheric plasma for treatment of NS is necessary.

The literature available on plasma-activated nutrient solution (PANS) is limited. PANS has shown an ability to aid in the plant growth of Japanese spinach (Takano et al., 2016), microbial inactivation of some plant diseases (Filipić et al., 2019; Set Madian Perez et al., 2019; Siddique, Hardy, & Bayliss, 2018; Siddique, St.J. Hardy, & Bayliss, 2019; Takahashi et al., 2018), and reduction of natural microflora of NS (Takahashi et al., 2018) in hydroponic farming.

PANS has been reported to increase plant growth and inactivate microorganisms. Takano et al. (2016) employed a plasma discharge bubbled into nutrient solution, which was used to grow Japanese mustard spinach (*Brassica rapa* var. *perviridis*). Since the concentration of nitrogen species was higher in PANS than in NS throughout the 42 days growing period, the researchers suggested that plasma treatment was creating reactive nitrogen species. The researchers also noted that that nitrogen was being depleted from NS and PANS, which suggested that the plants were absorbing the nutrients. Takano et al. (2016) observed a significant increase ($p < 0.01$) in height and a not statistically significant increase ($p < 0.05$) in weight of plants grown in PANS, up to 1.2 times taller and 1.2 times heavier than the control spinach. Their weight results were within experimental error of each other, which pointed out the variable effect of PANS. Also, Takahashi et al. (2018) observed a reduction in natural microflora present in nutrient solution treated by plasma: from 5 log CFU mL⁻¹ to undetectable levels after continuous treatment. The results were attributed to the presence of reactive oxygen species, in particular ozone, and nitric acid (Takahashi et al., 2018). Therefore, the reactive species from the plasma treatment may influence both plant growth improvement, and microbial population reduction.

Sweet basil is a crop that is best suited for soilless propagation (Hiltunen, 1999), such as hydroponics. Sweet basil is an annual herb from the family of Lamiaceae, and the genus *Ocimum* (Simon, 1985). Commercially, its most common uses are as a spice, essential oils for flavoring, and ornamentals (Simon, 1985). This plant may be affected by bacterial and fungal diseases, with *Fusarium* wilt being the most relevant infestation (Hiltunen, 1999). In addition, sweet basil containing products have been associated with contamination from food-borne disease related microorganisms such as *E. coli*, *Salmonella* and *L. monocytogenes* during the period of 2017-2018 (U. S. Food and Drug Administration, 2018). Hence, due to the susceptibilities in terms of microbial contamination and its market value, hydroponic sweet basil growers could benefit from improved treatment technologies.

The literature available on PANS has explored plant growth or microbial inactivation of some plant pathogens. There is a distinct lack of knowledge on the effect of PANS on the food quality. More research is needed in characterizing PANS, as well as its effect on plant growth, plant quality and microbial safety. The objectives of this study were,

1. To characterize PANS and PAW in terms of chemical composition including pH, electrical conductivity, oxidation-reduction potential, and concentration of long-lived reactive nitrogen species: nitrate-nitrogen and nitrite.
2. To compare the microbiological inactivation of *E. coli* DH5 α in planktonic solution between PAW and PANS treatments.
3. To apply PANS in hydroponic cultivation of sweet basil and understand its effects on yield, morphology, and quality compared to plants grown without plasma treatment.

3.3. Materials and methods

3.3.1. Plasma treatment

Non-thermal plasma is characterized by the ionized products of the plasma phase not being in thermodynamic equilibrium: electrons are at a higher temperature than protons and neutrons in the gas (Lewis et al., 2020). A non-thermal gliding arc plasma equipment (WTPS-V1, AA Plasma, PA, USA) was used to ionize the nitrogen and oxygen that composed the feed gas, compressed air (Figure 3.1). The gliding arc plasma equipment was used to treat tap water (TW) to create plasma-activated water (PAW), or nutrient solution (NS) to create plasma-activated nutrient solution (PANS). Reactive nitrogen species are produced at the gas-liquid interface (Lewis et al., 2020), which allows for these species to be contained in the plasma-treated solution, PAW or PANS. The gliding arc plasma equipment has been described in detail in previous publications (Chernets et al., 2012; Kim, Hyong S. et al., 2013; Lewis et al., 2020) by the C&J Nyheim Plasma Institute at Drexel University (NJ, USA).

The operating conditions were 2 Amp current, 10 kV to 30 kV voltage, $1.3 \text{ m}^3 \text{ hr}^{-1} \pm 0.08 \text{ m}^3 \text{ hr}^{-1}$ water flow rate, and $2.8 \text{ L hr}^{-1} \pm 1.0 \text{ L hr}^{-1}$ air flow rate. PAW and PANS were generated by treating 50 L of solution, either tap water or NS, until pH dropped 4 ± 0.5 . The treatment process time varied from 120 min to 180 min. After plasma treatment, the pH of NS was adjusted to 5.8 ± 0.04 by addition of 10% potassium hydroxide aqueous solution since this pH is ideal for sweet basil growth. Plasma treatment of NS was performed once at the beginning of the growing cycle.

3.3.2. Chemical characterization

PAW and PANS were characterized before and after plasma treatment. Throughout the treatment period the of oxidation-reduction potential (ORP), electrical conductivity (EC) and pH

were measured with standard laboratory equipment (Joshi et al., 2018). During that same period, the concentration of long-lived reactive species, nitrate and nitrite, was performed based on colorimetric assays that relied on modified Griess' reactions (Joshi et al., 2018; Judée et al., 2018). In addition, plant nutrient composition was analyzed in tap water (TW), PAW, NS, and PANS by the North Carolina Department of Agriculture (NCDA) days after treatment (Walters, Kellie Jean, 2015). The analyses aimed to assess the differences between PAW and PANS, and how plasma treatment affected the mineral content in NS after plasma treatment.

ORP, pH, and EC were quantified with an Orion A325 pH/Conductivity portable multiparameter meter (Thermo Scientific, USA) every 30 min during the generation period. In addition, RNS were measured every 60 min following the instructions of proprietary colorimetric tests kits (Merck Millipore, MA, USA). For nitrate measurement, 0.1 mL of sample was mixed in a reaction cell with 1 mL of the proprietary $\text{NO}_3\text{-K}$ reactant. The reaction was allowed to rest for 5 min, then the absorbance was measured at 340 nm (Nitrate Cell Test, Merck Millipore, MA, USA). For nitrite measurement, the reaction cell was prepared by mixing 8 mL of sample with 2 teaspoons of the proprietary $\text{NO}_2\text{-K}$ reactant in a reaction cell. After 20 min reaction time, nitrite was computed by measuring the absorbance of a reaction cell at a wavelength of 451 nm (Nitrite Cell Test, Merck Millipore, MA, USA). Standard curves were created for nitrite and nitrate from stock solutions made with sodium nitrate (Thermo Scientific, USA) and sodium nitrite (Thermo Scientific, USA).

The nutrients analyzed by the NCDA were Al, B, Ca, Cu, Fe, K, Na, Mg, Mn, P, S, Zn, inorganic nitrogen, ammonia nitrogen, and nitrate-nitrogen. A brief description of the methods followed by the NCDA is given. Concentration of ammonia-nitrogen was determined via a modified Berthelot reaction (US EPA method 350.1), inorganic nitrogen and nitrate-nitrogen

were quantified based on the hydrazine reduction method (US EPA method 353.1), and Chloride was tested based on the reaction of thiocyanate (US EPA Method 325.2); these methods relied on a segmented flow analyzer measuring at a wavelength of 660 nm, 540 nm and 490 nm, respectively. All other nutrients were analyzed by the NCDA through Inductively Coupled Plasma (ICP) Emission Spectroscopy, following the U.S. EPA Method 200.7.

3.3.3. Microbial inactivation

The ability of the plasma treatment to inactivate non-pathogenic *E. coli* DH5 α in planktonic solution using PANS and PAW was tested. The bacterial colonies of the untreated planktonic solution were compared to those of the experimental treatment units (PAW, PANS) to determine the logarithmic reduction (CFU mL⁻¹) capabilities of these treatments.

PAW and PANS were generated using the gliding arc plasma generator until pH dropped from their respective starting points until a pH of 4 ± 0.5 , about 120 to 180 min as described earlier. Then, 0.990 mL of PAW or PANS were used to treat 0.01 mL of *E. coli* DH5 α . The bacterium was incubated for 5 min, then serially diluted and plated on Remel Tryptic Soy Agar (Thermo Scientific, USA). The plates were incubated overnight at a temperature of 37 °C. Afterward, bacterial counts were performed.

3.3.4. Growth study of hydroponic sweet basil

The method of growing hydroponic sweet basil was adapted from Walters (2015) to meet the environmental conditions of the experimental design. Sweet basil (*O. basilicum*) variety Devotion DMR was used in this study. The seeds were propagated in rockwool blocks for 14 days at a temperature of 25 °C and 16 hr photoperiod. Once the seeds sprouted, they were watered with half-strength nutrient solution.

Full-strength nutrient solution (NS) was prepared by mixing 480 mL fertilizer (5 N - 12 P - 26 K Jack's Nutrients, JR Peters Inc., PA, USA) stock solution, and 480 mL Calcium nitrate stock solution (YaraLiva CALCINIT, Yara North America Inc., FL, USA), into 49 L of water to make a volume of 50 L. The pH was adjusted to 5.75 ± 0.05 by addition of 10% potassium hydroxide (Fisher Scientific International Inc., NH, USA) aqueous solution or 10% phosphoric acid (Fisher Scientific International Inc., NH, USA) aqueous solution during the growing cycle.

An ebb-and-flow hydroponic system was used to grow 14 plants for a cycle of three weeks. The plants were rotated weekly to ensure even light input to each plant. The hydroponic growing units had 4 fluorescent white lights (light temperature was 6500 K), which were at a height of 0.3 m from the flooding tray. The control unit was watered with NS for growing sweet basil, and the experimental unit was watered by PANS for growing sweet basil. Watering was scheduled for 8 cycles per day for 10 min each cycle. The growing units were enclosed in a hydroponic tent which enclosed a flooding tray where plants were grown, a water pump, environmental sensors, and four white fluorescent lamps as seen in figure 3.2. The experiments were performed in duplicate.

Sensors were employed to measure the environmental conditions within the growing chambers during each cycle. The sensors were used to measure average CO₂ (K33 ELG , Sensirion AG, CH), average air temperature and relative humidity (SHT31, Sensirion AG, CH), the temperature of the nutrient solution and the plants (HOBO 4-Channel, Onset Computer Corporation, MA, USA), and the photosynthetic photon flux (PPF) which was reported as daily light integral (DLI) (MQ-500, Apogee Instruments, Inc., UT, USA). The relative humidity, temperature, and CO₂ concentrations were not significantly different between either growing chamber ($p < 0.05$) as seen in table 3.1.

3.3.4.1. Yield assessment

The fresh weight was calculated by recording the aggregated mass of 9 sweet basil plants immediately after they were severed from the rockwool block. After morphology and quality assessments, the 9 cut basil plants were dried inside a forced air oven at 40 °C for 3 days, the dry mass was recorded afterward (Walters, Kellie Jean, 2015).

A previous study from Zahoranova (2018) suggested that plants grown with the aid of plasma systems could require less watering than the control plants. To assess this hypothesis, the moisture content of the sweet basil was calculated through the following formula:

$$\text{Moisture Content} = \frac{\text{Fresh Weight} - \text{Dry Weight}}{\text{Fresh Weight}} * 100$$

3.3.4.2. Morphology assessment

Nine randomly selected plants were sampled from each growing chamber (PANS, and NS) to measure the number of nodes and branches, plant height and width, node appearance rate, leaf area index, and chlorophyll fluorescence. Plant height and width were measured on the longest or widest part of each plant, respectively. The number of nodes and branches were quantified if the branches were longer than 0.01 m. The node appearance rate was calculated by the following formula:

$$\text{Node Appearance Rate} = \frac{\text{total number of nodes}}{\text{treatment time}}$$

Leaf area index was measured by a standard leaf scanner (Licor 3100c area meter, LICOR Biosciences Inc., NE, USA) on the aggregated leaves of the nine plants if the leaves were longer than 0.01 m across the adaxial side. The total leaf area for the entire flooding tray was determined by calculating the average based on 9 plants and then extrapolating it for 14 plants.

The total leaf area was compared to the growing area (depth and width) of the flooding tray.

Therefore, the following formula was used:

$$\text{LAI} = \frac{\text{leaf area of the experimental unit (cm}^2\text{)}}{\text{growing area of the experimental unit [412.9 cm}^2\text{]}}$$

Chlorophyll fluorescence was measured by a chlorophyll scanner (Dualex, ForceA Scientific, FR). The measurements relied on the difference in transmission of two wavelengths in the near infrared region, which would give a value of chlorophyll fluorescence between 5 to 80 $\mu\text{g cm}^{-2}$. The procedure consisted of measuring chlorophyll fluorescence in three random spots on the adaxial side of each leaf, while avoiding the veins on the leaf. The sample size for each measurement was 10 leaves.

3.3.4.3. Quality assessment

Quality was analyzed in terms of color, texture, aromatic oils, and leaf tissue mineral composition. From the 9 plants randomly sampled, the leaves from the second and third node were separated and mixed to sample 10 leaves randomly for color and texture. Three independent plants were sampled, and their aromatic oils were quantified via gas chromatography mass spectroscopy (GCMS) at Rutgers' University by Dr. James Simons from the Department of Plant Biology (NJ, USA). The leaf tissue mineral analysis was obtained from a single whole plant which was selected randomly, and the measurement was performed at the NCDA.

3.3.4.3.1. Color

Color was measured with a CM-700 handheld spectrophotometer (Konica Minolta Inc., JP). Calibration was performed using the manufacturer's standard D65 white plate (Y 93.5, x 0.3155, y 0.3320). The measurements were taken in triplicate for each leaf in three random locations: top, middle, or bottom of the leaf (Finten, Agüero, Jagus, & Niranjana, 2016). Color

measurements were taken on a 3 mm aperture, white background, and 8° observer. Color was assessed in the CIE LAB color space, where L* quantifies lightness and it ranges from black (L*= 0) to white (L*= 100), a* measures greenness (negative values) or redness (positive value), and b* shows yellow (positive values) and blue (negative values) color (Schuessler, 2016). Common quality defects on leafy produce can be perceived by color, such as chlorosis (yellowing) and necrosis (blackening) (Mitcham, Crisosto, & Kader, 2014).

3.3.4.3.2. Texture

Texture was measured by the maximum force required to fracture the leaf with a TA.XTplus texture analyzer (Stable micro Systems, UK) using a method from Gutierrez-Rodriguez et al. (2013) The leaf was held in place with a ring clamp (cross-sectional area of 0.012 m) enforced with sandpaper #80 to avoid leaf movement. A 1 mm diameter blunt tip probe was used to puncture a single basil leaf on the adaxial side, 1.5 mm away from the stem, right corner. The test speed of the blunt tip probe was 1 mm sec⁻¹. The pre-test speed was 2 mm sec⁻¹ pretest, and the post-test speed was 15 mm sec⁻¹. The trigger force was 0.005 kg_f, the load cell was 5 kg_f, and the end test distance was 30 mm.

3.3.4.3.3. Leaf tissue mineral analysis

All the leaves of a single plant were a random sample of the population within the flooding trays. Tests to quantify the nutrient contents of the leaves were conducted by NCDA based on the same US EPA Methods employed for nutrient solution analysis (methods 350.1, 353.1, 325.2, and 200.7). The nutrients quantified were nitrogen, potassium, copper, calcium, magnesium, nitrogen, phosphorus, sulphur, aluminum, sodium, iron, manganese, boron and zinc.

3.3.4.5. Statistics

A normal distribution was assumed, as well as unequal variances. The averages of the negative control (NS) and the experimental unit (PANS) were compared by a two tailed t-test in the data analysis software Excel (Microsoft Corporation, WA, USA).

3.4. Results and discussion

3.4.1. Comparison of PAW and PANS

3.4.1.1. Chemical characterization

Figure 3.3 and figure 3.4 shows that the final pH and ORP of PAW and PANS were not significantly different ($p < 0.05$), suggesting that the treatment for both solutions were consistently stopped at a similar end point. The pH was used as a sign to stop plasma treatment of water similarly to Lindsay et al. (2014) and Takano et al. (2016). However, as seen by the slopes of figure 3.3 and figure 3.4, the changes in pH and ORP during the plasma treatment (about 2 hours) were higher in PAW than in PANS initially. It is speculated that the slower change in pH and ORP is probably due to the additional minerals present in NS as plant nutrients, which may interact with the plasma discharge in a complex chemistry that could affect the rate of the acidification of nutrient solution compared to tap water.

There was no significant difference for EC for PANS before and after plasma treatment, while a significant difference was noted for PAW after plasma treatment (figure 3.5). The NS measured high EC at the beginning of the plasma treatment probably due to the presence of nutrients. This result suggests that ORP may be a more suitable parameter than EC to monitor plasma treatment for NS.

Nitrate-nitrogen and nitrite were measured during the plasma treatment process. For nitrate-nitrogen, at the end of the treatment the levels in PANS were not

significantly different from NS at the end of treatment (from $134.8 \text{ ppm} \pm 11.2$ to 131.5 ± 21.0), but they significantly increased in PAW from negligible levels to $29.6 \text{ ppm} \pm 2.7 \text{ ppm}$ (figure 3.6). In contrast, nitrite increased significantly from negligible levels to $33.9 \text{ ppm} \pm 2.6 \text{ ppm}$ in PANS, and $36.5 \text{ ppm} \pm 8.1 \text{ ppm}$ in PAW (figure 3.7). Takano et al. (2016) also observed the production of reactive nitrogen species in plasma-activated nutrient solution, which suggested that plasma treatment generated reactive nitrogen species that could be absorbed by the plants during the growth cycle.

Since PANS was used to grow the sweet basil plants, the end point pH (4.0 ± 0.5) was raised to pH of 5.8 by addition of 10% potassium hydroxide. It was observed that pH adjustment did not significantly affect the concentration of nitrate-nitrogen or nitrite immediately after plasma treatment (figure 3.9). In contrast, Lindsay et al. (2014) observed a change after adjusting the pH of PAW, where the addition of the base compound NaHCO_3 increased nitrate concentration and lowered nitrite concentration. A possibility for this interaction is that there were no initial nitrogen species in the water for Lindsay et al. (2014), while there was a high concentration of nitrogen compounds in NS, and this may affect the chemical reactions between the reactive species. There was no significant difference in the concentration of nitrite present in PAW and PANS immediately at the end of the treatment. However, the concentration of nitrogen species may have changed over time once it was measured at the NCDA.

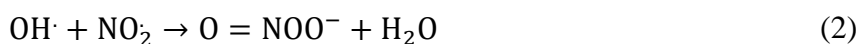
The concentration of plant nutrients was later analyzed by the NCDA in TW, PAW, NS, and PANS (figure 3.9 and figure 3.10). This measurement occurred days after plasma treatment. In TW and PAW, the concentration of total inorganic nitrogen and nitrate-nitrogen were significantly different. The concentrations of total inorganic nitrogen (which includes multiple forms of nitrogen such as ammonia-nitrogen, and nitrate-nitrogen), nitrate-nitrogen, zinc, and

copper were significantly higher in PANS than NS. All other plant nutrients (NH₄-N, P, K, Ca, Mg, S, Fe, Mn, B, Al, Na, and Cl) were not significantly different between the untreated and the treated solution. The nitrate-nitrogen concentrations in PANS (figure 3.8) measured later by the NCDA contradict the levels measured immediately after treatment in our lab (figure 3.6). A possible reason is that nitrite had degraded into nitrate (Lindsay et al., 2014) by the time that they were measured at the NCDA, which resulted in increased nitrate concentration in the solution. As seen on figure 3.9, PANS showed a significant increase in the concentration of zinc (from 0.16 ± 0.01 to 0.21 ± 0.01) and copper (from 0.15 ± 0.03 to 0.25 ± 0.03), although they did not reach harmful levels for plant growth. The increase in these metal ions was not observed in PAW. A researchers have reported an increase in metal ions such as zinc (31.6 ppm) and copper (51.7 ppm) in PAW (Chen, T. et al., 2018). The presence of metal ions was attributed to electrode corrosion induced by the plasma discharge.

3.4.1.2. Microbial inactivation efficacy of PAW and PANS

E. coli DH5 α in planktonic solution was treated with PAW or PANS for 5 min. As seen in figure 3.11, PAW was able to achieve a reduction of *E. coli* DH5 α below detection limit, whereas PANS achieved an average reduction of 3.5 ± 2.5 log CFU mL⁻¹. Shen et al. (2016) and Chen et al. (2018) observed a similar bacterial reduction in planktonic solutions treated with PAW, and Takahashi et al. (2018) observed a similar reduction in natural microflora present in nutrient solution treated by plasma. The current treatment achieved inactivation below detection level in PANS after 5 min compared to continuous operation over 8 days by Takahashi et al. (2018). The mechanism of bacterial inactivation may be complex due to multiple conditions in PAW and PANS creating an environment that leads to cell death. For instance, Chen et al. (2018) found out that the metal ions present from corrosion could have a synergistic

effect with low pH by degrading the cell wall. The increase in ORP may indicate the creation of an oxidizing environment for the bacteria, as well as the presence of reactive nitrogen species leading to the formation of nitric acid (Shen et al., 2016). The creation of reactive species within the gas-liquid interface also leads to the generation of peroxyxynitrite (OONO^- , ONOOH), for instance nitrite in PANS and PAW can lead to the generation of peroxyxynitrite *via* the following chemical reactions (Zhou et al., 2018):



Peroxyxynitrite which has been shown to be key for microbial inactivation through cell apoptosis and necrosis. This compound could move through the lipid bilayer of the cell membrane, then cause cell damage by lipid and protein peroxidation and nitration (Brisset & Pawlat, 2016). Peroxyxynitrite may also induce cell death by its accumulation inside the cell, and its degradation into other reactive species (nitronium (NO^+), nitrosonium (NO_2^+)) that can also cause intracellular oxidation (Brisset & Pawlat, 2016; Zhou et al., 2018).

The logarithmic reduction achieved by PANS presented a large experimental error, as shown by the standard deviation in figure 3.11. Previous studies on post-harvest treatment of produce by PAW have suggested that the treatment time could play important role in bacterial inactivation (Chen, T. et al., 2018; 2017a; Shen et al., 2016; Xiang et al., 2019). Longer incubation time may result in more consistent inactivation using PANS. Nevertheless, PANS did not achieve a statistically significantly different reduction ($p < 0.05$) than PAW. It is possible that plant nutrients could interact with the plasma discharge by interfering with the collisions and ionization of reactive oxygen and nitrogen species (Takano et al., 2016). Therefore, the

additional elements in PANS may affect its generation and microbial inactivation ability compared to PAW.

3.4.2. Effect of PANS on plant growth and quality

3.4.2.1. Yield assessment

There was no significant difference ($p < 0.05$) in average fresh weight, dry weight, or moisture content of sweet basil plants grown in NS or PANS (table 3.2). Therefore, it was concluded that PANS treatment did not affect plant innate yield while other studies have reported mixed results in terms of plant yield (Brar et al., 2016; Lindsay et al., 2014; Takano et al., 2016). A possible reason for the similar fresh weight of plants grown in PANS and NS would be a significant drop ($p < 0.05$) in relative humidity. Batch 2 observed a 20% drop in relative humidity compared to batch 1. The lower relative humidity created an environment that stressed the plants and caused stunted growth (Ku wagata et al., 2012). Nevertheless, the fresh weight and moisture content results were not significantly different between batches. In fact, the average dry weight of plants grown in PANS was 4% higher than the average weight of plants grown in NS, which suggested that an increase in sample size could also improve the sensitivity of the experiment to measure the effect of growing plants with PANS. Therefore, PANS did not affect the innate yield or moisture content of the sweet basil plants.

The results of this study are in accordance with Takano et al. (2016) and Brar et al. (2016) in terms of dry weight and moisture content, respectively. A possible reason for these results are the variable effect of PANS, which resulted in yield results were within experimental error of each other (Takano et al., 2016). Furthermore, Brar et al. (2016) noticed that when PAW was used to irrigate plants grown in soil, there was no significant difference in the absolute water content of leaves treated with control or plasma.

The results of this study may also be explained by the crop selected for this study: sweet basil. It is possible that PANS could influence the yield of certain crops while not on others. Lindsay et al. (2014) noted a significant increase ($p < 0.05$) in shoot dry mass for tomatoes and radishes, which were 1.7 to 2.2 larger for PAW than control, and no significant difference for marigolds. It is suggested that the long-lived reactive nitrogen species, nitrate and nitrite, could aid in plant metabolic processes (Lindsay et al., 2014). However, it is speculated that the results from this experiment were also affected by other factors that could influence these metabolic processes, such as the relative humidity, and concentration of carbon dioxide (Mohammed, 2018).

3.4.2.2. Morphology

The average height of plants grown in PANS was 7% higher than the average height of plants grown in NS, although this difference was not statistically significant. Despite the higher nutrient content present in PANS compared to NS, there was no significant difference ($p < 0.05$) in terms of width, leaf area index, and chlorophyll fluorescence (table 3.3). These results contrast the current literature in PAW, where significant increases in plant height (Brar et al., 2016; Judée et al., 2018; Takano et al., 2016) and leaf area (Brar et al., 2016) have been observed. A possible reason for this contradiction could be the significant change in relative humidity that occurred between replicate 1 and replicate 2, which may have stressed the plants and caused stunted growth (Ku wagata et al., 2012). However, the results are similar to Lindsay et al. (2014), who observed that the height of PAW treated plants were not significantly different ($p < 0.05$). It is possible that the length of the experiment may affect the appearance of the morphological changes present themselves. Takano et al. (2016) conducted their experiment for 42 days compared to the 21 days of the present study, and Lindsay et al. (2014) noted a significantly

larger leaf size in plants treated with PAW than control only after half of the growing period had passed. Walters et al. (2015) also observed that increased concentrations of nutrients in the nutrient solution did not significantly increase yield of sweet basil, and they attributed this difference to the short experimental growth cycle compared to the long growth cycles observed in commercial hydroponic farming.

Plant stress is signaled indirectly by the chlorophyll content in the leaves. Chlorophyll fluorescence was not significantly different, which showed that basil plants grown in PANS did not face a more significant stress than plants grown in NS. However, PANS had a significant increase ($p < 0.05$) number of branches and nodes, and a larger node appearance rate (figure 3.13), about 15%. The results agree with Walters et al. (2018), who observed similar height and number of nodes for sweet basil plants grown in hydroponics. However, Walters et al. (2018) also observed a smaller number of branches compared to our study, which is due to the difference in criteria of measuring branches; Walters et al. (2018) measured branches longer than 2.5 cm, while this study measured branches longer than 1 cm. These results suggest that the application of PANS to grow hydroponic sweet basil may incur in additional changes in plant morphology to those described in the literature of PAW (Brar et al., 2016; Judée et al., 2018; Lindsay et al., 2014; Takano et al., 2016). This study speculates that a possible reason for the increase in the number of nodes and branches in plants irrigated with PANS was that the reactive nitrogen species (RNS) present in PANS were used for the plant as signaling molecules. Although the mechanism is still an active area of research in the plant sciences literature, there are inherent RNS present within plant cells. These inherent RNS have been described as intermediary compounds in the regulation of oxidation-reduction processes, which follow enzymatic or non-enzymatic pathways (Kocsy et al., 2013). The oxidation-reduction processes

can be involved in the production of chloroplasts and nodes, plant growth, among others (Kocsy et al., 2013). Research on the application of plasma-activated water (PAW) on plant growth has quantified the increase of compounds present in the cell oxidation-reduction process, such as the enzyme nitrate reductase (Brar et al., 2016), and other non-enzymatic compounds such as ascorbate and asparagine (Eun-Jung et al., 2018). The researchers suggested that the increase of enzymatic and non-enzymatic compounds occurred due to the RNS from PAW being used in a similar manner as the inherent RNS. They also suggested that the participation of RNS from PAW in the plant cell processes of oxidation-reduction may be involved on their improved plant growth (Brar et al., 2016; Eun-Jung et al., 2018). This study speculates that the RNS from PANS could be assimilated by the sweet basil plants and employed as signaling molecules for the plant cells as part of the oxidation-reduction process, such that an increase in nodes and branches was observed. Therefore, further research on the mechanism of plant growth and how it is affected by plasma treatment is necessary before conclusions can be established. These results may be of importance when growing sweet basil plants for the fresh market, such as in ornamentals (Hiltunen, 1999).

3.4.2.3. Quality

There was no significant difference in the CIE LAB values of sweet basil leaves from plants grown either in NS or PANS (figure 3.14). Therefore, the leaves from treatment and control growing units had similar attributes in terms of lightness, greenness and yellowness. Changes in leaf color may be one of the first responses that the plant generates when it faces environmental stresses, such as chlorosis (yellowing) or necrosis (blackening) (Mitcham et al., 2014). Since the plants were already being irrigated with an optimum recipe of nutrients, it is possible that PANS did not negatively stress the sweet basil plants. These results contradicts the

visual assessment of Lindsay et al. (2014), who observed that plants treated with PAW had a healthy green color at the end of the experiment, while control plants were yellowing and withering. A possible reason for the discrepancy would be the experimental design, where their plants were grown in soil and watered with tap water. Therefore, the additional nitrogen from plasma treatment could aid the plants maintain their metabolism (Lindsay et al., 2014). In the case of PANS, the sweet basil plants were watered with optimum levels of nutrients, such that yellowing was not likely to happen from nutrient depletion. Another possible reason for the discrepancy would be the significant reduction in relative humidity that occurred between replicate 1 and replicate 2, which may have prevented the sweet basil plants from displaying superior color attributes compared to control, as well as the limited number of samples used for this study.

There was no significant difference in the texture of sweet basil leaves from plants grown either in NS or PANS (figure 3.15). The texture of vegetables may become less rigid after physiological changes in the cell wall or parenchyma cells (Gross, Wang, & Saltveit, 2004). In addition, elevated concentrations of nitrogen in the leaves lead to less force to be required to puncture them (Gutierrez-Rodriguez et al., 2013). These changes would make the basil leaves lose their viscoelastic behavior and reduce their quality. Although there was a significant increase in the levels of total inorganic nitrogen and nitrate-nitrogen in PANS compared to NS, it is possible to conclude that the additional nitrogen from the plasma treatment did not have an adverse effect on the basil texture. Since PANS did not negatively affect the crunchiness of sweet basil leaves, it is suggested that the texture results agreed with the chlorophyll fluorescence and the color results.

As seen on table 3.4, the aromatic oil compounds most associated with sweet basil were detected in plants grown in NS and PANS (Hiltunen, 1999). The aromatic oils were reported as a relative percentage (%) of total essential oils, which is determined by the peak area observed by GCMS. There was no significant difference in the relative percentage of the compound eucalyptol, linalool, and methyl chavicol. Although eucalyptol was 72% higher in plants grown in PANS than in NS, the results were within experimental error of each other. There was a significant difference ($p < 0.05$) in methyl eugenol, where plants grown in NS had a significantly higher content ($0.7\% \pm 0.1\%$ for plants grown in NS and $0.2\% \pm 0.1\%$ for plants grown in PANS). A single sample of sweet basil grown in NS was reported to contain the aromatic compound eugenol. The relative percentage of linalool and eucalyptol in plants grown in NS ($71.3\% \pm 9.5\%$ and $12.4\% \pm 5.9\%$) and PANS ($62.4\% \pm 2.0\%$ and $21.3\% \pm 3.6\%$) were higher than what has been reported in the literature (52.42% and 5.61%) (Purushothaman et al., 2018). Methyl eugenol ($0.7\% \pm 0.1\%$ for plant grown in NS, and $0.2\% \pm 0.1\%$ for plants grown in PANS) was lower than values previously reported in the literature (18.74%) (Purushothaman et al., 2018). The composition of aromatic compounds in plants may depend on the growing techniques, irrigation doses, mineral nutrition, plant density, or environmental conditions (Moghaddam & Mehdizadeh, 2017). A possible reason for the difference in aromatic compounds to the literature could be the relative humidity stress that the plants faced during the second replicate, the growing techniques (ebb-and-flow hydroponics compared to soil samples), or the nutrient solution provided to the plant.

As the plant grows, it consumes inorganic compounds and minerals from the nutrient solution. These nutrients must be within the effective range to avoid a loss of plant quality in the form of stunting or poisoning. Nutrients were measured in terms of mineral composition. There

was no significant difference in the mineral composition of NS and PANS in terms of the following minerals: NO₃-N, NH₄-N, P, K, Ca, Mg, S, Fe, Mn, B, Zn and Cu (Table 3.5). Although total inorganic nitrogen, nitrate-nitrogen, Zn and Cu were significantly higher in PANS than NS, the absorption of these nutrients was not significant in the plant tissue. The nutrient composition results are in concord with the chlorophyll fluorescence, color and texture assays. However, they contradict the results of Takano et al. (2016), who quantified a significant increase ($p < 0.01$) in nitrate-nitrogen concentration in the leaf tissue, and Walters et al. (2018), who observed that increased concentration of nutrients also lead to increased concentration of these nutrients on the leaf tissue. As was the case of yield and morphology, a possible reason for the contradicting results could be that PANS induced a different response depending on the crop, the environmental conditions, and the length of the experiment.

In conclusion, PANS treatment did not significantly change the quality of sweet basil leaves during the growing cycle of 3 weeks. Further research can proceed to focus on the effect of plasma treatment in recirculated nutrient solutions and its effect on vegetable quality.

3.5. Conclusion

Plasma is a promising treatment technology for nutrient solution in hydroponic systems. Plasma treatment resulted in the decrease of pH and increase of ORP in nutrient solution and tap water. The efficacy of PANS to inactivate *E. coli* DH5 α was not significantly different than that of PAW. Plants grown with PANS were not significantly different to those grown with NS in terms of yield and quality. However, PANS may have induced morphological changes in the sweet basil plants, such as increased number of nodes and branches, and a faster node appearance rate. PANS was able to combine the characteristics of PAW, in terms of microbial inactivation, and was comparable to NS based on plant growth. The advantages of PANS include

a combination of both PAW and NS, with a positive increase in morphological traits that may be useful in niche industries such as commerce of live plants. Further research is needed to understand the interaction between plasma and plant growth, as well as the effect of multiple plasma treatments on the sanitation of the growing environment.

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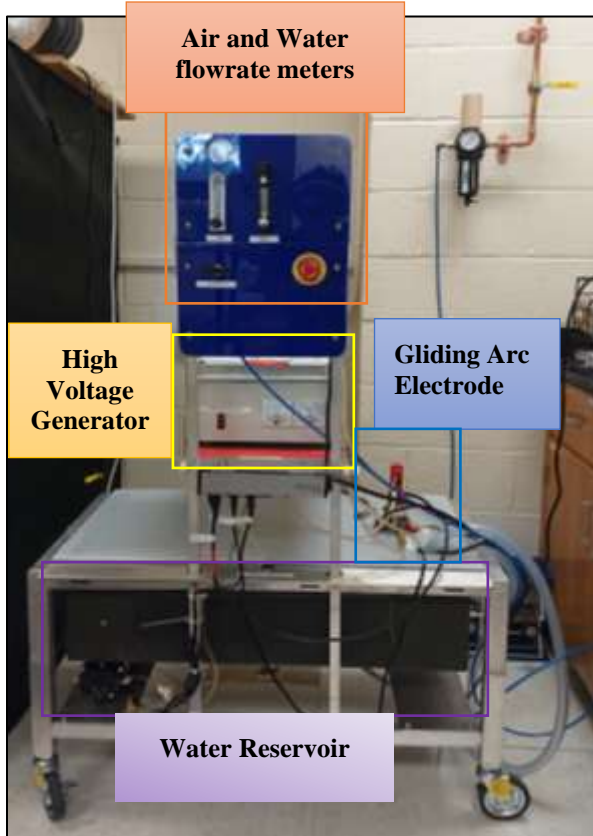


Figure 3.1: Gliding arc plasma generator.

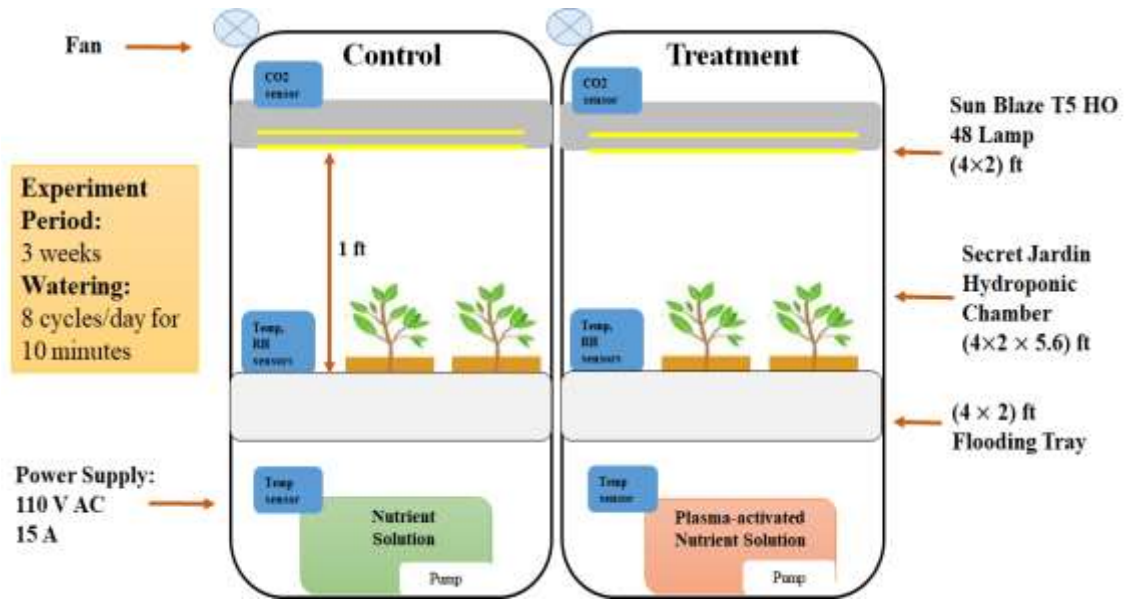


Figure 3.2: Schematic of experimental set-up for hydroponic growing units for sweet basil, NS (negative control) and PANS (experimental unit).

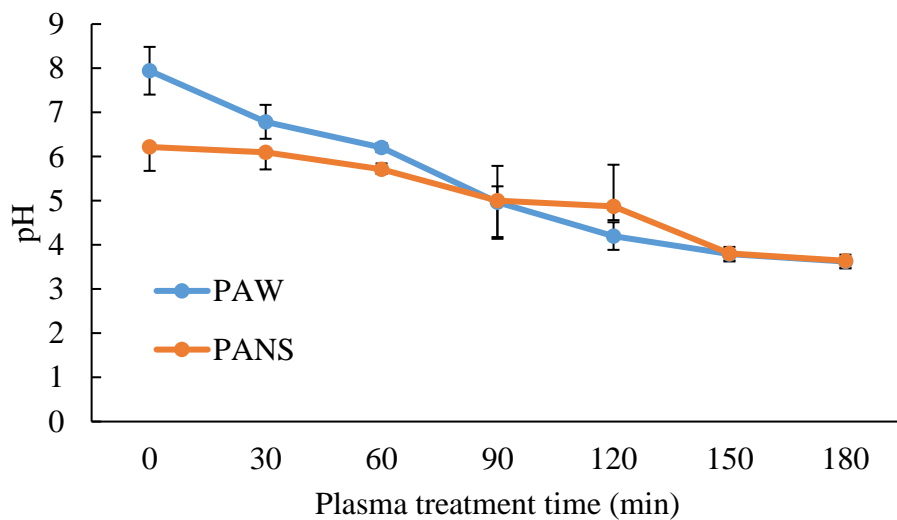


Figure 3.3: Change in pH as a function time for PAW and PANS during plasma treatment.

Notes: Each data point is the average of 3 measurements \pm standard deviation.

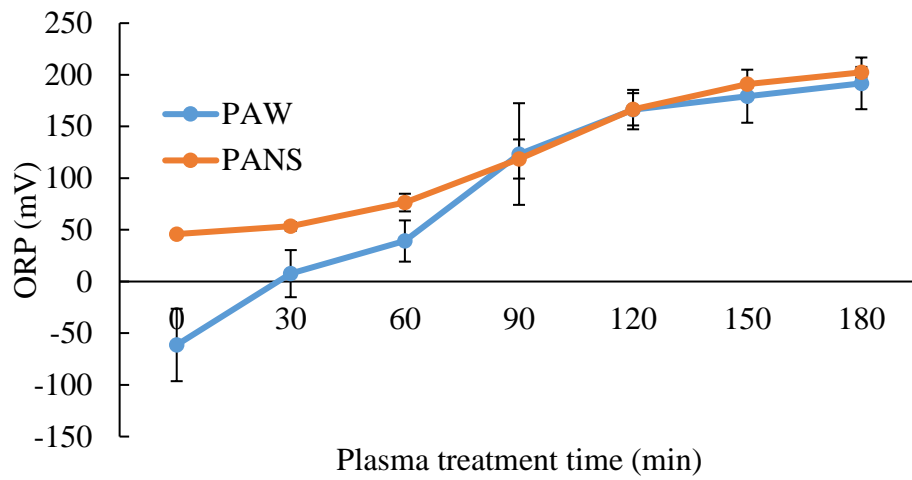


Figure3. 4: Change in oxidation-reduction potential (ORP) as a function of time for PAW and PANS during plasma treatment.

Notes: Each data point is the average of 3 measurements \pm standard deviation.

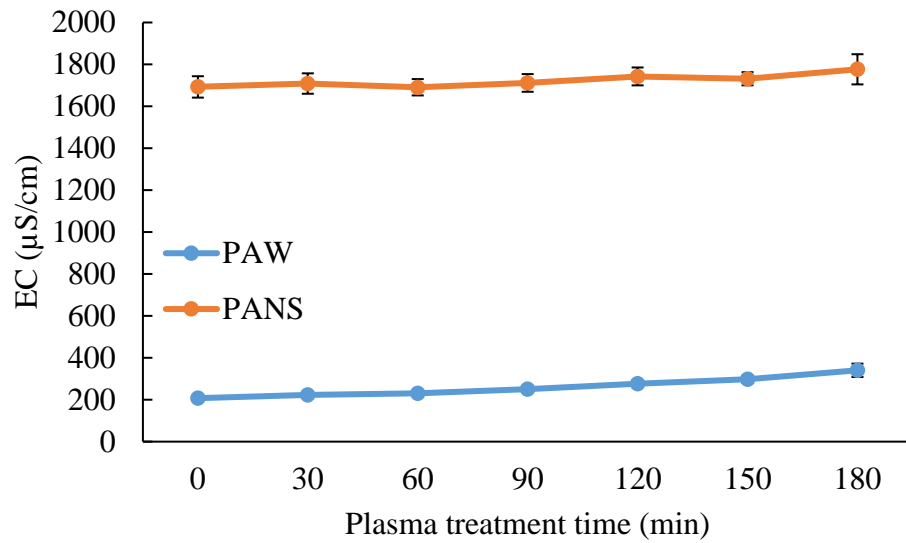


Figure 3.5: Change in electrical conductivity (EC) as a function of time for PAW and PANS during plasma treatment.

Notes: Each data point is the average of 3 measurements \pm standard deviation.

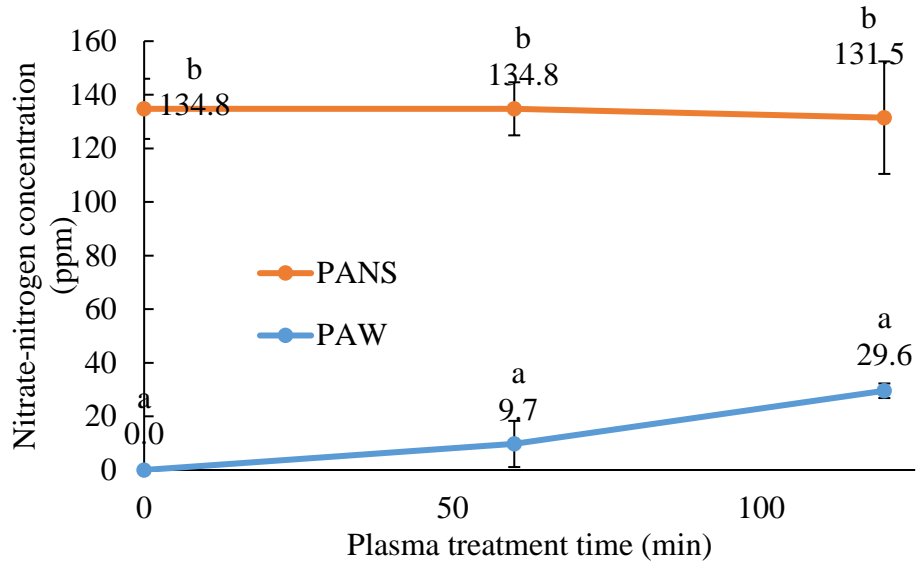


Figure 3.6: Change in the concentration of nitrate-nitrogen ($\text{NO}_3\text{-N}$) in plasma-activated water (PAW) and plasma-activated nutrient solution (PANS) over a period of 120 min.

Notes: Each data point is the average of 3 measurements \pm standard deviation. Different letters indicate statistical significance ($p < 0.05$).

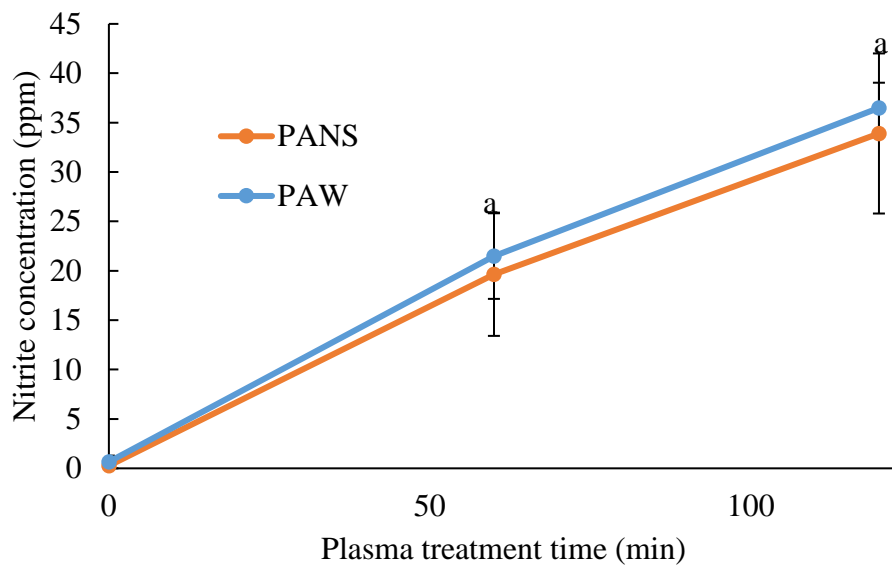


Figure 3.7: Change in concentration of nitrite (NO_2^-) in plasma-activated nutrient solution (PANS) and plasma-activated water (PAW) over a period of 120 min.

Notes: Each data point is the average of 3 measurements \pm standard deviation. Different letters indicate statistical significance ($p < 0.05$).

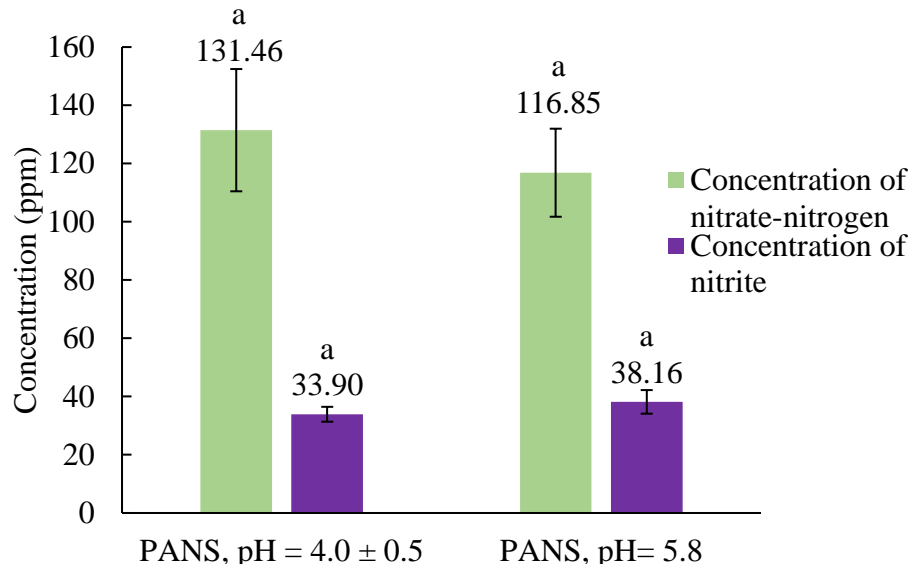


Figure 3.8: Comparison of concentration of RNS, nitrate-nitrogen ($\text{NO}_3\text{-N}$) and nitrite (NO_2^-), before and after the pH of plasma-activated nutrient solution (PANS) was adjusted by addition of 10% potassium hydroxide.

Notes: Each bar represents the average of 3 measurements \pm standard deviation. Different letters indicate statistical significance ($p < 0.05$).

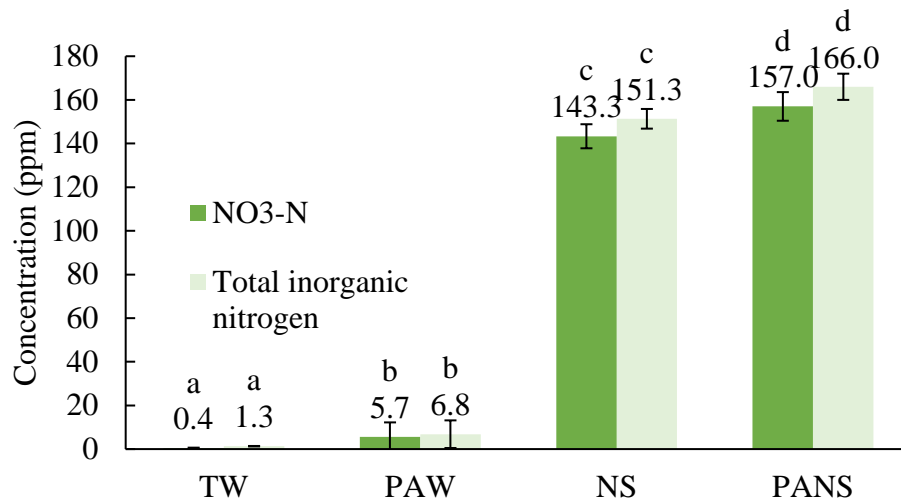


Figure 3.9: Total inorganic nitrogen (Inorganic N) and nitrate-nitrogen (NO₃-N) nutrient levels in tap water (TP), plasma-activated water (PAW), nutrient solution (NS), and plasma-activated nutrient solution (PANS). Levels of other nutrients: NH₄-N, P, K, Ca, Mg, S, Fe, Mn, B, Al, Na, and Cl were not significantly different.

Notes: Each bar represents the average of 3 measurements \pm standard deviation. Different letters indicate statistical significance ($p < 0.05$).

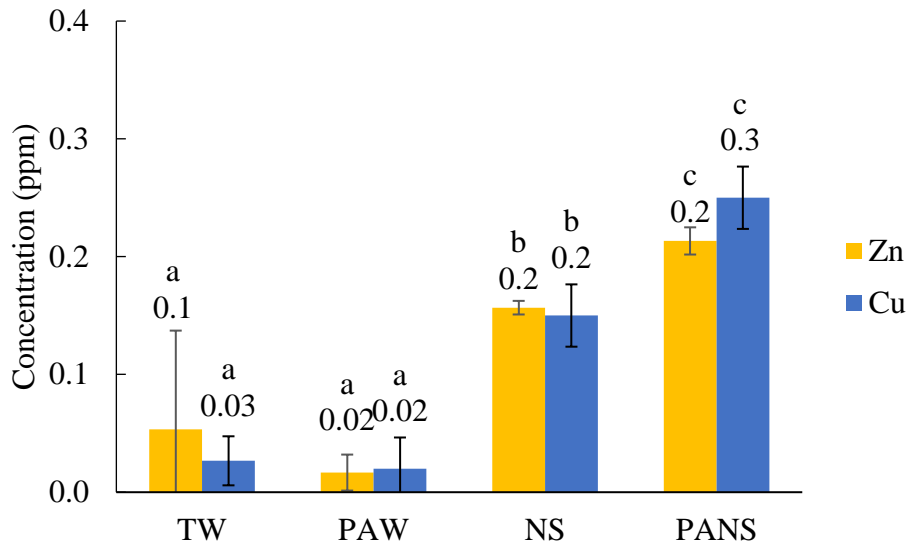


Figure 3.10: Copper (Cu) and Zinc (Zn) nutrient levels in tap water (TP), plasma-activated water (PAW), nutrient solution (NS), and plasma-activated nutrient solution (PANS). Levels of other nutrients: $\text{NH}_4\text{-N}$, P, K, Ca, Mg, S, Fe, Mn, B, Al, Na, and Cl were not significantly different ($p < 0.05$).

Notes: Each bar represents the average of 3 measurements \pm standard deviation. Different letters indicate statistical significance ($p < 0.05$).

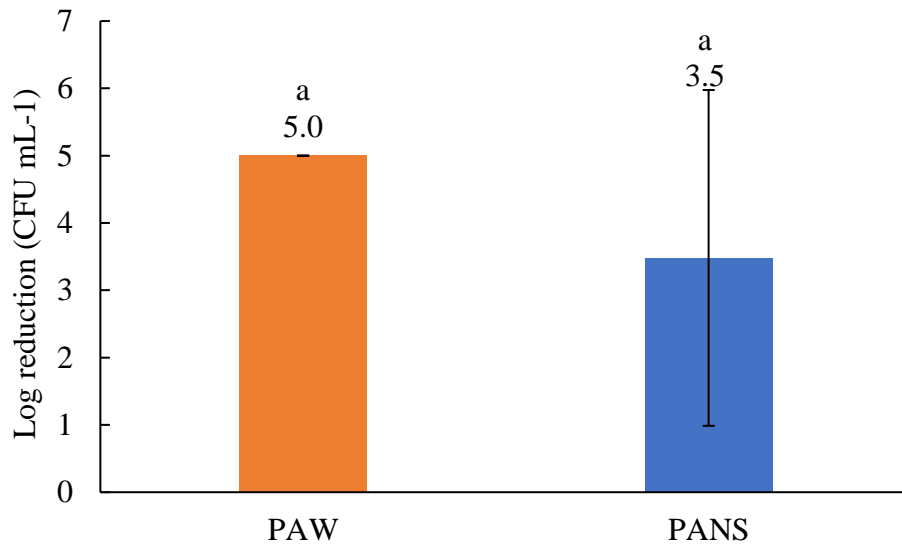


Figure 3.11: Inactivation of *E. coli* DH5 α after 5 minutes of incubation by plasma-activated water (PAW) or plasma-activated nutrient solution (PANS) after plasma treatment over a period of 120 min. The limit of detection was 1 log CFU mL⁻¹.

Notes: Each bar represents the average of 3 measurements \pm standard deviation. Different letters indicate statistical significance ($p < 0.05$).



Figure 3.12: Pictures of hydroponic sweet basil plants on week 3 of the growth cycle. On the left, control plants grown in nutrient solution (NS). On the right, plants grown in plasma-activated nutrient solution (PANS).

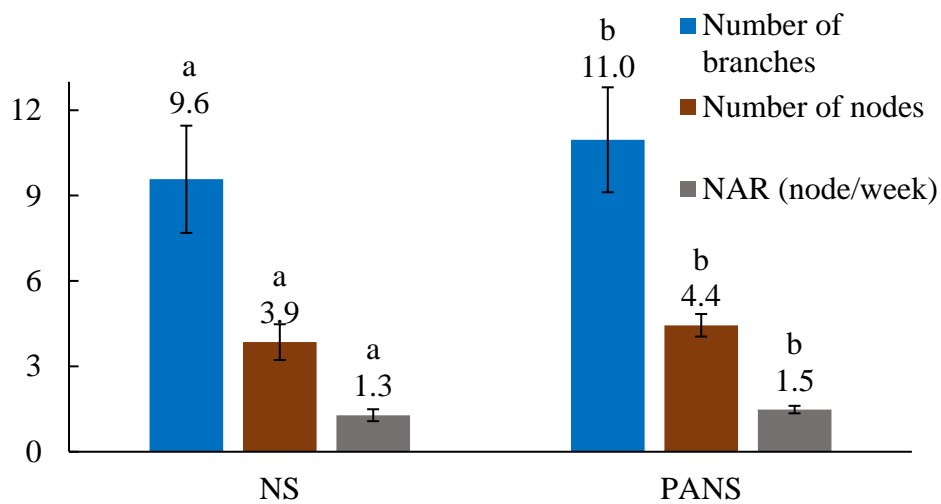


Figure 3.13: Number of branches, number of nodes, and node appearance rate of sweet basil plants grown in NS or PANS.

Notes: Each result represents the average of 2 measurements \pm standard deviation. Different letters indicate statistical significance ($p < 0.05$).

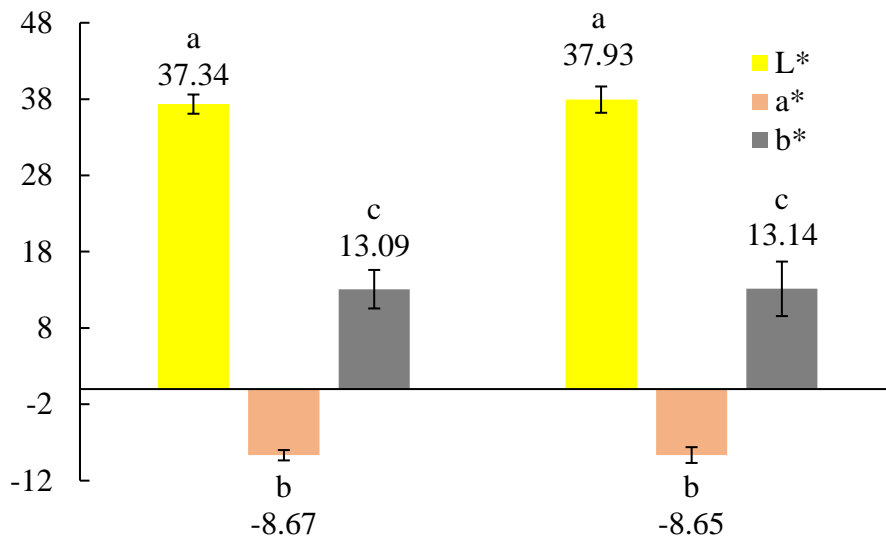


Figure 3.14: CIE LAB color values of sweet basil plants grown in NS or PANS.

Notes: Each bar represents the average of 2 separate batches of sweet basil \pm standard deviation.

Within each batch, 10 leaves were randomly sampled to obtain the average of that batch.

Different letters indicate statistical significance ($p < 0.05$).

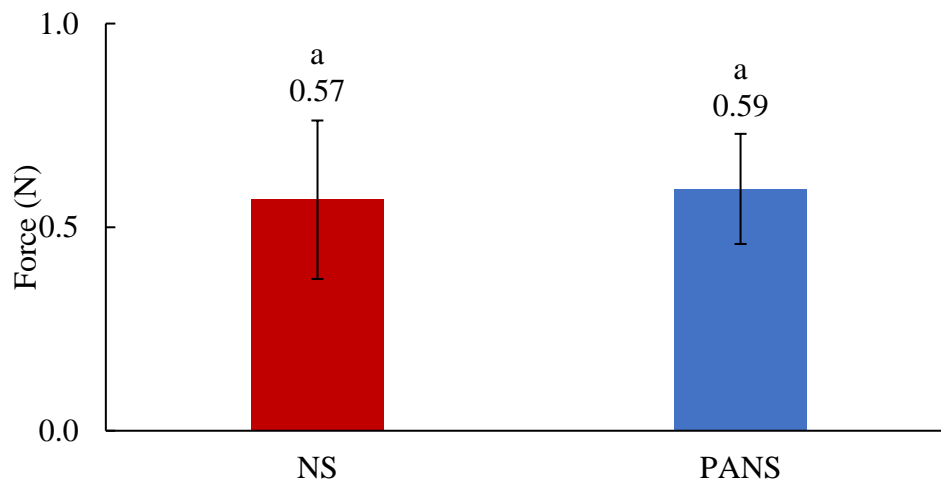


Figure 3.15: Force required to penetrate sweet basil plants grown in NS or PANS.

Notes: Each bar represents the average of 2 separate batches of sweet basil \pm standard deviation.

Within each batch, 10 leaves were randomly sampled to obtain the average of that batch.

Different letters indicate statistical significance ($p < 0.05$).

Table 3.1: Summary of environmental conditions for experimental (PANS) and control (NS) growing units used to grow sweet basil plants for a period of 3 weeks.

Notes: Different letters indicate significant difference ($p < 0.05$)

Parameters	NS	PANS
Relative humidity (%)	42.6 ± 11.9 ^a	42.1 ± 13.4 ^a
CO ₂ concentration (ppm)	456.2 ± 31.3 ^a	455.5 ± 16.4 ^a
Temperature – Plant (°C)	22.5 ± 0.6 ^a	22.1 ± 1.0 ^a
Temperature – Nutrient Solution (°C)	22.03 ± 0.9 ^a	21.9 ± 0.4 ^a
DLI (mol m ⁻² day ⁻¹)	18.2 ± 1.0	

Table 3.2: Average values of fresh weight (FW), dry weight (DW), and moisture content (MC) of sweet basil plants grown in experimental (PANS) and control (NS) growing chambers after a period of 3 weeks.

Notes: Each result represents the average of 2 measurements \pm standard deviation. Different letters indicate statistical significance ($p < 0.05$)

Parameters	NS	PANS
Fresh Weight (kg)	0.474 ± 0.06^a	0.477 ± 0.05^a
Dry Weight (kg)	0.0428 ± 0.006^a	0.0443 ± 0.008^a
Moisture Content (%)	91.0 ± 0.01^a	90.7 ± 0.6^a

Table 3.3: Average values of height, width, leaf area index (LAI), and chlorophyll fluorescence of sweet basil plants from experimental (PANS) and control (NS) growing chambers used after a period of 3 weeks.

Notes: Each result represents the average of 2 measurements \pm standard deviation. Different letters indicate statistical significance ($p < 0.05$)

Parameters	NS	PANS
Height (cm)	15.3 \pm 1.5 ^a	16.3 \pm 1.8 ^a
Width (cm)	16.2 \pm 2.1 ^a	16.1 \pm 1.7 ^a
Leaf Area Index (LAI)	1.5 \pm 0.0 ^a	1.6 \pm 0.04 ^a
Chlorophyll Fluorescence ($\mu\text{g cm}^{-2}$)	32.2 \pm 4.5 ^a	29.6 \pm 5.4 ^a

Table 3.4: Area of aromatic oils for experimental (PANS) and control (NS) growing chambers used to grow sweet basil plants for a period of 3 weeks.

Notes: Each result represents the average of 2 measurements \pm standard deviation. Different letters indicate statistical significance ($p < 0.05$)

Aromatic Oil Compound	NS	PANS
	Average area (%)	Average area (%)
Eucalyptol	12.4 ± 5.9^a	21.3 ± 3.6^a
Linalool	71.3 ± 9.5^a	62.4 ± 2.0^a
Methyl chavicol	0.6 ± 0.3^a	0.5 ± 0.1^a
Methyl eugenol	0.7 ± 0.1^a	0.2 ± 0.1^b
Eugenol	0.1	-

Table 3.5: Leaf nutrient analysis for experimental (PANS) and control (NS) growing chambers used to grow sweet basil plants for a period of 3 weeks.

Notes: Each result represents the average of 3 independent samples \pm standard deviation.

Different letters indicate statistical significance ($p < 0.05$)

Plant Nutrients	NS	PANS
N (%)	5.9 \pm 0.5 ^a	5.3 \pm 0.3 ^a
P (%)	0.9 \pm 0.2 ^a	0.7 \pm 0.03 ^a
K (%)	7.1 \pm 0.9 ^a	6.1 \pm 1.6 ^a
Ca (%)	1.8 \pm 0.4 ^a	1.5 \pm 0.2 ^a
Mg (%)	0.8 \pm 0.05 ^a	0.7 \pm 0.2 ^a
S (%)	0.6 \pm 0.04 ^a	0.5 \pm 0.03 ^a
Fe	101.2 \pm 12.5 ^a	89.1 \pm 2.3 ^a
Mn	96.5 \pm 21.5 ^a	54.9 \pm 11.9 ^a
Zn	34.2 \pm 13.1 ^a	34.3 \pm 11.7 ^a
Cu	10.4 \pm 2.03 ^a	11.6 \pm 3.2 ^a
B	69.8 \pm 20.9 ^a	73.6 \pm 6.4 ^a
Al	5.4 \pm 7.6 ^a	6.3 \pm 8.8 ^a
Na	0.06 \pm 0.03 ^a	0.06 \pm 0.0 ^a

CHAPTER 4: Effect of Plasma-activated Water on Microbiological and Quality Characteristics of Alfalfa Sprouts, Broccoli Sprouts, and Clover Sprouts

4.1. Abstract

Sprouts have been commonly associated with outbreaks of foodborne illness. Current chlorine-based sanitizers used for decontamination can leave chemical residue. In contrast, plasma-activated water (PAW) could provide an effective decontamination without chemical residue. In this study, we investigated application of PAW on alfalfa sprouts, broccoli sprouts, and clover sprouts for microbial inactivation. Deionized water was treated with atmospheric pressure plasma jet for 15 minutes (PAW-15). PAW-15 was characterized in terms of nitrate and nitrite. Inactivation efficacy of PAW-15 was investigated on inoculated *E. coli* DH5 α and aerobic mesophilic microorganisms in alfalfa sprouts, broccoli sprouts, and clover sprouts. Chlorine (Cl) (200 ppm) and deionized water (DI water) were used as controls. Quality changes of sprouts were assessed based on color change (ΔE) and electrolyte leakage (EL). Nitrate and nitrite concentrations of PAW-15 were 861.00 ± 67.99 ppm and 90.74 ± 13.14 ppm, respectively. On alfalfa sprouts, DI water and PAW-15 was able to reduce *E. coli* by $1.3 \log \text{CFU g}^{-1} \pm 0.2 \log \text{CFU g}^{-1}$ and $3.5 \log \text{CFU g}^{-1} \pm 0.9 \log \text{CFU g}^{-1}$, which were significantly lower compared to reduction by Cl ($7.1 \log \text{CFU g}^{-1} \pm 0.3 \log \text{CFU g}^{-1}$). On broccoli sprouts, PAW-15 ($1.8 \log \text{CFU g}^{-1} \pm 0.5 \log \text{CFU g}^{-1}$) and Cl ($2.2 \log \text{CFU g}^{-1} \pm 0.2 \log \text{CFU g}^{-1}$) achieved statistically similar reduction, and DI water achieved a significantly lower reduction ($0.9 \log \text{CFU g}^{-1} \pm 0.2 \log \text{CFU g}^{-1}$). On clover sprouts, PAW-15 ($1.4 \log \text{CFU g}^{-1} \pm 0.4 \log \text{CFU g}^{-1}$) and Cl ($1.7 \log \text{CFU g}^{-1} \pm 0.3 \log \text{CFU g}^{-1}$) achieved statistically similar reduction and DI water achieved a significantly lower reduction ($0.4 \log \text{CFU g}^{-1} \pm 0.5 \log \text{CFU g}^{-1}$). On alfalfa sprouts and clover sprouts, each sanitizing solution (DI water, Cl, or PAW-15) was effective in significantly reducing between ~ 1

log CFU g⁻¹ to 2 log CFU g⁻¹ in total plate counts. On broccoli sprouts, no sanitizing solutions had a significantly different number in total plate count to untreated broccoli sprouts. No significant difference was observed on ΔE between PAW-15 and Cl, suggesting no visual color difference between PAW-15 and Cl washed sprouts. EL, an indicator of tissue damage, was similar for PAW-15 and Cl washed alfalfa sprouts, broccoli sprouts, and clover sprouts, suggesting no difference in tissue damage between PAW-15 and Cl washed sprouts. PAW is a promising alternative to chlorine-based sanitizers for washing sprouts.

4.2. Introduction

Farmers favor sprouts due to their ability to grow under controlled environmental conditions, short output cycles, and reduced cropland costs. On the customer side, sprouts are sought after because of their high nutritional content (antioxidant compounds, as well as vitamins A and C, iron and calcium), protein and dietary fiber compounds, and their sensory characteristics such as flavor and crunchy texture (Davis, 2016). Since sprouts are generally consumed raw in the western diet, microbial reduction before consumption is of utmost importance.

Sprouts are commonly associated with outbreaks of food-borne illness. The contamination of sprouts can occur at various stages: germination, pre-harvest, or post-harvest. During these stages, the environmental conditions such as time, temperature, water activity, pH, and available nutrients are ideal for bacterial growth, then sprouts are prone to bacterial proliferation at each of those stages (U.S. Food and Drug Administration, 2019). From the period of 2010 to 2017, sprouts accounted for 27.6% of multistate vegetable outbreaks (Carstens, Salazar, & Darkoh, 2019). From 1996 until 2016, most sprout outbreaks were attributed to alfalfa, clover, mung bean, and chia, while the most common pathogens were *Salmonella*, *E. coli*, and *Listeria* (Gensheimer & Gubernot, 2016). Due to the broad consequences of foodborne illness and the consumer preference for raw sprouts, the FDA released a guidance document for sprouts farmers in 2015 to improve their sanitation and avoid possible sources of contamination during each stage (U.S. Food and Drug Administration, 2019).

A traditional sanitation step involves a post-harvest washing using a chlorine-based sanitizer such as bleach. Current chlorine-based sanitizers are the industry standard for decontamination. Still, the possibility of the generation of harmful disinfection by-products

above EPA allowed levels and maintaining the concentration of residual chlorine necessary to remove the pathogens altogether, are the challenges faced by the processor (Chen, X. & Hung, 2018). Alternative chemical sanitation treatments have been researched. Kim et al. (2009) studied the post-harvest sanitation with combination treatments of UV-C and fumaric acid or ClO₂ and fumaric acid in alfalfa sprouts and clover sprouts. The treatments were the most effective against total aerobic bacteria (3.2 log CFU g⁻¹), *E. coli* O157:H7 (4.1 log CFU g⁻¹), *S. typhimurium* (3.6 log CFU g⁻¹), and *L. monocytogenes* (3.7 log CFU g⁻¹). Peroxyacetic acid (PAA) was compared to sodium hypochlorite (chlorine) in varying concentrations for the inactivation of *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* spp., and natural microflora on mung bean sprouts; the longer incubation time (2 min) and highest concentration (170 ppm chlorine or 70 ppm PAA) achieved a reduction of 1 to 2 log CFU g⁻¹ (Neo et al., 2013). Phua et al. (2014) investigated a variety of sanitizers on mung bean sprouts: acidic electrolyzed water (75 ppm available chlorine, pH 2.3), acidified sodium chlorite (1200 ppm), cetylpyridinium chloride (2%), ozonated water (2 ppm), trisodium phosphate (10%) or hot water (70 °C). The researchers observed that hot water (70 °C) was the most effective treatment since it decreased *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* spp., and natural microflora by almost 5 log CFU g⁻¹ after an incubation time of 2 min, but it had the most significant adverse effect on the color and firmness of sprouts. Non-thermal technologies have been explored for sanitation of sprouts. Millan-Sango et al. (2017) examined the effect of ultrasound exposure and aqueous ClO₂ on alfalfa sprouts and mung bean sprouts for an incubation time of 5 min. They observed that ClO₂ was better at reducing *E. coli* and *S. enteritidis*, while ultrasound reduced these microorganisms by 1.1 log CFU g⁻¹ ± 0.3 log CFU g⁻¹ and 1.2 log CFU g⁻¹ ± 0.4 log CFU g⁻¹ on alfalfa sprouts, and 1.4 log CFU g⁻¹ ± 0.4 log CFU g⁻¹ and 1.9 log CFU g⁻¹ ± 0.5 log CFU g⁻¹ on

mung bean sprouts. Microbial reduction in mung bean sprouts have been studied by combination treatment with 200 ppm sodium hypochlorite and 12 kGy gamma radiation; the treatment resulted in a decrease in a total plate, spore, yeast, and mold counts for the sprouts to less than 10 CFU g⁻¹ (Nagar, Pansare Godambe, & Shashidhar, 2016). Mung, matki, chickpea, and garden pea sprouts were radiated at a dose of 1 kGy and 2 kGy, which achieved a decrease in *S. Typhimurium* by 4 log CFU g⁻¹ and *L. monocytogenes* by 3 log CFU g⁻¹ (Saroj et al., 2006). Although the novel treatments were effective in various degrees in reducing the microbial load on sprouts, they involved drawbacks. For instance, technology accessibility is a challenge with gamma radiation, since there are few radiation facilities in the U.S. (U. S. Nuclear Regulatory Commission, 2016). A combination of non-thermal or chemical treatments may involve additional post-harvest steps and management of these sanitizers. Finally, there may be drawbacks in sprouts quality as a more significant reduction is achieved, which may cause consumers not to find the sprouts attractive. For instance, *Phua et al.* (2014) observed that hot water was the most effective sanitizer, but it also had the most significant impact in the color and firmness quality of the mung bean sprouts.

In contrast to chlorine-based sanitizers, plasma-activated water (PAW) can provide effective decontamination without chemical residue. Plasma is the fourth state of matter, achieved by adding energy to the gas phase. During the phase transition, reactive species are produced. Plasma can be employed to generate plasma-activated water (PAW) by diffusion of the reactive species from the plasma phase into the liquid phase. This effect can be achieved by surface plasma treatment or by bubbling the plasma discharge into the liquid. The impact of plasma-activated water for microbial inactivation has been investigated. Inactivation of *E. coli* was found below the detection limit in planktonic solution after 30 minutes of PAW

generation (Traylor et al., 2011). Treatment of mung bean sprouts with PAW generated by 30 seconds plasma jet exposure resulted in a reduction of 2.3 log CFU g⁻¹ for total aerobic bacteria and 2.8 log CFU g⁻¹ for yeasts and molds after 30 min of immersion; the nutritional quality in terms of flavonoids, antioxidants, and total phenolic content was unaffected by the plasma treatment (Xiang et al., 2019). In another study, treatment of mung bean sprouts with PAW generated by 50 seconds microwave plasma exposure resulted in a reduction of ~4 log CFU g⁻¹ of *E. coli*, and limited effects on the product's quality (Schnabel, Sydow, Schlüter, Andrasch, & Ehlbeck, 2015). The literature on the application of PAW on sprouts is limited due to the novelty of the research in plasma-activated solutions. There is a need for a detailed study on the effect of PAW on a variety of sprouts in short washing times.

In this study, the objectives were to investigate the application of PAW in the microbial inactivation on alfalfa sprouts, broccoli sprouts, and clover sprouts, and its effect on the quality of these sprouts.

4.3. Materials and methods

4.3.1. PAW generation

Plasma was generated using an atmospheric pressure plasma jet (Plasmacreat Inc., IL, USA) at the following operating parameters: 295 V voltage and 22.5 kHz frequency. Compressed air (1190 mBar) was the feed gas, which allowed for diffusion of reactive species into the water. The height of the plasma jet from the water surface was 5 cm, as seen on figure 4.1. Deionized water (200 mL) was treated by plasma for 15 min (PAW-15). PAW-15 was characterized in terms of nitrate (NO₃⁻) and nitrite (NO₂⁻) concentration, pH, electrical conductivity (EC), and oxidation-reduction potential (ORP).

4.3.2. PAW characterization

Plasma treatment generated RNS. Long-lived species, nitrate (NO_3^-) and nitrite (NO_2^-) were quantified immediately after plasma treatment by a chemical assay based on Griess reaction (Miranda, Espey, & Wink, 2001). EC ($\mu\text{S}/\text{cm}$), pH, and ORP (mV) were measured with Orion A325 pH/Conductivity portable multiparameter meter (Thermo Scientific, USA).

4.3.3. Sample preparation

Alfalfa sprouts, clover sprouts, and broccoli sprouts were obtained from a local supermarket. Sprouts were randomly sampled from the same container for each measurement. Sanitizers used were chlorine at a concentration of 200 ppm (Cl), and PAW-15. Cl and deionized water (DI) were used as controls. Sprouts were washed by immersing 1 gr of sprouts in 19 mL of solution. Samples were shaken at 150 RPM for an incubation time of 5 minutes. After treatment, the samples were rinsed with deionized water for 5 seconds.

4.3.4. Quality assessment

The effect on sprouts quality was characterized by color values, the total color difference (ΔE), and electrolyte leakage (EL). These characteristics were assessed after post-harvest treatment by washing.

4.3.4.1. Color

The color attributes were quantified with a CM-700 (Konica Minolta Inc., JP) handheld spectrophotometer on treated (DI water, Cl and PAW-15) and unwashed sprouts. Samples were air-dried for 30 minutes after treatment in a laminar flow fume hood. Afterward, they were packaged in transparent bags for color measurement (Fan & Thayer, 2001). The CIE L^* , a^* , and b^* color attributes were evaluated against a black background. Then, the color characteristics of

treated sprouts were compared to unwashed sprouts by calculating the total color difference (ΔE):

$$\Delta E = \sqrt{((L_1^* - L_2^*)^2 + (a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2)}$$

4.3.4.2. Electrolyte leakage

Electrolyte Leakage was determined by measuring electrical conductivity ($\mu\text{S}/\text{cm}$) with standard laboratory equipment. Plant cells contain electrolytes (i.e., calcium, potassium) for regulating cell processes; physical or chemical treatments can damage the plant tissue, which causes the cells to leak the electrolytes (Kou et al., 2014). Electrolyte leakage has been correlated with senescence and shelf-life of sprouts (Chandra, Kim, & Kim, 2012). Treated and unwashed samples were immersed in 50 mL of deionized water at room temperature for 30 minutes (Chandra et al., 2012), electrical conductivity was measured afterward and it was used as the electrolyte leakage values.

4.3.5. Microbial inactivation

The reduction microbial counts were assessed by total plate counts and inactivation of *E. coli* DH5 α .

4.3.5.1. Total plate counts (TPC)

TPC assay was performed on treated and unwashed sprouts to quantify the reduction of natural microflora. One gram of sprouts was immersed in 19 mL of sterile DI water in a whirl bag, and they were stomached for 2 minutes at 260 RPM (Xiang et al., 2019). The liquid in which the sprouts were suspended was serially diluted, spot plated in plate count agar (Difco, USA), and incubated for 24 hr at 37 °C for colony enumeration.

4.3.5.2. Microbial inactivation of *E. coli* DH5 α

4.3.5.2.1. Bacterial strain and inoculum preparation

E. coli DH5 α inoculum was kindly provided by Prof. Sophia Kathariou from the Department of Food, Bioprocessing, and Nutrition Sciences at North Carolina State University. The bacterium was selected as a model of pathogenic *E. coli* O157:H7 due to its phenotypical characteristics while being unable to generate toxins. Stock cultures were initially stored at -80 C in tryptic soy broth (TSB) (Difco, NJ) supplemented with 20% glycerol. A sample of the stock culture was re-activated by inoculating in a plate of tryptic soy agar (TSA) (Difco, USA), which were stored at 37 °C for 24 hr, the transferred to storage at 4 °C. The plated culture was re-cultured monthly. The inoculum of *E. coli* DH5 α for experimentation was prepared by transferring a single colony to 10 mL of TSB (Difco, Franklin Lakes, NJ), which was incubated for 18-20 hr at 37 °C to obtain a bacterial population in the stationary phase.

4.3.5.2.2. Microbial inactivation of *E. coli* DH5 α

The incubated bacteria *E. coli* DH5 α was centrifuged for 10 minutes at room temperature at 10 000 x g, washed twice, and resuspended in DI water to obtain an initial concentration of 8.6 log CFU mL⁻¹ \pm 0.2 log CFU mL⁻¹. Sprouts were sanitized by immersing for 15 minutes in 70% ethanol (Millan-Sango et al., 2017) while being shaken at 150 RPM, and then they were rinsed for 2 minutes in sterile DI water. Afterward, sprouts were air-dried at room temperature in a conventional laboratory fume hood for 30 minutes. The inoculate of *E. coli* was prepared by mixing 15 mL sterile DI water and 0.5 mL of bacteria (Millan-Sango et al., 2017). Sprouts were immersed in the inoculate for 15 minutes while being shaken at 150 RPM. Next, the sprouts were drained and dried in a laminar flow fume hood for 60 minutes. At this point the samples were ready for treatment by DI water, Cl and PAW-15 for 5 min as described previously on the

Sample Preparation section. After treatment the samples were stomached, serially diluted, plated on Tryptic Soy Agar (TSA), and incubated for 24 hr at 37 °C for enumeration of bacterial colonies.

4.3.6. Statistics

The sample size was six for each measurement. The means of experimental groups were compared through an all pairs Tukey's HSD test (Xiang et al., 2019), and its significance was assessed against a p-value of 0.05 by using JMP software (SAS Inc., NC). Normal distribution and unequal variances were assumed.

4.4. Results and discussion

4.4.1. PAW characterization

PAW was characterized in term of pH, ORP, and EC. These properties were measured in DI water and PAW-15. Oxidation-reduction potential (ORP) measures the oxidizing or reducing ability of a solution, where positive values indicate an oxidizing state (The University of Kansas, 2019). Therefore, the more positive ORP value may indicate that PAW can accept electrons and induce oxidation. ORP increased from $-76.5 \text{ mV} \pm 32.0 \text{ mV}$ to $284.2 \text{ mV} \pm 1.6 \text{ mV}$, which indicated that oxidative reactive species increased. Electrical conductivity (EC) quantifies the ability to conduct electrical current from ions present in the solution. EC increased from $1.6 \text{ } \mu\text{S/cm} \pm 0.6 \text{ } \mu\text{S/cm}$ to $1210.3 \text{ } \mu\text{S/cm} \pm 160.8 \text{ } \mu\text{S/cm}$, signaling the increase of dissolved ions in the solution.

In addition, pH dropped from 8.4 ± 0.6 to 2.6 ± 0.01 . In the plasma-activated water (PAW-15) used in this study, the changes in pH, EC, and ORP were attributed to the presence of reactive nitrogen species (RNS), measured in terms of concentrations of NO_2^- and NO_3^- . These species were present in a negligible amount in DI water. After plasma treatment, RNS increased

to $90.7 \text{ ppm} \pm 13.1 \text{ ppm}$ of NO_2^- , and $861.0 \text{ ppm} \pm 68.0 \text{ ppm}$ of NO_3^- from initial undetectable levels in DI water. The current plasma set up, as seen in figure 4.1, does not generate long lived reactive oxygen species. Preliminary testing of long-lived reactive oxygen species through test strips and colorimetric assays revealed that the concentrations of oxides and hydrogen peroxide were undetectable. Hence, it was assumed that there were no long-lived reactive oxygen species of interest in PAW-15.

These results were consistent with the results of Xiang et al. (2019), Traylor et al. (2011) and Oehmigen et al. (2010). The acidification phenomenon is assumed to correlate with the production of reactive species, such as nitrite and peroxyxynitrite (Zhou et al., 2018), and the reactive species in PAW associated with antimicrobial capabilities along with low pH. The pH drop decrease may indicate the presence of protons, which are associated with bactericidal effects of PAW (Xiang et al., 2019). ORP observed a significant increase, which has been associated with the presence of reactive species (Joshi et al., 2018). The increase in EC could also be associated with the production of reactive species, in conjunction with the increase of nitrates and nitrites concentration (Xiang et al., 2019).

4.4.2. Quality assessment

4.4.2.1. Color

Color of alfalfa sprouts, clover sprouts, and broccoli sprouts was measured after 5 min exposure to a washing solution, and without washing (i.e. unwashed: UW). The color of whole sprout samples was measured in the CIE LAB color space. The results showed a general trend for all three types of sprouts in L^* values closer to 100, which indicated a whiter sample, a^* values were negative since the leaves of the sprouts are a green color, and b^* values were positive hence the samples were yellower in color (table 4.1). L^* , a^* , and b^* results were varied.

For L* values, there was no significant difference in alfalfa sprouts between treatments and control, in broccoli sprouts DI water and PAW-15 were significantly different while CI and UW were not significantly different. In clover sprouts, CI and PAW-15 did not have a significantly different L*, but UW and DI water were significantly different. In the case of a* values, there was a significant difference in the greenness of PAW-15 in alfalfa sprouts. At the same time, there was no significant difference in greenness either in broccoli sprouts or clover sprouts. Finally, b* values for PAW-15 and UW were more similar to each other than to b* values for DI water and CI in alfalfa sprouts, in broccoli sprouts there was a significant difference in yellowness between UW and PAW-15, and there was no significant difference in clover sprouts. In general, the results were variable when comparing treatments to the negative control, UW. Washing treatments seemed to have the most significant effect on the L* and b* values, while a* values were not significant except for PAW-15 in alfalfa sprouts.

A possible reason for the reduction in color quality of the sprouts throughout the treatments could be physical injury. Phua et al. (2014) observed a color change on mung bean sprouts from the temperature of the treatment agent. In this case, the damage could come from exposure to the chemical species present in either chlorine or PAW-15. Also, a* and b* values may present a high fluctuation due to the delicate structure of the sprouts (Phua et al., 2014).

The L*, a*, and b* results were employed to determine the total color difference (ΔE) between unwashed samples (UW) and treated samples for alfalfa sprouts, broccoli sprouts, and clover sprouts as seen in figure 4.2. The total color difference of all sprouts treated by PAW-15, CI, or DI water was higher than or equal to 2. Hence the human eye would be capable of noticing the color difference between the washed sprouts and unwashed sprouts (Schuessler, 2016). This result contradicts Xiang et al. (2019) and Schnabel et al. (2015), who observed a similar visual

color quality between mung bean sprouts unwashed and mung bean sprouts washed by sterile DI water or PAW30. However, this assessment was not quantified via colorimetric analysis. In figure 4.3, there is a visual representation of the sprouts after treatment.

Although washing treatments could not preserve the initial color quality of sprouts, there was no significant difference ($p < 0.05$) between the values of total color difference for sprouts washed with DI water, Cl or PAW-15. Although the treatment by PAW-15 decreased the color quality of sprouts, the reduction in color quality was similar compared to the other sanitizing treatments.

4.4.2.2. Electrolyte leakage

Alfalfa sprouts, broccoli sprouts, and clover sprouts were treated by DI water, Cl or PAW-15 for 5 min. Afterward, electrolyte leakage was measured by electrical conductivity after 30 min of soaking in DI water. This measurement assesses the tissue damage due to sanitation treatments. As seen in figure 4.4, the electrolyte leakage caused by treatments with PAW-15 and Cl was not significantly different ($p < 0.05$) in broccoli sprouts, alfalfa sprouts, and clover sprouts. The electrical conductivity of sprouts washed by DI water was measured to ensure that mechanical damage from washing was not a confounding variable. Electrolyte leakage of unwashed or washed sprouts with DI water was not significantly different. However, for alfalfa sprouts and clover sprouts, the electrolyte leakage due to PAW-15 and Cl washes was more significant than electrolyte leakage in sprouts that were unwashed or washed by DI water. In broccoli sprouts, electrolyte leakage was significantly different between PAW-15 and unwashed, while Cl and DI water were not significantly different from unwashed broccoli sprouts. In general, PAW-15 achieved similar tissue damage, as measured by electrolyte leakage, as the

industry standard of 200 ppm active chlorine solution in alfalfa sprouts, broccoli sprouts, and clover sprouts.

The increase in electrolyte leakage after treatment agrees with the results from Chandra et al. (2012), who observed the highest electrolyte leakage from Cl treatment. In contrast, a lower electrolyte leakage has been observed for treatments that involved electrolytes required for cell metabolism (Kou et al., 2014). A decline in electrolyte leakage compared to controls may be due to membrane damage recovery process, while increased electrolyte leakage may indicate irreversible membrane damage (Chandra et al., 2012). In turn, changes in electrolyte leakage could be inversely correlated to senescence of plant cells (Kou et al., 2014).

Therefore, electrolyte leakage is sensitive to a variety of treatments, where some treatments may cause damage to the plant cell tissue, and other treatments may help repair the cell membrane. In general, PAW-15 had a similar increase in electrolyte leakage to Cl, such that the sanitizing treatments damaged plant cell tissue in a similar manner. DI water was the mildest treatment since it had a similar electrolyte leakage than unwashed samples.

4.4.3. Microbial inactivation

4.4.3.1. Total plate counts (TPC)

As seen in figure 4.5, there was a noticeable variance between trials for the same experimental group. Total plate counts quantify the presence of natural microflora on the sprouts, such as aerobic bacteria, yeasts, molds, and fungi at the incubation temperature. The variance within each experimental group suggested that bacterial attachment of natural microflora on the sprouts was not uniformly distributed across the sampled population of alfalfa sprouts, broccoli sprouts, and clover sprouts. Each sanitizing solution (DI water, Cl, or PAW-15) was effective in significantly reducing between $\sim 1 \log \text{CFU gr}^{-1}$ to $2 \log \text{CFU gr}^{-1}$ in total plate counts in alfalfa

sprouts and broccoli sprouts. For alfalfa sprouts and clover sprouts, the reduction in TPC was not significantly different between Cl and PAW-15, while both sanitizers achieved a similar or higher microbial reduction than DI water. However, no treatment (DI water, Cl or PAW-15) achieved a significant decrease the natural microflora in broccoli sprouts.

Similar results have been reported by Neo et al. (2013). A possible reason for these results were explained as natural microflora being more resistant to sanitizing treatments than inoculated microorganisms due to surface biofilms. The natural microflora adheres to the produce surface during the time taken to farm it and transport it, so it may be more firmly attached to the surface compared to model microorganisms, which have only interacted with the surface for a short period (Neo et al., 2013). In addition, biofilms could form during the farming of the sprouts, and natural microflora be present within the biofilm, which protects it from the sanitizers (Neo et al., 2013). In their follow-up study, a higher microbial inactivation was achieved after using a treatment that had been reported effective in decreasing biofilm on food surfaces (Phua et al., 2014).

Another possible reason for the limited efficacy of the PAW-15 and Cl treatments in decreasing the total plate counts of the sprouts (broccoli, clover, and alfalfa) could be the treatment time. For instance, Xiang et al. (2019) found a significant reduction of 2.3 log CFU gr⁻¹ in total plate counts after incubating mung bean sprouts in PAW for 30 min. Also, the combination of long-lived reactive oxygen and nitrogen species (Guo, J. et al., 2017; Ma et al., 2015; Xiang et al., 2019), in comparison to long-lived reactive nitrogen species in this study, could have a significant effect on the bactericidal ability of PAW, and it is a topic that deserves further future research.

Overall, PAW-15 is a comparable sanitizer to Cl in terms of total plate counts. Further research is needed to assess the ability of PAW-15 combined with temperature to decrease natural microflora. The effect of temperature, acidification, and the presence of long-lived reactive nitrogen species needs to be studied.

4.4.3.2. Microbial inactivation of *E. coli* DH5a

Alfalfa sprouts, broccoli sprouts, and clover sprouts were inoculated with *E. coli* DH5a. The sprouts were washed with DI water, Cl, or PAW-15 for 5 min to simulate post-harvest processing of the sprouts (figure 4.6). In clover sprouts, DI water had a similar microbial count than the unwashed samples. DI water reduced significantly ($p < 0.05$) the amount of *E. coli* by $0.9 \log \text{CFU gr}^{-1} \pm 0.2 \log \text{CFU gr}^{-1}$ in broccoli sprouts, and $1.3 \log \text{CFU gr}^{-1} \pm 0.2 \log \text{CFU gr}^{-1}$ in alfalfa sprouts. The results indicated that the process of post-harvest washing could decrease the bacterial load. An increase in the inactivation of *E. coli* was observed by treatment by Cl or PAW-15 in broccoli sprouts or clover sprouts, where these treatments achieved a reduction of 1 $\log \text{CFU gr}^{-1}$ to 2 $\log \text{CFU gr}^{-1}$. In addition, there was no significant difference between the inactivation results obtained by Cl and PAW-15 in broccoli sprouts and clover sprouts.

The mechanism of bacterial inactivation by PAW-15 could be a synergistic interaction between reactive species and pH drop (Oehmigen et al., 2010). A synergistic effect between the acidification of PAW and reactive species (i.e. peroxyntrous acid, peroxyntrite, nitrite), may lead to the disruption of the cell membrane of *E. coli* (Oehmigen et al., 2010). Peroxyntrite in PAW could be key for microbial inactivation, since this compound can cross the lipid bilayer of the cell membrane, then cause cell damage by lipid and protein peroxidation and nitration (Brisset & Pawlat, 2016; Zhou et al., 2018). The antibacterial activity of PAW could also come from the decomposition of reactive nitrogen species into oxidizing species, such as nitrous acid

to generate nitric oxide, and nitrate and nitrite anions (Traylor et al., 2011). Therefore, multiple components of PAW may aid in the ability of PAW to induce microbial inactivation.

Although Oehmigen (2010) and Traylor et al. (2011) achieved an inactivation of *E. coli* below detection levels, it is important to note that both investigated ability of PAW to inactivate *E. coli* in planktonic solution. Therefore, it is within reason that inactivation in the produce matrix would be lower. Although their PAW generation occurred also through plasma treatment of the liquid surface, they were performed at a much closer distance to the water surface, 5 mm. Finally, the authors had a significant concentration of hydrogen peroxide. This may have a synergistic effect with the reactive nitrogen species, for instance by reacting nitrite and hydrogen peroxide into peroxyxynitrite (Zhou et al., 2018). Further research may focus on reducing the distance between the water surface and the plasma jet to assess the presence of hydrogen peroxide or other reactive oxygen species, and their effect in improving microbial inactivation.

In the case of alfalfa sprouts, PAW-15 was also effective at decreasing the population of *E. coli* by $3.5 \log \text{CFU gr}^{-1} \pm 0.9 \log \text{CFU gr}^{-1}$, but Cl achieved the most considerable reduction ($7.1 \log \text{CFU gr}^{-1} \pm 0.3 \log \text{CFU gr}^{-1}$). Although unexpected, the results are consistent within the sample size and experimental error ($n=6$). The effect of PAW on alfalfa sprouts was similar to the results of Schnabel et al. (2015), who observed up to $4 \log \text{CFU gr}^{-1}$ reduction of *E. coli* on mung bean sprouts after a treatment time of 5 min. It was speculated that bacterial attachment might be weaker in alfalfa sprouts, compared to broccoli sprouts and clover sprouts, due to alfalfa sprouts having the least rough surface compared to the other sprouts. The effectiveness of PAW may depend on surface characteristics of the produce (Joshi et al., 2018). The theory was supported by the results of Fransisca et al. (2012), who observed that when seeds of alfalfa sprouts and seeds of broccoli sprouts were compared, the seeds of alfalfa sprouts had a smaller

surface roughness value and displayed a higher inactivation of *E. coli* after treatment with different sanitizers.

To investigate this theory, Confocal Laser Scanning Microscopy was used to look at unwashed samples of sprouts. The pictures indicated that the surface of alfalfa sprouts, broccoli sprouts, and clover sprouts were similar in appearance (figure 4.7). Therefore, other factors may be a stronger influence on the inactivation of *E. coli* on alfalfa sprouts. For instance, Joshi et al. (2018) pointed out that material hydrophobicity and the presence of cuticular waxes on the experimental samples might affect bacterial attachment. Also, it is speculated that a difference in the presence of biofilm could lead to the difference in reduction levels achieved by Cl and PAW within alfalfa sprouts. Biofilm has been detected on all parts of alfalfa sprouts, broccoli sprouts and clover sprouts from commercial sources, where the biofilms seemed to be rod-shaped bacteria (Fett, 2000). In that case, the bacteria within the biofilm would be much harder to remove and the effect of the treatments would be reduced (Center for Disease Control and Prevention, 2016). Increasing the plasma generation time could increase the concentration of reactive species in PAW (Chen, T. et al., 2018; Xiang et al., 2019), which would make it more comparable to Cl at a concentration of 200 ppm. Future research is needed to look into the presence of biofilm on alfalfa sprouts, broccoli sprouts, and clover sprouts by Scanning Electron Microscopy after treatment with PAW and Cl (Neo et al., 2013).

In a similar manner to the effect of wash treatments on total plate counts, Cl and PAW-15 had a significant inactivation effect on *E. coli* DH5 α . Although the act of washing with water could help reduce the incidence of *E. coli* on sprouts, the addition of a sanitizer agent significantly improved microbial reduction. Finally, PAW-15 had a similar sanitizing effect on

broccoli sprouts and clover sprouts, while still achieving a significant microbial reduction on alfalfa sprouts.

4.5. Conclusion

Although limited in clover sprouts, in the case of alfalfa sprouts and broccoli sprouts, PAW-15 achieved a significant reduction in total plate counts. The ability of each sanitizing solution (DI water, Cl, and PAW-15) to reduce the microbial counts of *E. coli* DH5 α was significantly different depending on the variety of sprout. However, there was no significant difference in ability of PAW-15 to inactivate *E. coli* DH5 α in broccoli sprouts and clover sprouts when compared to chlorine, and PAW-15 achieved a significant reduction of *E. coli* DH5 α in alfalfa sprouts. In general, PAW-15 treated sprouts did not show a significantly different effect on sprout quality compared to Cl treated sprouts in terms of total color difference and electrolyte leakage. PAW is a promising alternative to chlorine-based sanitizers for washing sprouts.

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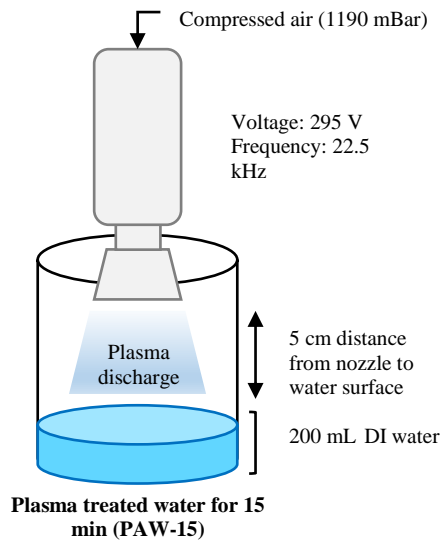


Figure 4.1: Schematic diagram of atmospheric pressure plasma jet (Plasmatreat Inc., IL, USA) to generate plasma-activated water.

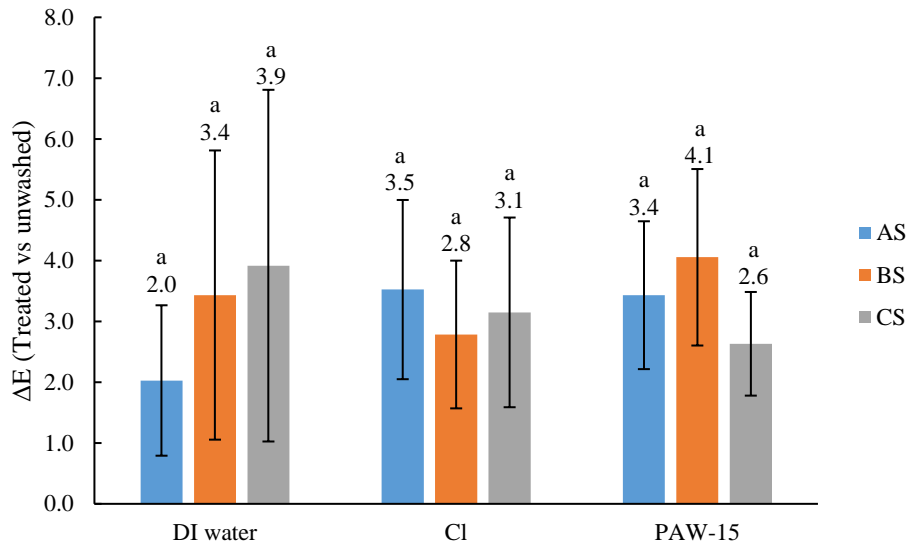


Figure 4.2: Total color difference between unwashed (UW) alfalfa sprouts (AS), broccoli sprouts (BS), and clover sprouts (CS) and sprouts treated by either DI water, Cl, or PAW-15 respectively.

Notes: Measurements were quantified in the CIE LAB color space. Each data point is an average of six measurements \pm standard deviation. Data that do not share the same letter are significantly different from each other (Tukey test, $p < 0.05$).

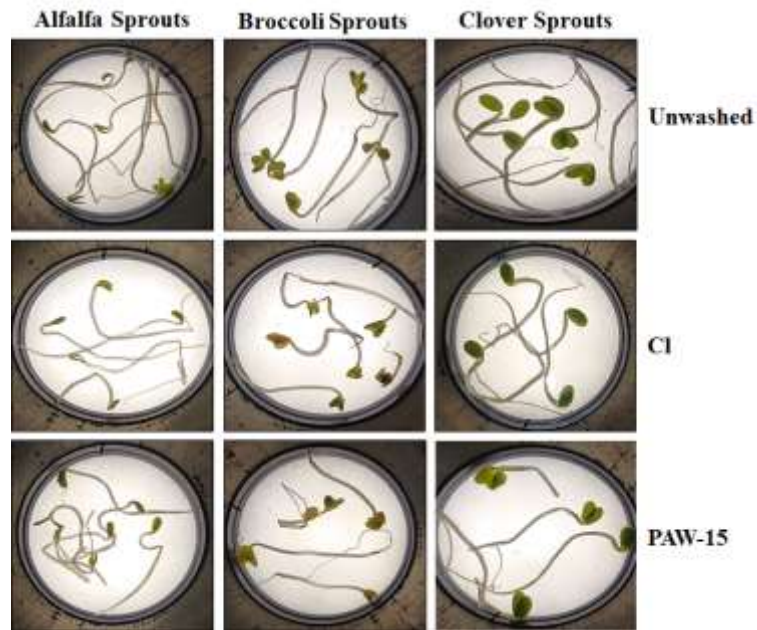


Figure 4.3: Pictures of alfalfa sprouts (AS), broccoli sprouts (BS), and clover sprouts (CS) that were unwashed, or washed with PAW-15 or Cl.

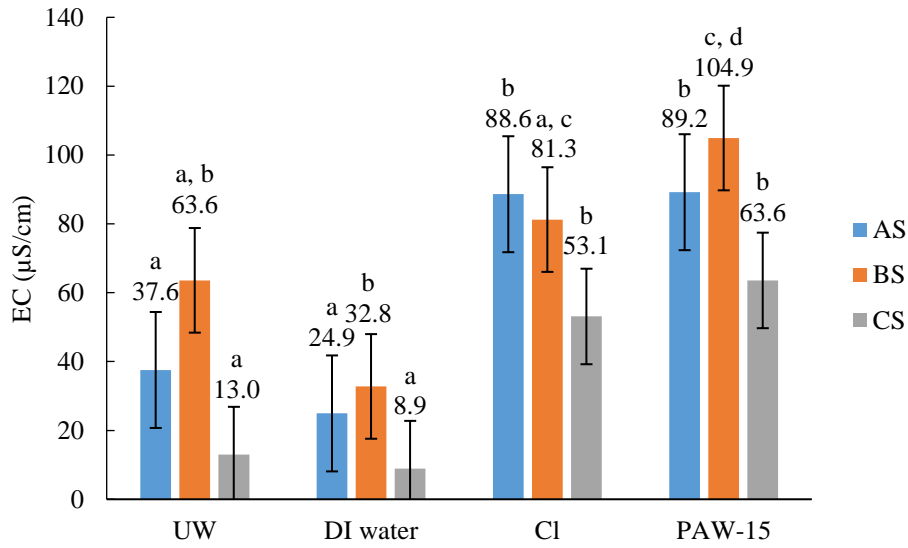


Figure 4.4: Electrolyte leakage measured by EC for unwashed (UW) alfalfa sprouts (AS), broccoli sprouts (BS), and clover sprouts (CS), as well as sprouts treated by DI water, Cl or PAW-15.

Notes: Each data point is an average of six measurements \pm standard deviation. Data that do not share the same letter are significantly different from each other (Tukey test, $p < 0.05$).

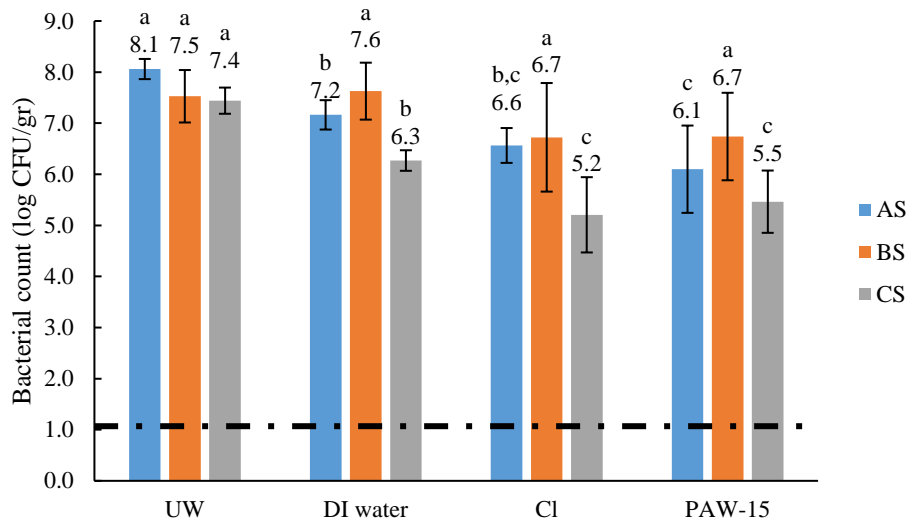


Figure 4.5: Logarithmic count of total plate counts for unwashed (UW) alfalfa sprouts (AS), broccoli sprouts (BS), and clover sprouts (CS), as well as sprouts treated by DI water, Cl or PAW-15.

Notes: Each data point is an average of six measurements \pm standard deviation. Data that do not share the same letter are significantly different from each other (Tukey test, $p < 0.05$). The dotted line indicates limit of detection (LOD 1 log CFU g^{-1}).

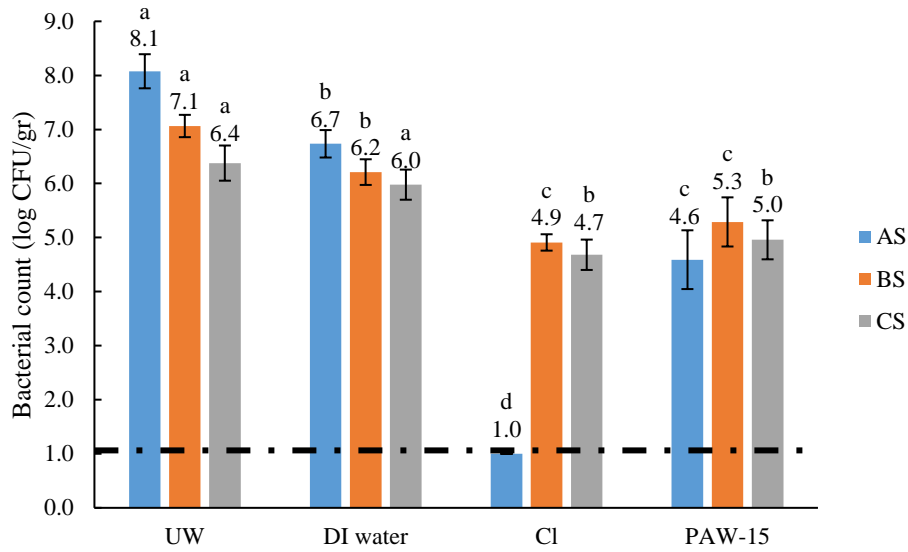


Figure 4.6: Logarithmic count of *E. coli* DH5 α for unwashed (UW) alfalfa sprouts (AS), broccoli sprouts (BS), and clover sprouts (CS), as well as sprouts treated by DI water, Cl or PAW-15.

Notes: Each data point is an average of six measurements \pm standard deviation. Data that do not share the same letter are significantly different from each other (Tukey test, $p < 0.05$). The dotted line indicates limit of detection (LOD 1 log CFU g^{-1}).

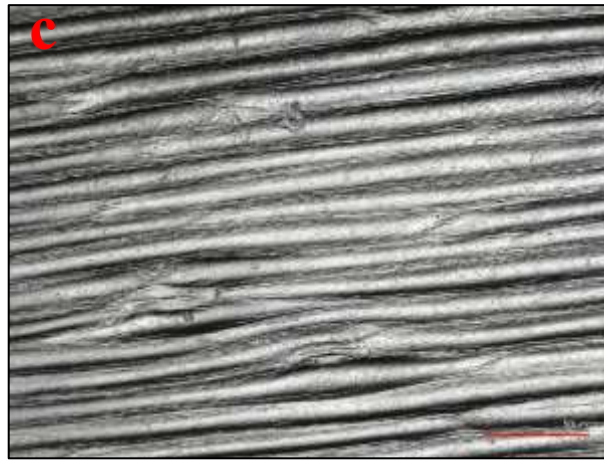


Figure 4.7: Pictures obtained from confocal Laser Scanning Microscopy of unwashed (UW) alfalfa sprouts (a), broccoli sprouts (b), and clover sprouts (c) at a scale of 50 μm .

Table 4.1: Summary of color measurements for unwashed (UW) broccoli sprouts, alfalfa sprouts, and clover sprouts, as well as sprouts treated by DI water, Cl or PAW-15. Measurement was quantified in the CIE LAB color space.

Notes: Each data point is an average of six measurements \pm standard deviation. Letters referred to statistical differences regarding to a $p < 0.05$, where items not connected by the same letter are significantly different.

Treatment	Sprout sample	CIE value		
		L*	a*	b*
UW	Alfalfa	67.3 \pm 1.0 ^a	-1.3 \pm 0.3 ^a	13.7 \pm 0.6 ^{a,b}
DI water	Alfalfa	66.2 \pm 1.5 ^a	-1.3 \pm 0.1 ^a	12.4 \pm 0.6 ^b
Cl	Alfalfa	67.9 \pm 1.7 ^a	-1.0 \pm 0.7 ^a	15.3 \pm 2.2 ^a
PAW-15	Alfalfa	68.3 \pm 2.3 ^a	-2.1 \pm 0.4 ^b	14.4 \pm 2.0 ^{a,b}
UW	Broccoli	64.6 \pm 1.2 ^{a,b}	-1.2 \pm 0.5 ^a	12.9 \pm 2.3 ^a
DI water	Broccoli	63.1 \pm 0.5 ^b	-0.9 \pm 0.7 ^a	13.1 \pm 2.1 ^a
Cl	Broccoli	63.7 \pm 2.3 ^{a,b}	-1.0 \pm 0.4 ^a	12.2 \pm 1.1 ^{a,b}
PAW-15	Broccoli	65.9 \pm 1.0 ^a	-1.4 \pm 0.6 ^a	10.0 \pm 1.5 ^b
UW	Clover	67.1 \pm 1.9 ^a	-2.6 \pm 0.5 ^a	13.8 \pm 1.5 ^a
DI water	Clover	64.5 \pm 1.7 ^b	-2.1 \pm 0.9 ^a	13.4 \pm 2.2 ^a
Cl	Clover	66.4 \pm 0.4 ^{a,b}	-1.9 \pm 0.7 ^a	14.8 \pm 1.6 ^a
PAW-15	Clover	66.7 \pm 1.5 ^{a,b}	-3.0 \pm 0.7 ^a	14.0 \pm 1.7 ^a

CHAPTER 5: Conclusions

This research focused on the optimization of soil-less farming of sweet basil with plasma-activated nutrient solution (PANS), and the optimization of post-harvest treatments of alfalfa sprouts, broccoli sprouts, and clover sprouts with plasma-activated water (PAW).

The effect of PANS on sweet basil growth in an ebb-and-flow hydroponic system in terms of yield, morphology, and quality was evaluated. PAW and PANS were generated using a gliding arc plasma generator, where tap water (TW) and nutrient solution (NS) were treated until pH dropped to 4 ± 0.5 . PAW and PANS contained a similar level of nitrite, a reactive nitrogen species produced by the plasma treatment. PAW and PANS were effective in reducing *E. coli* DH5 α by 5 log CFU mL⁻¹. The results suggest that PANS could imitate the antimicrobial ability of PAW, therefore plasma treatment could be adapted to hydroponic production of sweet basil to address microbial contamination in nutrient solutions. NS, the control solution, and PANS were used to grow sweet basil plants for 3 weeks in hydroponic chambers. The plants grown in PANS had a significantly higher number of nodes, number of branches, and node appearance rate after harvest. Also, the yield and quality of the plants was preserved. It is speculated that the experimental group did not show an improvement in yield or quality due to a drop in relative humidity between replicates 1 and 2. These results suggested that PANS could be applied in the hydroponic industry to enhance plant growth while maintaining microbial sanitation. This study contributes to the body of literature because it investigated the application of PANS to hydroponic sweet basil in terms of microbial inactivation, plant growth, and plant quality.

The application of PAW in the microbial inactivation of *E. coli* DH5 α and aerobic mesophilic bacteria on alfalfa sprouts, broccoli sprouts, and clover sprouts, and its effect on the

quality of these sprouts were investigated. The results suggest PAW and Cl (control sanitizer) achieved similar effectiveness on reducing *E. coli* DH5 α on broccoli sprouts and clover sprouts, which was better than the effectiveness of a wash with DI water. In the case of aerobic mesophilic bacteria, each sanitizing solution (DI water, Cl, and PAW) was effective in reducing the microbial load ($\sim 1 \log \text{CFU g}^{-1}$ to $2 \log \text{CFU g}^{-1}$). Also, PAW and Cl had a similar effect on the quality of the sprouts. However, the microbial reduction of *E. coli* DH5 α observed on alfalfa sprouts treated by PAW and Cl was significantly different. The results suggest that PAW was a superior sanitizing solution than DI water, since plasma treatment generated reactive nitrogen species and increased the acidity of the solution. PAW was also comparable to Cl in terms of microbial inactivation and quality suggesting PAW as a promising alternative to chlorine-based sanitizers for washing sprouts. This study contributes to the existing literature on the application of PAW to the inactivation of aerobic mesophilic microorganisms and a model non-pathogenic microorganism.

In conclusion, plasma treatment has shown promising results in the hydroponic farming of fresh produce at the pre-harvest stage as a treatment of nutrient solutions, or in the post-harvest stage for washing of fresh produce.

CHAPTER 6: Recommendations for Future Work

This study characterized PAW and PANS generated from the same plasma source. PAW and PANS were compared in terms of microbial inactivation for a treatment time of 5 min, and they were not tested against a control treatment. Further research should focus on comparing the effectiveness of PAW and PANS to a sanitizing agent currently used in the hydroponic industry, such as UV, chlorine, or heat. Furthermore, the sanitizing treatments should be tested on the recycled nutrient solution at different treatment times, in order to evaluate the ability of plasma treatment to reduce microbial contamination over time in the presence of organic matter. Likewise, the technologies could be tested for other microorganisms (i.e. plant pathogens or algae) to test their ability against the range of microbial contamination possible in the hydroponic industry.

Although the application of PAW to enhance plant growth has been widely researched, reporting of the environment in which the plants are grown is limited, and the data reported is not consistent in the literature. This study reported on the CO₂ concentration, temperature of the plants and nutrient solution, light conditions, and relative humidity of the environment that the plants experienced through the growth cycle. A significant drop in relative humidity between replicates 1 and 2 was found, which may have affected the development of the plants. Therefore, this study recommends the reporting of environmental conditions in research involving the application of PAW and PANS for plant growth. It is also recommended to conduct experiments under controlled environment conditions, such that the effects of the environmental variables could be mitigated between replicates.

Regarding the effect of PANS on plant growth, this research was a first step in validating the effect of PANS for the agriculture and food sciences. Further research should investigate the

mechanisms within the plant that are involved in the enhancement of plant growth. It is suggested that reactive species from plasma treatment are responsible for the increase in morphological traits in sweet basil, but more research is needed to understand the specific compounds involved and their interaction within the plant cells. A deeper understanding of plant metabolism will aid in further optimizing plasma treatment times, frequency, and operating parameters for nutrient solutions.

In the case of sprouts sanitation, further research should focus on characterizing surface roughness and natural microflora present on the sprouts. It was speculated that the differences in surface roughness between each type of sprout may have contributed to differences in effectiveness of the sanitizing solutions. In addition, further research should investigate the combinations of the plasma generation times, the distance between plasma discharge and water surface, and initial water volume, to optimize the PAW improve microbial reduction. Finally, it was speculated that biofilm could be present on the sprouts due to the warm and wet environment that they are grown in. Further research should observe unwashed sprouts and washed sprouts (DI water, Cl, and PAW) under Scanning Electron Microscopy (SEM), to examine the presence of biofilm and the effect of the sanitizing solutions on reducing this biofilm. Further research would lead to a deeper understanding of the differences in the effect of PAW on similar produce, such as types of sprouts, and expand the available literature.

APPENDICES

Appendix A

Appendix A: Daily light integral (DLI) or photosynthetic photon flux (PPF) at a height of 1 ft from the flooding tray to the light source, which were fluorescent white lights at a temperature of 6500 K.

Seedling Position	PPF ($\mu\text{mol m}^{-2} \text{sec}^{-1}$)	DLI ($\text{mol m}^{-2} \text{day}^{-1}$)
1	207	17.9
2	219	18.9
3	224	19.4
4	214	18.5
5	202	17.5
6	218	18.8
7	225	19.4
8	226	19.5
9	211	18.2
10	199	17.2
11	210	18.1
12	210	18.1
13	200	17.3
14	184	15.9

Appendix B

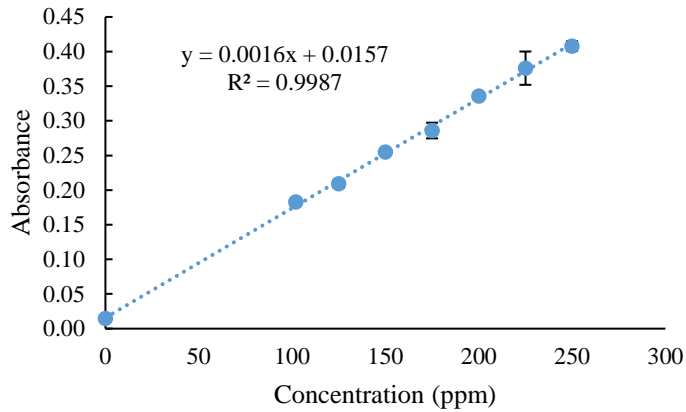
Appendix B: Solution nutrient analysis for experimental (PANS) and control (NS) growing chambers used to grow sweet basil plants for a period of 3 weeks.

Notes: Each result represents the average of 3 independent samples \pm standard deviation.

Different letters indicate statistical significance ($p < 0.05$)

Plant Nutrients	NS	PANS
Inorganic Nitrogen	156.0 \pm 1.4 ^a	173.5 \pm 0.7 ^b
NH ₄ -N	8.9 \pm 0.7 ^a	9.5 \pm 0.4 ^a
NO ₃ -N	147.5 \pm 0.7 ^a	164.0 \pm 1.4 ^b
P	66.0 \pm 6.2 ^a	53.7 \pm 3.1 ^a
K	213.5 \pm 0.7 ^a	240.5 \pm 9.2 ^a
Ca	123.5 \pm 2.1 ^a	123.5 \pm 3.5 ^a
Mg	54.0 \pm 0.8 ^a	53.8 \pm 1.6 ^a
S	88.0 \pm 3.8 ^a	87.9 \pm 2.9 ^a
Fe	2.7 \pm 0.02 ^a	2.8 \pm 0.0 ^a
Mn	0.43 \pm 0.01 ^a	0.46 \pm 0.02 ^a
Zn	0.17 \pm 0.01 ^a	0.23 \pm 0.01 ^b
Cu	0.18 \pm 0.01 ^a	0.27 \pm 0.01 ^b
B	0.54 \pm 0.04 ^a	0.54 \pm 0.03 ^a
Al	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a
Na	27.0 \pm 3.0 ^a	27.0 \pm 2.8 ^a
Cl	13.5 \pm 0.17 ^a	16.0 \pm 3.6 ^a

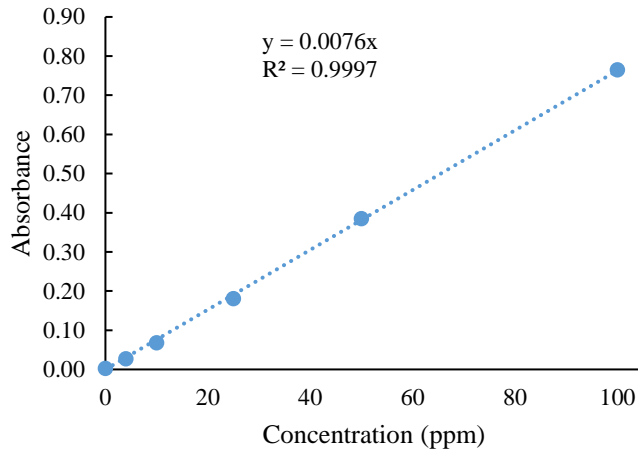
Appendix C



Appendix C: Standard curve to calculate the content of nitrate-nitrogen ($\text{NO}_3\text{-N}$) in water and nutrient solution based on the EMD Millipore Nitrate Cell Test (1.00614.001 Nitrate Cell Test, Merck Millipore, MA, USA).

Notes: Each bar represents the average of 3 independent samples \pm standard deviation.

Appendix D



Appendix D: Standard curve to calculate the content of nitrite (NO_2) in water and nutrient solution based on the EMD Millipore Nitrate Cell Test (1.00609.001 Nitrite Cell Test, Merck Millipore, MA, USA).

Notes: Each bar represents the average of 3 independent samples \pm standard deviation.

Appendix E

Appendix E: Solution nutrient analysis for experimental (PANS) and control (NS) growing chambers used to grow sweet basil plants. The analyses were performed on nutrient solution at the beginning (initial) and at the end (final) of the growing cycle of 3 weeks. R1 and R2 indicate replicate 1 and replicate 2.

Notes: At the end of replicate 1 there was a significant drop in relative humidity, which caused increased evaporation of the nutrient solution and concentration of the nutrients.

Plant	R1	R2	R1	R2	R1	R2	R1	R2
	NS	NS	PANS	PANS	NS	NS	PANS	PANS
Nutrients	Initial	Initial	Initial	Initial	Final	Final	Final	Final
	Plant nutrient concentration (ppm)							
Inorganic Nitrogen								
NH ₄ -N	157	155	232	138	173	174	327	140
NH ₄ -N	9.41	8.44	11	7.07	9.8	9.24	13	5.57
NO ₃ -N	148	147	221	131	163	165	315	134
P	61.6	70.3	189	92.8	51.5	55.9	137	72.4
K	214	213	421	212	234	247	518	232
Ca	125	122	191	124	126	121	252	110
Mg	54.5	53.4	93.7	61.2	54.9	52.6	135	59.8
S	85.3	90.7	171	104	85.8	89.8	246	113
Fe	2.73	2.7	3.29	2.72	2.77	2.77	4.1	1.78
Mn	0.42	0.44	0.6	0.35	0.44	0.47	0.82	0.28

Appendix E (cont.): Solution nutrient analysis for experimental (PANS) and control (NS) growing chambers used to grow sweet basil plants. The analyses were performed on nutrient solution at the beginning (initial) and at the end (final) of the growing cycle of 3 weeks. R1 and R2 indicate replicate 1 and replicate 2.

Notes: At the end of replicate 1 there was a significant drop in relative humidity, which caused increased evaporation of the nutrient solution and concentration of the nutrients.

Plant Nutrients	R1	R2	R1	R2	R1	R2	R1	R2
	NS	NS	PANS	PANS	NS	NS	PANS	PANS
	Initial	Initial	Initial	Initial	Final	Final	Final	Final
	Plant nutrient concentration (ppm)							
Zn	0.19	0.17	0.29	0.19	0.22	0.23	0.58	0.29
Cu	0.18	0.17	0.35	0.32	0.26	0.27	0.76	0.55
B	0.51	0.56	0.97	0.6	0.52	0.56	1.35	0.59
Al	0	0	0	0	0	0	0	0
Na	29.1	24.9	49.3	47.7	29	25	74.9	64.1
Cl	13.4	13.6	28.1	24.5	13.4	18.5	38.3	30.8

Appendix F

Appendix F: Solution nutrient analysis for experimental (PANS) and control (NS) growing chambers used to grow sweet basil plants. The analyses were performed on nutrient solution at the beginning (initial) and at the end (final) of the growing cycle of 3 weeks.

Notes: Each result represents the average of 2 independent samples \pm standard deviation.

Different letters indicate statistical significance ($p < 0.05$)

Plant Nutrient	NS	PANS	NS	PANS
	Initial	Initial	Final	Final
Plant nutrient concentration (ppm)				
Inorganic Nitrogen	156 \pm 1.4 ^a	173.5 \pm 0.7 ^a	185.0 \pm 66.5 ^a	233.5 \pm 132.2 ^a
NH ₄ -N	8.9 \pm 0.7 ^a	9.5 \pm 0.4 ^a	9.0 \pm 2.8 ^a	9.3 \pm 5.3 ^a
NO ₃ -N	147.5 \pm 0.7 ^a	164.0 \pm 1.4 ^a	176.0 \pm 63.6 ^a	224.5 \pm 128.0 ^a
P	66.0 \pm 6.2 ^a	53.7 \pm 3.1 ^a	140.9 \pm 68.0 ^a	104.7 \pm 45.7 ^a
K	213.5 \pm 0.7 ^a	240.5 \pm 9.2 ^a	316.5 \pm 147.8 ^a	375.0 \pm 202.2 ^a
Ca	123.5 \pm 2.1 ^a	123.5 \pm 3.5 ^a	157.5 \pm 47.4 ^a	181.0 \pm 100.4 ^a
Mg	54.0 \pm 0.8 ^a	53.8 \pm 1.6 ^a	77.5 \pm 23.0 ^a	97.4 \pm 53.2 ^a
S	88.0 \pm 3.8 ^a	87.8 \pm 2.8 ^a	137.5 \pm 47.4 ^a	179.5 \pm 94.0 ^a
Fe	2.7 \pm 0.0 ^a	2.8 \pm 0.0 ^a	3.0 \pm 0.4 ^a	2.9 \pm 1.6 ^a
Mn	0.4 \pm 0.0 ^a	0.5 \pm 0.0 ^a	0.5 \pm 0.2 ^a	0.6 \pm 0.4 ^a
Zn	0.18 \pm 0.01 ^a	0.23 \pm 0.01 ^a	0.24 \pm 0.07 ^a	0.4 \pm 0.21 ^a
Cu	0.18 \pm 0.01 ^a	0.27 \pm 0.01 ^b	0.34 \pm 0.02 ^a	0.7 \pm 0.2 ^a

Appendix F (cont.): Solution nutrient analysis for experimental (PANS) and control (NS) growing chambers used to grow sweet basil plants. The analyses were performed on nutrient solution at the beginning and at the end of the growing cycle of 3 weeks.

Notes: Each result represents the average of 2 independent samples \pm standard deviation.

Different letters indicate statistical significance ($p < 0.05$)

Plant Nutrient	NS	PANS	NS	PANS
	Initial	Initial	Final	Final
Plant nutrient concentration (ppm)				
B	0.5 ± 0.0^a	0.5 ± 0.0^a	0.8 ± 0.3^a	1.0 ± 0.5^a
Al	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Na	27.0 ± 3.0^a	27.0 ± 2.8^b	48.5 ± 1.1^a	69.5 ± 7.6^a
Cl	13.5 ± 0.1^a	16.0 ± 3.6^a	26.3 ± 2.5^a	34.6 ± 5.3^a